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TOWARD SOLUTIONS FOR STRESSED LANDS

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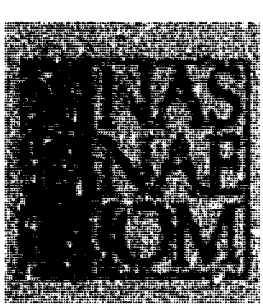

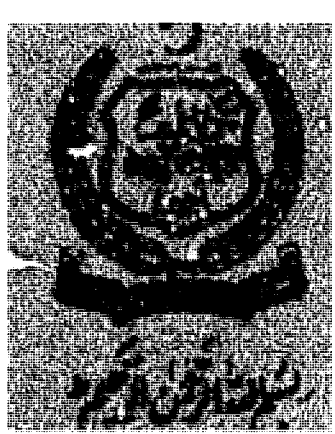

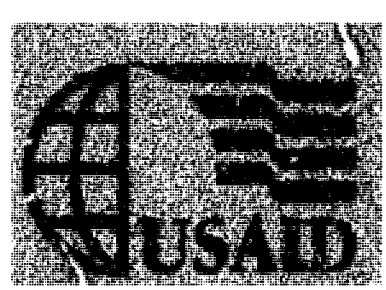

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TOWARD SOLUTIONS FOR STRESSED LANDS

Research Manuscripts

THE BOSTID-PARC RESEARCH PROGRAM

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TOWARD SOLUTIONS FOR STRESSED LANDS

THE BOSTID/PARC RESEARCH GRANTS PROGRAM

Board on Science and Technology for International Development (BOSTID)
Pakistan Agricultural Research Council (PARC)

The manuscripts in this collection present research conducted under the BOSTID-PARC Research Grants Program, a joint U.S.-Pakistan endeavor to strengthen collaborative and competitive science in Pakistan. Funded by the U.S. Agency for International Development (USAID) in Islamabad, this program focused on one of Pakistan's most significant challenges: improving the productivity of agriculture stressed by heat, drought, flood, pests, and poor soils.

At the beginning of the program, Pakistani scientists were requested to submit research proposals to a joint Committee on Pakistan Agricultural Research. An impressive 117 responses were received. After initial review, candidates with promising proposals received visits from U.S. scientists. This allowed these researchers to discuss and refine their proposed research, and to consider comments and ideas from external reviews of their proposals. After further revision and review, 25 proposals were selected by the Committee; these were funded by USAID. The research projects were organized into five groups: stress tolerance, soil fertility, water and range mangement, molecular biology, and integrated pest management. A senior U.S. scientist advised each group. On the following page is a roster of advisors, committee, and staff.

The researchers carried out their studies over a two-year period, during which they began their experiments, acquired new equipment, made research visits to the United States, and were visited in turn by U.S. experts in specific areas. Grantees also participated in meetings of all grantees and of the five advisory groups. In addition, they presented their work at international conferences and at a final conference of BOSTID-PARC grantees. Moreover, they have prepared drafts of research results for ultimate publication in international scientific journals.

It is these manuscripts that are in this volume. It is to the credit of the researchers that they have been able to bring their work together in as short a time as two years. Each draft has undergone external peer review in the United States, and eventually many papers will be further revised to include data taken over a longer time period. Because the documents are in various stages of revision and often present only initial findings, **the consent of the author is necessary before citing any manuscript.**

All research teams made significant progress in the quest to improve agriculture in the stressed lands of Pakistan. Despite difficulties and limitations, much innovative and exciting research was performed. Many operational and scientific obstacles were overcome. Most project teams are dedicated to continue, strengthen, and expand their research efforts. The transfer of science to practice unfolds slowly, but I believe the world will benefit in the years ahead from research performed under USAID's BOSTID-PARC Research Program.

Judith Bale, Director
Pakistan Research Program

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Comparative physiology and root anatomy of two wheat varieties exhibiting differential growth response to salinity and hypoxia

Shafqat Nawaz*, R. H. Qureshi*, R. G. Wyn Jones** and M. Aslam*

*Saline Agriculture Research Cell, Dept. of Soil Science, University of Agriculture, Faisalabad, Pakistan.
**Science and Policy Development Countryside Council for Wales Plas Penrhos, Bangor Gwynedd, LL57 2LQ, UK.

Key words: Salinity, hypoxia, wheat, tolerant, enzyme, ions.

ABSTRACT

Wheat is the major cereal sown in saline-waterlogged, sodic and compact saline soils and is likely to be faced with waterlogging during winter. Under waterlogged conditions the effects of salinity stress are expected to be accentuated further. This experiment was laid out in matrix culture at two salinity levels (0 and 100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$) and two aeration levels (aerobic and waterlogged) by applying modified Phostrogen nutrient solution to determine the mechanisms of tolerance to the combined stress of salinity and waterlogging. Waterlogged condition were developed by keeping the solution standing in the root zone. The tolerant variety (Blue Silver) produced comparatively more shoot and root fresh weight than the sensitive variety (7-Cerros) while shoot:root ratio was not affected. Accumulation of Na^+ was more in the three young leaves of the sensitive variety compared with the tolerant variety whereas the concentration of K^+ showed the opposite trend. Na^+ concentration increased and that of K^+ decreased as the age of the leaf increased. CO_2 assimilation rate was higher in Blue Silver while transpiration rate showed no differences under saline-waterlogged condition. Simultaneously, the tolerant variety induced more aerenchyma in the root up to its tip and the sensitive variety induced very few aerenchyma near the root tip. The activity of lactate dehydrogenase (LDH) was higher and that of alcohol dehydrogenase (ADH) was lower in the roots of Blue Silver, indicating a favourable biochemical pathway followed by this variety.

INTRODUCTION

Restricted drainage conditions cause waterlogging not only in high rainfall areas, but also in semi-arid regions when seasonal rains may cause localized flooding (Rickman and Klepper, 1980). Poor drainage affects perhaps as much as one half of the world's irrigated land (Donman and Houston, 1967), while 400×10^6 hectares are affected by excess of salts (Mudie, 1974). Excess of water in the root zone coupled with high soil temperature can rapidly deplete soil oxygen and damage crops (Hodgson, 1982). Salinity superimposed on waterlogging imposes additional stress (see Barrett Lennard, 1986).

Plant breeders and geneticists seek to modify plants to suit adverse soil conditions while maintaining reasonable and reliable yields. This approach has recently received more attention as a possible means of utilizing saline lands and waters (Epstein and Rains, 1987; Qureshi et al., 1990).

Anaerobic conditions, like the presence of an uncoupling agent, almost immediately inhibit uptake from solution and radial transport of nutrient ions by roots of non-wetland species (Cheeseman et al., 1980). This indicates that anaerobic metabolism provides energy insufficient to maintain the activity of ion pumps so that the transfer of some ions from the rooting medium to the shoot may only take place passively; ions move with little selectivity across damaged cell membrane by mass flow with the transpirational stream (Trought and Drew, 1980). Thus a higher concentration of certain ions in the outer medium can offset the anoxia-induced inhibition of transport to the shoot (Trought and Drew, 1981).

A range of tolerance to the stress is the requirement of breeding programmes focussing on the development of salt tolerant varieties.

However, the basic information regarding various mechanisms involved in tolerance to a certain stress can greatly facilitate the process of breeding for stress tolerance. Especially, there is a need to know about heritability of a given trait and how traits interact with saline areas as these are very heterogenous.

Our earlier studies on wheat showed that different varieties responded differently to salinity (see Rashid, 1986; Qureshi et al., 1990), hypoxia (Jackson and Drew, 1984) and the dual stress which provided a basis for a future systematic study of the mechanisms of tolerance of wheat to these stresses.

This experiment was designed, on the basis of the results described earlier (Nawaz et al., 1992) to analyse some of the different physiological, biochemical and anatomical mechanisms involved in wheat for tolerance to salinity and hypoxia.

Materials and Methods

Two varieties of wheat, Blue Silver (tolerant to both salinity and hypoxia) and 7-Cerros (moderately tolerant to salinity but sensitive to hypoxia) were selected. Seedlings were grown under greenhouse conditions (photoperiod 16 hrs, day temp. 25°C, night temp. 20°C and PAR 400-700 nm) in matrix culture (mixture of gravel, compost and vermiculite) in plastic pots (30 x 20 x 15 cm^3). Treatments applied were control (non-saline aerobic), hypoxic (non-saline anaerobic), saline (100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$ with aerobic condition) and saline hypoxic (100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$) but anaerobic condition). Twenty replications were considered each of a single plant and a completely rendomized design was adopted. Nutrition was supplied by adding Phostrogen (a 10-10-27 blend of NPK fertilizer containing 1.3% Mg^{2+}, 0.4% Fe^{2+} and 200 ppm Mn^{2+}) @ 1 g L^{-1} and micronutrients as used in Hoagland's solution (0.5 mL L^{-1}). Salinity was developed stepwise over 4 days (25 mol m^{-3} NaCl

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+ 2.5 mol m^{-3} $CaCl_2$ per day) when the plants were at the two leaf stage. Hypoxia stress was superimposed on the day after the final salinity had been achieved. Nutrient solutions (non-saline and saline) were added and immediately drained daily in the case of the aerobic treatments while for the hypoxic treatments solutions were changed first fortnightly and then weekly after bubbling excess of N_2 gas through the solution to remove dissolved O_2.

During the fourth week of stress, data for net CO_2 assimilation rate, stomatal conductance and transpiration rate from the youngest fully expanded leaf were recorded by means of an Infra-Red Gas Analyzer (LCA 2, The Analytical Development Co. Ltd. Pindar Road, Hoddesdon, Herts, England). At the end of the fourth week the plants were harvested and fresh weight of shoot and root were recorded. The youngest first, second and third leaves were separated to determine the concentration of Na^+ and K^+ (flame photometrically) in the tissue sap after appropriate dilution (Richards, 1954). Root samples were preserved for measuring the activities of enzymes (alcohol dehydrogenase and lactate dehydrogenase), and for determining the extent of aerenchyma (Hoffman et al., 1986).

Data were analyzed statistically by Analysis of Variance for three factors (salinity, hypoxia and variety) and their interactions by using MSTATC statistical package (Crop and Soil Science Department, Michigan State University) using ANOVA to assess significant differences ($P<0.05$) between means.

RESULTS AND DISCUSSION

The main effects of salinity and hypoxia and their interaction on varieties were statistically significant ($P<0.05$) for most of the parameters. This means that the effects of salinity x hypoxia were different on different varieties. Hence, in the

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results, emphasis was placed upon determining the relative and absolute performance of the two wheat varieties under the various stress conditions: means were compared in rows as well in columns.

Genotypic differences in growth response of wheat to salinity and hypoxia

Salinity and hypoxia individually and in combination reduced the fresh weight of shoot significantly compared with the control (Table 1). Hypoxia caused a mean reduction of 49.2% in shoot fresh weight compared with the control while the reduction was 81.1% due to salinity stress and 86.8 % for the combined stress of salinity and hypoxia. This suggested that the effects of salinity and hypoxia were non-additive.

There were no significant differences between the two varieties in the control treatment. The fresh weight of both varieties decreased significantly due to hypoxia, salinity or the combined stress, but cv. 7-Cerros was more adversely affected than cv. Blue Silver and had a significantly lower fresh shoot weight than cv. Blue Silver under all stresses.

Hypoxia had no significant effect on mean root fresh weight under both non-saline and saline conditions, but salinity significantly reduced the root growth under both aerobic and anaerobic conditions (Table 2). Hypoxia significantly reduced the fresh weight of roots under non-saline conditions in cv. 7-Cerros while in the case of Blue Silver, root weight increased, although this was non-significant. Under saline conditions there was no significant effect of hypoxia on either variety. Salinity decreased the fresh weight of root significantly in both the varieties under both aerobic and hypoxic conditions. Blue Silver had significantly greater root fresh weight than 7-Cerros under hypoxic and saline-hypoxic conditions. Thus the

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genotypic differences of the two varieties to various stresses are apparent, Blue Silver being more tolerant than 7-Cerros. The main reasons for growth depression of plants due to salinity and hypoxia are well documented (see Greenway and Munns, 1980; Kozlowski, 1984)

Ionic relations of wheat under salinity and hypoxia

Salinity significantly increased the accumulation of Na^+ compared with the control (Table 3). Hypoxia alone did not increase the Na^+ concentration significantly compared with control in all the three leaves, as might be expected as no additional Na^+ was available to the plants. The combined stress of salinity and hypoxia significantly increased Na^+ concentration compared with all other treatments.

Hypoxia caused a significant increase in Na^+ concentration under saline conditions while the difference under non-saline conditions was non-significant. On the other hand, salinity significantly increased Na^+ concentration in all the leaves studied, under both aerobic and hypoxic conditions.

Comparison of the two varieties under different treatments indicated that Na^+ concentration differed significantly only under salinity alone in which 7-Cerros accumulated significantly more Na^+ than Blue Silver in all the three leaves. Blue Silver could control Na^+ accumulation better under saline condition than 7-Cerros but under saline-hypoxic condition this control mechanism via the energy dependent selective uptake of Na^+ was apparently disrupted.

The slope of the regression line drawn between Na^+ concentration in the youngest fully expanded leaf and shoot fresh weight under both saline-aerobic and saline-hypoxic treatments (Fig. 1) showed that under saline-hypoxic conditions the toxic

effect of the tissue Na^+ was lesser than in the case of saline-aerobic conditions [apparently more Na^+ concentration should depress the yield to a greater extent, (see Rashid, 1986; Flowers et al., 1991)]. This could be due to greater accumulation of some compatible osmotica along with effective intracellular compartmentation under this treatment. Perhaps higher LDH activity in the root under saline-hypoxic conditions (see later) plays a role in this, but this needs further studies for elucidation.

Salinity increased K^+ concentration significantly compared with control (non-saline aerobic), hypoxic and saline-hypoxic treatments in all the three leaves, while in the case of second and third youngest leaves, hypoxia alone accumulated significantly less K^+ than the other treatments (Table 4). Hypoxia decreased K^+ concentration under both saline and non-saline conditions for all comparisons except for the youngest fully expanded leaf under non-saline conditions.

A significantly higher accumulation of K^+ was observed in Blue Silver than in 7-Cerros in the second and third youngest leaves under saline treatments, while in other treatments such differences were non-significant.

The increased concentration of K^+ under salinity may be related to decreased growth while the decreased concentration may be due to reduced selectivity at the root membrane level due to reduced availability of energy (Cheeseman and Hanson, 1979).

Hypoxia and salinity individually reduced K^+:Na^+ ratio compared with control (non-saline aerobic) (Table 5). The combined stress of salinity and hypoxia further decreased this parameter but the difference was significant only for the third leaf. The decrease in K^+:Na^+ ratio was greater under salinity stress and differed

significantly compared with hypoxic stress. Salinity reduced $K^+:Na^+$ ratio significantly under both aerobic and hypoxic conditions. Blue Silver showed Na^+ concentration similar to that of 7-Cerros but due to higher K^+ concentration it maintained a relatively higher K^+ : Na^+ ratio in all the three leaves. Higher K^+ or $K^+:Na^+$ in leaves conformed to the better tolerance of Blue Silver than 7-Cerros as the relationship between salt tolerance and K^+ : Na^+ ratio has been indicated by a number of workers (see Rashid, 1986; Wolf et al., 1991).

CO_2 assimilation and transpiration rates

CO_2 assimilation rate was significantly lower under all the stresses compared with control, although the differences between the stress treatments were non-significant (Table 6). Hypoxia did not reduce CO_2 accumulation rate significantly in the individual varieties under both non-saline and saline conditions. On the other hand, salinity significantly decreased CO_2 assimilation rate in 7-Cerros under saline and saline hypoxic conditions, and this was significantly lower than in Blue Silver under comparable conditions.

All the stresses caused a significant reduction in the stomatal conductance (Table 7) the maximum reduction was observed under the saline-hypoxic treatment and was of the same order in both the varieties (83.6 and 85.4 % in Blue Silver and 7-Cerros, respectively). It was interesting to note that stomatal conductance decreased to a higher extent than the decrease in CO_2 assimilation rate in the stress treatments.

Blue Silver showed a significantly lower transpiration rate under the saline-hypoxic treatment compared with the control (non-saline aerobic) (Table 8). However, 7-Cerros did not show any significant difference in transpiration rate in stress treatments. Blue Silver had a significantly higher transpiration rate than 7-Cerros

under saline aerobic condition. The combined stress of salinity and hypoxia depressed CO_2 assimilation rate, transpiration rate and stomatal conductance. Under saline and saline-hypoxic conditions, Blue Silver showed significantly higher rates of CO_2 assimilation and transpiration (saline) than 7-Cerros, indicating a more successful production of photosynthates and hence the ability to generate tolerance against the dual stress of salinity and hypoxia.

Specific enzymes

Hypoxia significantly increased the activity of LDH under both non-saline (both varieties) and saline (Blue Silver only) conditions (Table 9). Salinity, on the other hand, significantly increased LDH activity of under aerobic conditions (both varieties) but had no significant effect under hypoxic conditions. In general, LDH activity in the wheat root was more due to hypoxia than salinity. Blue Silver showed significantly higher LDH activity in the roots compared with 7-Cerros under saline hypoxic treatment only.

Hypoxia had a non-significant effect on ADH activity under both non-saline and saline conditions (Table 10). Salinity, on the other hand, significantly decreased the activity of ADH under both aerobic and hypoxic conditions.

Comparison of the varieties for root ADH activity under different treatments indicated that under both the aerobic conditions (non-saline and saline), there was no significant difference between the varieties, but under both the hypoxic conditions, 7-Cerros showed significantly higher ADH activity than Blue Silver. Under hypoxia, the mode of respiration is changed from aerobic to anaerobic. Under anaerobic respiration pyruvate, the end-product of glycolysis, can be converted into ethanol or lactate and NAD^+ is regenerated which can help to continue

glycolysis. The enzymes involved in these reactions are alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH), respectively. In theory flood tolerance of a number of herbaceous species is related to the capacity to avoid the generation of potentially toxic ethanol at the end of the glycolytic pathway (Crawford, 1967).

Hypoxia significantly increased the activity of LDH under both non-saline and saline conditions in Blue Silver which had significantly higher LDH activity than 7-Cerros under saline hypoxic stress. Although the differences were non-significant, ADH activity showed a decreasing tendency in Blue Silver under both saline and non-saline conditions, while in the case of 7-Cerros it increased. Comparison of the varieties under hypoxic conditions showed a significantly higher activity of ADH in the roots of 7-Cerros than that of Blue Silver indicating the adverse effects of the alcohol that might be accumulated as a result of higher ADH activity. The results suggest that LDH was the preferred pathway in responding to hypoxia in these cultivars, and indicate that Blue Silver can adapt to hypoxic conditions better than 7-Cerros as conversion of pyruvate into lactate was dominant over the ethanol production. It was expected that in the case of 7-Cerros the pathway was shifted more towards the production of ethanol than lactate and possibly this ethanol became toxic to the root cells. The adverse effects of ethanol under flooded conditions have been mentioned by Fulton and Erikson (1964), Crawford (1967) and Smith and apRees (1979). Nevertheless, contrary to these findings, rice and the relatively flood-tolerant winter wheat and rye synthesize appreciable amounts of ethanol under anaerobic conditions (Bertani et al., 1980).

Root anatomy

Information on the effect of hypoxia on the aerenchyma formation in wheat under saline and non-saline conditions is presented in Figure 2 and the sketched diagram

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in Figure 3. As these are individual values, although developed from five to seven observations, statistical analysis was not possible. No aerenchyma were observed in any variety under aerobic conditions (non-saline or saline). In Blue Silver, the extent of aerenchyma increased from the root-shoot junction (17.6%) towards the root tip (35.2%) under hypoxia alone. Under saline hypoxic conditions the trend was somewhat different as the maximum extent of the aerenchyma (22.2%) was observed in the middle portion of the root. In the case of 7-Cerros the cross-sectional area of root occupied by aerenchyma, generated under hypoxia, decreased from the root-shoot junction towards the root tip under both non-saline and saline conditions.

Under saline-hypoxic treatment the extent of the aerenchyma was again at a maximum near the root-shoot junction in 7-Cerros and decreased towards the tip, while in Blue Silver the maximum cross-sectional area of aerenchyma was observed at the middle of the root and decreased towards both, root tip and the root-shoot junction. In general, the development of aerenchyma was more pronounced in Blue Silver at the middle of the root and near the root tip than in 7-Cerros.

In many wetland species e.g. rice and beetle grass, the development of aerenchymatous tissue, which arises by cell separation or by partial breakdown of the cortex, provides continuous gas-filled lacunae. The formation of aerenchyma is under genetic control in wetland species but restricted aeration can encourage further increase in the internal porosity of the roots. In many non-wetland herbaceous species aerenchyma may also develop in response to hypoxia in especially adapted roots (Trought and Drew, 1980). These roots functionally replace the original root system which is inhibited by the lack of oxygen.

The first adverse effect of hypoxia occur on the root tips as these are the tissues farthest from the shoot and the oxygen supply. At the same time the oxygen requirement of the apical meristem tissue is 25 times higher than the root cortex (Webb and Armstrong, 1983) because of the maximum biochemical activity of the apical meristematic region.

The relative distribution of aerenchyma in various parts of the root indicated a fast radial leakage of O_2 but an impeded longitudinal diffusion in 7-Cerros, so very little O_2 reached the meristematic tissues. On the other hand, in Blue Silver it may be hypothesized that sufficient O_2 may be reaching the meristematic tissues so that O_2 supply might not be a limiting factor as in 7-Cerros. These findings are in agreement with the mechanisms found by other workers (Armstrong, 1979; Trought and Drew, 1980) who reported that the concentration of O_2 reaching the apical meristem depends on the diffusion pathway length from the aerial parts, the extent of aerenchyma, the respiratory use of O_2 and radial leakage. In these studies the root growth ceased when the internal O_2 partial pressure declined to 0.03 - 0.05 atmosphere (Jackson, 1982).

Conclusion

Tolerance to salinity and hypoxia in wheat was related to ionic relations (the greater the accumulation of K^+ and $K^+:Na^+$ in the tissue sap, the higher was their tolerance), biochemistry of roots (a higher level of LDH and a lower level of ADH was associated with better tolerance to hypoxia) and root anatomy (better aerenchyma development in the root meristem was related to better tolerance to hypoxia).

Nevertheless, it appears that root anatomy in terms of aerenchyma development was the overriding factor in increasing tolerance to hypoxia, while the better LDH/low

ADH activity and control over Na^+ accumulation were probably secondary effects of increased O_2 supply to the root through the aerenchyma.

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LITERATURE CITED:

Armstrong, W. 1979. Aeration in higher plants. Adv. Bot. Res. 7:225-331.

Barrett-Lennard, E. G. 1986. Effects of waterlogging on the growth and NaCl uptake by vascular plants under saline conditions. Reclamation and Revegetation Research. 5:245-261.

Bertani, A., I. Brambilla and F. Menegas, 1980. Effect of anaerobiosis of rice seedlings: growth, metabolic rate and fate of fermentation products. J. Expt. Bot. 31:325-331.

Cheeseman, J. M. and J. B. Hanson. 1979. Energy-linked potassium influx as related to cell potential in corn roots. Plant Physiol. 64:842-45.

Cheeseman, J. M., P. R. LaFaytte, J. W. Gronewald and J. B. Hanson. 1980. Effect of ATPase inhibitors on cell potential and K^+ influx in corn roots. Plant Physiol. 65:1139-1145.

Crawford, R. M. M. 1967. Alcohol dehydrogenase activity in relation to flooding tolerance in roots. J. Expt. Bot. 18:458-464.

Donman, W. W. and C. E. Houston. 1967. Drainage related to irrigation management. In: R. W. Hagan, H. R. Haise and T. W. Edminster (Eds.) Drainage of Agricultural Lands. American Society of Agronomy. pp. 974-987.

Epstein, E. and D. W. Rains. 1987. Advances in plant tolerance. Plant and Soil. 99:17-29.

Flowers, T. J., M. A. Hajibagheri and A. R. Yeo. 1991. Ion accumulation in the cell walls of rice plants growing under saline conditions: evidence for the Oertli hypothesis. Plant Cell and Environ. 14:319-325.

Fulton, J. M. and A. E. Erickson. 1964. Relation between soil aeration and alcohol accumulation in xylem exudates of tomatoes. Soil Sci. Soc. Am. Proc. 28:610-614.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in non-halophytes. Ann. Rev. Plant Physiol. 31:149-90.

Hodgson, A. S. 1982. The effects of duration , timing and chemical amelioration short-term waterlogging during furrow irrigation of cotton in a cracking clay. Aust. J. Agric. Res. 33:1019-1028.

Hoffman, N. E., A. F. Bent and A. D. Hanson. 1986. Induction of Lactate Dehyd rogenase Isozymes by oxygen deficit in barley root tissue. Plant Physiol. 82:658-663.

Jackson, M. B. 1982. Ethylene as a growth promoting hormone under flooded conditions. In: P. F. Waring (Ed.) Plant Growth Substances. Academic Press, London. pp. 291-301.

Kozlowski, T. T. 1984. Flooding and Plant Growth. Academic Press, New York.

Mudie, P. J. 1974. The potential economic uses of halophytes. In: R. J. Rienold and W. H. Queens (Eds.) Ecology of Halophytes. Academic Press Inc., New York. pp. 565-97.

Nawaz, S., R. H. Qureshi, M. Aslam and S. Parveen. 1992. Comparative performance of different wheat varieties under salinity and waterlogg. I. Growth parameters. Pak. J. Agri. Sci. 29(4):376- 80.

Qureshi, R. H., A. Rashid and N. Ahmad. 1990. A procedure for quick screening of wheat cultivars for salt tolerance. In: N. ElBassam, M. Damborth and B. C. Laughman (Eds.) Genetic Aspects of Plant Mineral Nutrition, Kluwer Academic Publishers, Netherland. pp. 315-324.

Rashid, A. 1986. Mechanisms of salt tolerance in wheat (Triticum aestivum L.). Ph. D. Thesis, Univ, of Agirc. Faisalabad, Pakistan.

Richards, L. A. 1954. Diagnosis and Improvement of Saline and Alkali Soils. USDA HandBook No. 60, U. S. Govt. Printing Office, Washington DC.

Rickman, R. W. and B. L. Klepper. 1980. Wet season aeration problems beneath surface mulches in dry land winter wheat production. Agron J. 72:733-736.

Smith, A. M. and T. apRees. 1979. Effects of anaerobiosis on carbohydrate oxidation by roots of Pisum sativum. Phytochemistry. 18:1453-1458.

Trought , M. C. T. and M. C. Drew. 1980. The development of waterlogging damage in wheat seedlings (Triticum aestivum L.) I. Shoot and root growth in relation to changes in the concentrations of dissolved gas and solutes in the soil solution. Plant Soil. 54:77-94.

Trought, M. C. T. and M. C. Drew. 1981. Alleviation injury to young wheat plants in anaerobic solution cultures in relation to the supply of nitrate and other inorganic nutrients. J. Expt. Bot. 32:509-522.

Webb, T. and W. Armstrong. 1983. The effects of anoxia and carbohydrates on growth and viability of rice, pea and pumpkin roots. J. Expt. Bot. 34:579-603.

Wolf, O., R. Munns, M. L. Tonnet and W. D. Jeschke. 1991. The role of the stemi the partitioning of Na^+ and K^+ in salt-treated barley. J. Expt. Bot. 42(239): 697-704.

Table : 1 Effect of hypoxia x salinity interactions on fresh weight of shoot (g plant^{-1}) in two wheat varieties.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	8.31^{*a}	5.15^{b}_{a}	1.92^{c}_{a}	1.30^{c}_{a}
7-Cerros	8.51^{a}	3.39^{b}_{b}	1.27^{c}_{b}	0.91^{c}_{b}
Mean	8.41 A	4.27 B	1.59 C	1.11 C

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 20

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)
Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table: 2 **Effect of hypoxia x salinity interactions on fresh weight of root (g plant^{-1}) in two wheat varieties.**

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	1.62*^{a}	1.73 $^{a}_{a}$	0.48 ^{b}	0.48 $^{b}_{a}$
7-Cerros	1.59 ^{a}	1.12 $^{b}_{b}$	0.40 ^{c}	0.37 $^{c}_{b}$
Mean	1.61 A	1.42 A	0.44 B	0.43 B

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 20

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)
Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table: 3 Effect of hypoxia x salinity interactions on Na^+ (mol m^{-3}) in different leaves in two wheat varieties.

A: The youngest fully expanded leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	1.78 ^{c*}	1.85 ^{c}	29.49 $^{b}_{b}$	147.68 ^{a}
7-Cerros	2.21 ^{c}	4.88 ^{c}	69.10 $^{b}_{a}$	151.36 ^{a}
Mean	1.99 C	3.37 C	49.29 B	149. 2 A

B: The second youngest leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	4.83* ^{c}	3.72 ^{c}	41.98 $^{b}_{b}$	163.59 ^{a}
7-Cerros	3.28 ^{c}	6.94 ^{c}	86.57 $^{b}_{a}$	172.16 ^{a}
Mean	4.05 C	5.33 C	64.27 B	167.87 A

C: The third youngest leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	6.89* ^{c}	6.65 ^{c}	59.00 $^{b}_{b}$	185.92 ^{a}
7-Cerros	5.08 ^{c}	8.30 ^{c}	133.27 $^{b}_{a}$	197.42 ^{a}
Mean	5.99 C	7.48 C	96.13 B	191.67 A

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 20

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)
Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table: 4 Effect of hypoxia x salinity interactions on K^+ (mol m^{-3}) in different leaves in two wheat varieties.

A: The youngest fully expanded leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	216.3*^{b}	204.0^{bc}	256.7^{a}	216.3^{b}
7-Cerros	196.0^{b}	187.7^{b}	244.7^{a}	187.9^{b}
Mean	206.1 B	195.9 B	251.2 A	202.1 B

B: The second youngest leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	200.7*^{c}	188.1^{cd}	249.8$^{a}_{a}$	216.1$^{b}_{a}$
7-Cerros	183.0^{ab}	171.1^{bc}	196.6$^{a}_{b}$	164.3$^{c}_{b}$
Mean	191.9 B	179.6 C	223.2 A	190.2 B

C: The third youngest leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	181.0*^{c}	161.6^{d}	214.0$^{a}_{a}$	200.5$^{b}_{a}$
7-Cerros	160.6^{a}	149.5^{ab}	162.3$^{a}_{b}$	147.1$^{b}_{b}$
Mean	170.8 B	155.5 C	88.2 A	173.8 B

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 20

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)
Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table: 5 Effect of hypoxia x salinity interactions on $K^+:Na^+$ ratio in different leaves in two wheat varieties.

A: The youngest fully expanded leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	126^{a}_{a}*	121^{a}_{a}	11^{b}_{a}	1.8^{b}_{a}
7-Cerros	98^{a}_{b}	41^{b}_{b}	4^{c}_{b}	1.4^{c}_{b}
Mean	112 A	81 B	7 C	1.6 C

B: The second youngest leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	43^{b}_{b}*	54^{a}_{a}	8^{c}_{a}	1.7^{c}_{a}
7-Cerros	61^{a}_{a}	25^{b}_{b}	3^{c}_{b}	1.1^{c}_{b}
Mean	52 A	40 B	6 C	1.4 C

C: The third youngest leaf.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	27^{a}*	26^{a}_{a}	5^{b}_{a}	1.3^{c}_{a}
7-Cerros	34^{a}	18^{b}_{b}	2^{c}_{b}	0.9^{c}_{b}
Mean	30 A	22 B	4 C	1.1 D

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 20

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)
Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table: 6 Effect of hypoxia x salinity interactions on CO_2 assimilation rate (μ mol m^{-2} s^{-1})of two wheat varieties.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	12.60 *	10.72	11.51_{a}	8.83^{NS}_{a}
7-Cerros	15.04^{a}	10.48^{abc}	9.48^{bc}_{b}	6.95^{c}_{b}
Mean	13.82 A	10.60 B	10.50 B	7.89 B

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 2

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table: 7 Effect of hypoxia x salinity interactions on stomatal conductance (mmol m^{-2} s^{-1}) of two wheat varieties.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	539.0^{a*}	259.3^{ab}_{b}	188.3^{b}_{a}	88.3^{b}
7-Cerros	564.3^{a}	391.3^{ab}_{a}	119.3^{b}_{b}	82.3^{b}
Mean	551.7 A	325.3 B	153.8 BC	85.3 C

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 2

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table: 8 **Effect of hypoxia x salinity interaction on Transpiration rate (mmol m^{-2} s^{-1}) of two wheat varieties.**

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	51.67 ^{a*}	35.00 ^{ab}	48.67 $^{ab}_{a}$	31.67 ^{b}
7-Cerros	41.67	34.00	33.33 $_{b}$	31.33 ^{NS}
Mean	46.67 A	34.50 B	41.00 AB	31.50 B

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 2

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table 9 Effect of hypoxia x salinity interactions on LDH activity (IU mg protein^{-1}) in root of two wheat varieties.

Varieties	Control	Hypoxic	#Saline	#Saline hypoxic
Blue Silver	0.23 ^{c*}	0.53 ^{ab}	0.41 ^{b}	0.62 $^{a}_{a}$
7-Cerros	0.18 ^{b}	0.49 ^{a}	0.40 ^{a}	0.54 $^{a}_{b}$
Mean	0.21 C	0.51 A	0.41 B	0.58 A

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 2

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Table 10 Effect of hypoxia x salinity interactions on ADH activity (IU mg protein^{-1}) in root of two wheat varieties.

Varieties	Control	Hypoxic	#Saline	#Saline Hypoxic
Blue Silver	3.8^{a*}	3.3^{a}_{b}	2.3^{b}	2.2^{b}_{b}
7-Cerros	3.9^{ab}	4.4^{a}_{a}	2.8^{c}	3.4^{bc}_{a}
Mean	3.85 A	3.85 A	2.55 B	2.80 B

100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$

* n = 2

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Letters in superscript indicate the differences among means in rows while in subscript indicate the differences between means in columns.

Effect of salinity and hypoxia on wheat growth and ionic relations: a greenhouse study.

Riaz H. Qureshi*, A. Qayyum**, S. Nawaz* and M. Aslam*

* Saline Agriculture Research Cell, Dept. of Soil Science, University of Agriculture, Faisalabad.
** Directorate of Agriculture, Muzafarabad, Azad Kashmir.

Key Words: Salinity, hypoxia, tolerant, stress, wheat.

ABSTRACT

Salinity and waterlogging are probably the most important agricultural problems of the irrigated areas of the world. The effect of these stresses can be mitigated by selecting and/or developing salt tolerant plant species and genotypes of cultivated crops. Wheat exhibits genotypic differences in tolerance to salinity but such differences in response to hypoxia have not been much explored. The response of five wheat genotypes (lines/varieties) to hypoxia and moderate salinity alone and in combination were studied in solution culture (shoot growth, root growth, tillering capacity and ionic relations) and soil culture (tillering capacity and grain yield). Significant differences among the genotypes were recorded for salinity, hypoxia and the combined stress; the variety Pb-85 being the most tolerant and 7-Cerros being the most sensitive one to all stresses. This trend existed for shoot growth (solution culture) and grain yield (soil culture). The overall trend in solution culture for shoot growth had a positive correlation with grain yield in soil culture. However, salinity had more adverse effect than hypoxia on shoot fresh weight in solution culture while the opposite was true for grain yield in the soil culture. Plant growth was negatively correlated with the Na^+ and Cl^- concentration in the sap of the youngest fully expanded leaf while K^+ was not correlated with grain yield.

INTRODUCTION

Salinity and waterlogging are amongst the most prominent problems facing agriculture in the irrigated areas of the world, but most of the agricultural crops tolerate only moderate levels of salinity and hypoxia. Superimposing waterlogging

over salinity further reduces crop yield below the reduction due to salinity alone. In dense saline as well as in saline-sodic soils with low water permeability, crops face surface ponding (or temporary waterlogging) at the time of flood irrigation or after heavy rain and this proves very injurious to crop yield.

Wheat is an important crop of such lands in Pakistan but is poorly adapted to waterlogged conditions compared with rice and other hygrophytes. It has relatively poorly developed aerenchyma or biochemical mechanisms for efficient anaerobic metabolism such as described by Crawford (1966).

Utilization of salt-affected soils through growing crops or varieties tolerant to stress is a relatively new idea. Many workers are engaged in screening and breeding wheat varieties tolerant to salinity stress (see Kingsbury and Epstein, 1984; Shannon, 1985; Qureshi et al., 1990), but much less work has been reported on the response of wheat to hypoxia and combined hypoxia and salinity stress (see Barrett-Lennard, 1986).

Wheat genotypes exhibit differences in ionic relations which are related to salt tolerance (Rashid, 1986) and such differences may be under genetic control (Salam, 1993). Such detailed information is lacking with respect to tolerance to waterlogging. Results are reported here regarding the response of 5 wheat genotypes (varieties and lines) to salinity, waterlogging and their combination. The selected varieties may be used directly on the lands faced with these problems or may be studied further to determine the mechanisms involved in tolerance against these stresses.

MATERIALS AND METHODS

The experiment was conducted in two growth media, solution culture and soil culture. The 4 treatments in both te cases were: non-saline aerobic, non-saline hypoxic, saline aerobic and saline hypoxic and these were applied to five wheat varieties or lines, Blue Silver, Tchere T, 7-Cerros, SARC-1 and Pb-85, in a net houe with glass roof. Ther was no control over temperature, humidity or light intensity, and only protection from bird attack was required. In the solution culture, seedlings were grown in gravel by applying canal water (EC 0.25 dS m^{-1}) for irrigation. At the two leaf stage, they were transplanted into the holes in a thermopal sheet floating on modified Phostrogen (a 10-10-27 blend of NPK fertilizer containing 1.3% Mg^{2+}, 0.4% Fe^{2+} and 200 ppm Mn^{2+}, used @ 1 g L^{-1} and micronutrients as is used in Hoagland's solution) solution (Phostrogen Limited, Corwen, UK.). Each variety was repeated in five holes and every hole had two plants. Two days after transplanting salinity was developed stepwise by adding 25 mol m^{-3} NaCl + 2.5 mol m^{-3} $CaCl_2$ daily for four days (final salinity 100 mol m^{-3} NaCl + 10 mol m^{-3} $CaCl_2$). The pH of the solution was maintained daily between 6.0 and 6.5 with HCl or NaOH solution. Hypoxic conditions were developed in two tubs (one non-saline and one saline) by stopping aeration and sealing the surface with a polythene sheet to minimize air contact with the solution on the day after the required salinity level was attained. Solutions were replenished the first time after a fortnight and then weekly. Four weeks after the saline-hypoxic stress was imposed, the plants were harvested and data for physical parameters were recorded. The youngest fully expanded leaf of each plant of each variety was preserved at freezing temperature for extraction of tissue sap. The sap was diluted and Na^+ and K^+ were determined by means of a flamephotometer (Jenway PFP 7) while Cl^- was determined on a chloride analyser (Corning 925).

In the soil culture, 60 plastic pots were each filled with 5 kg of soil. The soil was deficient in N and P, while pHs was 8.5 with ECe 1.25 dS m^{-1}. The pots were grouped according to the treatments as in the case of the solution culture. NaCl was mixed with the soil for the saline treatments in order to obtain an ECe of 12.5 dS m^{-1}. Seeds were sown when the soil was at field capacity. After seedling establishment three uniform plants were left in each pot to mature. Waterlogging stress was applied before the tillering stage by applying excess water, so that water stood on the surface of the soil. This stress was continued until ear formation, after which it was relieved. At maturity, grain yield and number of tillers were recorded.

Both experiments were laid out as completely randomised designs. The data were analysed statistically using MSTATC (Crop and Soil Sciences Department, Michigan State University), and significant differences between treatments ($P<0.05$) obtained using Duncan's Multiple Range Test.

RESULTS

Plant growth (Solution culture)

Salinity and hypoxia individually as well as in combination significantly decreased the shoot fresh weight compared with the control (Table 1). Hypoxia caused a mean reduction of 33 % in the shoot fresh weight, and salinity showed a reduction of 71 % while a 74 % reduction was observed due to their combination. This indicated that the effects of salinity and hypoxia were non-additive on the fresh weight of the shoot in wheat.

Under hypoxic and saline-hypoxic treatments 7-Cerros produced the lowest relative (26.9 and 13.8 % of control, respectively) and absolute shoot fresh weights, while Pb-85 gave the maximum values for these parameters. Under the saline treatment

Tchere T had the lowest relative shoot fresh weight and 7-Cerros the lowest absolut shoot fresh weight, while Pb-85 had the highest relative and absolute shoot fresl weights.

Root fresh weight was also significantly reduced by salinity, hypoxia and their combination compared with the control (Table 2). Here again the effects of salinity and hypoxia were non-additive as hypoxia produced a reduction of 30 % and salinity 49 %, while their combination caused a reduction of 61 %. However, the effect of salinity alone was less pronounced on root fresh weight than on the shoot fresh weight. In all treatments Pb-85 produced the highest root fresh weight while 7-Cerros showed the least root fresh weight.

Tillering capacity (Solution culture)

Tillering capacity was significantly reduced by salinity and hypoxia individually as well as in combination compared with the non-saline aerobic treatment (Table 3). Hypoxia under non-saline conditions significantly reduced the number of tillers per plant but under saline conditions there was no significant increase in the number of tillers per plant.

In soil culture, salinity decreased tillering capacity under both aerobic and hypoxic conditions and similarly hypoxia significantly decreased tillering capacity under both non-saline and saline conditions (Table 4). Among the varieties Tchere T followed by Pb-85 produced the highest and 7-Cerros the lowest mean number of tillers per plant. Under all stress treatments 7-Cerros produced the lowest number of tillers while Pb-85 produced the maximum number of tillers under both the hypoxic conditions (non-saline and saline). However, in the saline aerobic treatment, Tchere T produced the highest number of tillers per plant.

Grain yield

Grain yield of wheat was significantly reduced by the imposition of hypoxia and salinity both individually and in combination (Table 5). Under hypoxic treatments (non-saline and saline) Pb-85 produced the highest grain yield and 7-Cerros the lowest.

Ionic concentration

Salinity under aerobic as well as hypoxic conditions significantly increased Na^+ and Cl^- concentrations in the youngest fully expanded leaf (Tables 6 & 7). Salinity and hypoxia individually and in combination decreased the K^+ concentration in the youngest fully expanded leaf (Table 8). Hypoxia significantly decreased the K^+ concentration under non-saline conditions, while under saline condition the decrease was non-significant. On the other hand salinity under both the conditions (aerobic and hypoxic) significantly decreased the concentration of K^+ in the youngest fully expanded leaf. Overall Pb-85 had the maximum and 7-Cerros the lowest concentration of K^+ in the youngest fully expanded leaf. A similar trend was observed under the two saline treatments (aerobic and hypoxic).

Discussion

The sensitivity of a crop plant to abiotic stress is usually manifested in reduced plant growth. The relative shoot fresh weight of a particular wheat genotype under salinity compared with the control (no stress) has been considered the best criterion to determine the salt tolerance (Qureshi et al., 1990), while higher economic yield under such adverse conditions is the ultimate goal (LeHouerou, 1986). In this study salinity and hypoxia both individually and in combination decreased the shoot fresh weight compared with the respective controls in all cultivars but the extent of the response was different in different varieties. It was apparent that salinity and

hypoxia acting together caused damage in excess of that found with either salinity or hypoxia alone, but the adverse effect of both the stresses was non-additive. This is partly due to the fact that both the stresses acting individually, among other things, caused a decrease in water uptake by plants, allowing osmotic adjustment mechanisms to work. Thus superimposing one stress over the other did not accentuate the adverse effects in a completely additive manner.

The effect of salinity and hypoxia on the above mentioned growth parameters indicated that genotypic differences existed in wheat in terms of response to these stresses. Among the varieties Pb-85 showed the minimum relative reduction in growth and therefore is considered relatively more tolerant to the stresses under study while 7-Cerros was found sensitive as it showed the maximum reduction in growth due to the stresses. The other varieties were intermediate. A similar pattern was observed on the basis of grain yield. However, the severity of damage by hypoxia in terms of grain yield was much higher than that to shoot fresh weight, root fresh weight and tillering capacity. Grain yield was reduced by 90.9 % by hypoxia, 41.6 % by salinity and 97.9 % by the combined stress of salinity and hypoxia in the soil culture while, under similar conditions in the solution culture, the reductions in shoot fresh weight were 33, 71 and 74 %, respectively. This indicated that salinity had more adverse effects than hypoxia on shoot fresh weight in solution culture. In contrast, in the soil culture, hypoxia produced more drastic effects on grain yield than salinity.

Exposure of plant roots to salinity and hypoxia reduces transpiration which may affect the supply of ions to the growing apex of stressed plants. It is well established that under saline waterlogged conditions, many plants take up Na^+ and Cl^- to the shoot at increased rates, and that this may lead to shoot senescence and

adverse effects on shoot growth (Barrett-Lennard, 1986). Such a response could be related to the increased permeability for Na^+ intake and decreased selectivity against Na^+ at root membrane level due to decreased energy available because of depressed aerobic respiration. Although it may be argued that reduced transpiration will reduce Na^+ transport to the leaves, it is assumed that the net balance may still result in increased Na^+ accumulation in leaves. Our results suggested that the variety (Pb-85) taking up the least amounts of these toxic ions was relatively tolerant to this stress. The relationship between grain yield in the soil culture and growth parameters (shoot fresh weight, root fresh weight and tiller number of five wheat varieties grown in solution culture) showed a significant positive correlation (Table 6). On the other hand the concentration of toxic ions (Na^+ and Cl^-) in the youngest fully expanded leaf showed a significant negative correlation with grain yield. Potassium concentration in the leaf was not significantly correlated with grain yield. The ion concentrations in the youngest fully expanded leaf also showed similar correlations with shoot fresh weight.

A variety tolerant to salinity generally maintains better selectivity for K^+ over Na^+ (Gorham, 1990). This process requires biochemical energy which is deficient under hypoxic conditions so that the uptake of K^+ is disturbed greatly under hypoxic conditions. In the present study, the varieties Blue Silver, Tchere T and 7-Cerros showed better control over K^+ selectivity under saline-hypoxic conditions.

LITERATURE CITED

Barrett-Lennard, E. G. 1986. Effects of waterlogging on the growth and NaCl uptake by vascular plants under saline conditions. Reclam. and Reveg. Res. 5:245-261.

Crawford, R. M. M. 1966. The control of anaerobic respiration as a determining factor in the distribution of the genus Senecio. J. Ecol. 54:403-413.

Gorham, J. 1990. Salt tolerance in Triticeae: K/Na discrimination in synthetic hexaploid wheats. J. Expt. Bot. 41:623-627.

Kingsbury, R. W. and E. Epstein. 1984. Selection of salt-resistant spring wheat. Crop Sci. 24:310-315.

Le Houerou, H. N. Salt tolerant plants of economic value in the mediterranean basin. Reclam. and Reveg. Res. 5:319-341.

Qureshi, R. H., A. Rashid and N. Ahmad. 1990. A procedure for quick screening of wheat cultivars for salt tolerance. In: N. ElBassam, M. Damborth and B. C. Laughman (Eds.) Genetic Aspects of Plant Mineral Nutrition, Kluwer Academic Publishers, Netherland. pp. 315-324.

Rashid, A. 1986. Mechanisms of salt tolerance in wheat (Triticum aestivum L.). Ph. D. Thesis, Univ, of Agirc. Faisalabad, Pakistan.

Salam, A. 1993. Physiological/Genetical Studies on the aspects of salt tolerance in Wheat (Triticum aestivum L.). Ph.D. Thesis, School of Biological Sciences, Univ. Wales, Bangor, UK.

Shannon. M. C. 1985. Principles and strategies in breeding for higher salt tolerance. Plant and Soil. 89: 227-241.

Table 1. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and hypoxia on shoot fresh weight (g $plant^{-1}$) (solution culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	26.75 ab	16.32 cd	6.58 e-h	5.01 fgh
Tchere T	32.47 a	11.30 d-g	4.68 fgh	6.85 e-h
7-Cerros	17.30 cd	4.65 fgh	4.29 gh	2.38 h
SARC-1	21.43 bc	17.61 cd	6.00 fgh	5.01 fgh
Pb-85	25.87 ab	32.84 a	14.24 cde	12.92 def
Mean	24.76 A	16.55 B	7.16 C	6.43 C

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Table 2. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and hypoxia on root fresh weight (g $plant^{-1}$) (solution culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	10.32 bc	7.14 ef	4.64 e-h	2.68 gh
Tchere T	13.84 a	6.44 def	4.36 e-h	4.81 e-h
7-Cerros	9.29 cd	4.01 fgh	3.54 fgh	1.73 h
SARC-1	10.87 bc	8.91 cd	4.92 efg	3.51 fgh
Pb-85	11.58 abc	12.48 ab	10.80 bc	8.95 cd
Mean	11.18 A	7.79 B	5.65 C	4.34 D

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Table 3. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and waterlogging on number of tillers $plant^{-1}$ (solution culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	7.2 bc	4.8 de	2.2 f	2.2 f
Tchere T	8.6 b	6.0 cd	1.8 f	3.2 f
7-Cerros	7.0 bc	2.6 f	1.8 f	2.0 f
SARC-1	7.6 bc	6.2 cd	2.6 f	2.0 f
Pb-85	13.2 a	15.2 a	7.8 bc	8.0 bc
Mean	8.72 A	6.96 B	3.24 C	3.48 C

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Table 4. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and waterlogging on number of tillers $plant^{-1}$ (soil culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	3.78 cde	2.67 d-h	2.89 d-g	0.56 j
Tchere T	5.56 a	3.56 c-f	5.22 ab	1.45 hij
7-Cerros	4.00 bcd	2.44 e-i	1.33 hij	0.22 j
SARC-1	3.45 def	4.00 bcd	2.33 f-i	1.22 ij
Pb-85	4.89 abc	5.22 ab	2.56 e-i	2.00 ghi
Mean	4.33 A	3.58 B	2.87 C	1.09 D

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Table 5. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and waterlogging on grain yield (g pot^{-1}) (soil culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	15.17 c	1.14 h	8.96 ef	0.00 h
Tchere T	12.61 d	0.37 h	12.21 d	0.00 h
7-Cerros	16.70 bc	0.02 h	6.89 f	0.00 h
SARC-1	19.48 a	2.03 gh	9.67 e	0.23 h
Pb-85	18.59 ab	3.93 g	10.53 de	1.54 h
Mean	16.51 A	1.50 C	9.65 B	0.35 D

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

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Table 6. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and waterlogging on Na^+ concentration in the leaf sap (mol m^{-3}) (solution culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	6.48 f	6.62 f	46.43 cde	131.25 a
Tchere T	10.93 f	6.32 f	60.41 c	92.27 b
7-Cerros	8.34 f	8.06 f	60.61 c	143.05 a
SARC-1	6.45 f	6.37 f	52.78 cd	116.63 ab
Pb-85	7.85 f	8.26 f	17.29 ef	25.48 def
Mean	8.01 C	7.13 C	47.50 B	101.74 A

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Table 7. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and waterlogging on Cl^- concentration in leaf sap (mol m^{-3}) (solution culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	28.8 e	29.8 e	88.6 cd	180.0 a
Tchere T	23.4 e	16.8 e	81.4 d	142.4 ab
7-Cerros	17.4 e	21.4 e	93.4 cd	182.2 a
SARC-1	18.8 e	22.0 e	79.4 d	126.2 bc
Pb-85	25.0 e	24.2 e	53.8 de	54.2 de
Mean	22.7 C	22.8 C	79.3 B	137.0 A

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Table 8. Effect of salinity (100 mol m^{-3} Na Cl + 10 mol m^{-3} $CaCl_2$) and waterlogging on K^+ concentration in leaf sap (mol m^{-3}) (solution culture).

Varieties	Non-saline		Saline	
	Aerobic	Hypoxic	Aerobic	Hypoxic
Blue Silver	201.6 a	107.7 c	57.2 fg	102.8 e
Tchere T	173.4 bc	152.2 cd	54.4 fg	48.99 fg
7-Cerros	169.7 bcd	172.2 bc	41.3 g	47.3 g
SARC-1	158.7 bcd	176.7 b	114.8 e	49.0 fg
Pb-85	210.5 a	162.0 bcd	150.0 d	69.5 f
Mean	182.8 A	154.1 B	83.5 C	63.5 C

Mean values followed by different letters differ significantly according to Duncan's Multiple Range Test (P = 0.05)

Table 9. Relationship between grain yield and ionic concentration in the leaf tissue and growth parameters of five wheat varieties grown under saline-hypoxic condition.

Variables	Intercept	Slope	Correlation coefficient	Probability
G.Y. vs. SFW	-0.64	0.154	0.910	0.019
G.Y. vs. RFW	-0.59	0.218	0.916	0.017
G.Y. vs. Till	-0.51	0.250	0.959	0.004
G.Y. vs. Na^+	1.69	-0.013	-0.911	0.019
G.Y. vs. K^+	0.19	0.003	0.092	0.881
G.Y. vs. Cl^-	1.99	-0.012	-0.929	0.012
SFW vs. Na^+	176.53	-11.625	-0.987	0.000
SFW vs. K^+	57.03	1.008	0.168	0.783
SFW vs. Cl^-	215.35	-12.177	-0.924	0.014

G.Y. = Grain yield in soil culture

SFW = Shoot fresh weight in solution culture

RFW = Root fresh weight in solution culture

Till = Number of tillers in solution culture

Na^+, K^+ and Cl^- concentration in solution culture

Production and evaluation of salt-tolerant wheat germplasm derived through crosses between wheat (*Triticum aestivum* L.) and *Aegilops cylindrica* I. Production of salt-tolerant wheat germplasm.

S. Farooq, M. Asghar, E. Askari and T. M. Shah

Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Key Words: Wide hybridization, K/Na discrimination, genetic variability, backcross derivatives, hydroponics.

Abstract

To produce salt-tolerant wheat germplasm through wide hybridization, eight different salt-tolerant accessions of *Ae. cylindrica* were crossed with three hexaploid wheat cultivars: Pak-81, LU-26, and Shalimar-88. Frequency of seed set ranged between 1.72% (Shalimar-88 x *Ae. cylindrica* acc 38-53) and 52.6% (Pak-81 x *Ae. cylindrica* G.). The performance of wheat cultivars LU-26 and Shalimar-88 as female parent for F_1 hybrid production was poor. Frequency of BC_1 seed production ranged between 0.53% (Pak-81 x *Ae. cylindrica*// LU-26) and 8.92% (Shalimar-88 x *Ae. cylindrica*// LU-26). BC_1 plants with different chromosome numbers were selfed to produce BC_1F_1 derivatives. Seven BC_1 combinations produced selfed seeds ranging between 1 and 200. Six BC_1 combinations did not produce selfed seeds and were backcrossed again with LU-26 to produce BC_2 seeds, which ranged between 1 and 12 seeds per BC_1 combination. Of the 30 different BC_2 seeds produced in different combinations, only 19 produced BC_2F_1 selfed seeds. All the BC_2F_1 and

BC_1F_2 seeds were tested for germination under saline solutions of EC 2.5 (control) 15, 20, and 25 dS/m. The effects of salinity on root and shoot length and seedling fresh weight were also recorded. In all the test material, salinity induced reduction and delay in seed germination; reduction in shoot and root length and in seedling fresh weight was observed with considerable variation among different salinity levels and among different genotypes at one salinity level. The results have been discussed with reference to the wide hybridization approach and its potential use in the production of genetic variability for polygenically controlled characters such as salt-tolerance.

Introduction

Hexaploid wheat (*Triticum aestivum*) is reported as moderately tolerant to salinity (Kingsbury and Epstein, 1982, 1984), with adequate genetic variability for salt-tolerance (Qureshi et al 1980; 1985; Kingsbury and Epstein, 1984). However, intervarietal differences in salt-tolerance in wheat generally fall within a narrow range of EC 6-10 dS/m (Rana, 1986). Therefore, improvement in the tolerance level of cultivated wheat beyond this tolerance limit may not be achieved through any varietal combination. However, such tolerant germplasm has been produced by transferring salt-tolerance genes from perennial and annual salt-tolerant wild wheatgrasses (Dewey, 1960; McGuire and Dvorak, 1981; Farooq *et al.*, 1988, 1989a) to cultivated wheat. The transfer of salt-tolerance genes from perennial wild wheatgrasses to cultivated wheat has generally been achieved through production of amphiploids (Storey *et al.*, 1985;

Foster *et al.*, 1987) or addition lines (Dvorak *et al.* 1985; and Farooq *et al.*, 1993a), where as gene(s) from annual goat grass species (*Aegilops squarrosa or Ae. cylindrica*) are transferred through direct crossing over between chromosomes of wheat and the alien species (Farooq *et al.*, 1992). Salt-tolerance evaluation of the germplasm produced through crosses of wheat with *Ae. cylindrica*, conducted under saline fields, indicated its salt-tolerance potential (Farooq *et al.*, 1993b) and encouraged use of other identified salt-tolerant accessions of *Ae. cylindrica* (Farooq *et al.*, 1989) as donors of salt-tolerance gene(s) to cultivated wheats. Results obtained during hybridization of these accessions with cultivated wheats are being reported here.

Materials and Methods

Materials used in this study comprised eight salt-tolerant accessions of *Ae. cylindrica* (Farooq *et al.* 1989a), and three different cultivars of hexaploid wheat, viz., Pak-81, LU-26, and Shalimar-88. Plant material used in hybridization was planted in plastic pots in the net house in November 1990 (average day and night temperature: 20±5 ^{0}C/10±5 ^{0}C, 8-10 hours natural light, and 50-60% relative humidity). The pots were filled with 6 kg normal soil to which nitrogen and phosphorus fertilizer was applied in the form of ammonium nitrate and superphosphate in a ratio of 100:75 kg/ha (Anonymous, 1993). At flowering, cultivated wheat varieties were emasculated and pollinated with pollen from different accessions of *Ae. cylindrica*. F_1 seeds were left on the plant and harvested at maturity. F_1 hybrids, confirmed through mitotic cytology, were

backcrossed with wheat cultivars Pak-81 and LU-26 during 1991-92. Before backcrossing, immature spikes of all the F_1 hybrid combinations were fixed in a mixture of alcohol, acetic acid and chloroform (6:3:1). Acetocarmine squash preparations were made and analyzed for the meiotic chromosome associations at metaphase-I.

BC_1 plants produced from different BC_1 seeds were selfed to produce BC_1F_1 self-fertile derivatives. Some of the combinations that did not produce selfed seeds were again backcrossed with the same wheat varieties to produce BC_2 seeds. All the BC_1 and BC_2 combinations that produced more than 100 selfed seeds were tested for germination in petriplates lined with filter papers soaked in artificially saline solutions of EC 2.5 (control), 15, 20, and 25 dS/m created by mixing $NaSO_4$, $MgCl_2$, $CaCl_2$, and NaCl (Qureshi *et al.*, 1977) in a ratio of 10:1:5:4 in Hoagland nutrient solution (Hoagland and Arron, 1950). Five seeds per treatment per combination were tested in three replications. Data on root length, shoot length and seedling fresh weight were taken 2 weeks after seed germination. After 2 weeks, seedlings were removed from the petriplates. They were briefly washed in three changes of distilled water to remove any salt crystals attached on the leaf surfaces. The seedlings were dried at 70 ^{o}C for 72 hours, ground, and digested with concentrated nitric acid (HNO3). The concentration of K and Na in the digest was analyzed by flamephotometer (Anonymous, 1954). All the data were subjected to statistical analysis using Duncan's Multiple Range Test.

Results

Of the eight different salt-tolerant accessions of *Ae. cylindrica* used as pollen sources, only six could be crossed with wheat cultivar Pak-81, three with Shalimar-88, and two with LU-26 (Table 1). Observed seed set in all the combinations ranged between 1.69% (Pak-81 x *Ae. cylindrica* acc 21-87) and 52.6% (Pak-81 x *Ae. cylindrica* acc G). As female parent in F_1 hybrid production, LU-26 performed poorly as compared with Pak-81 and produced only two F_1 seeds.

Significant variations were observed in mean meiotic chromosomal associations at metaphase-I in different hybrid combinations (Table 2). Minimum chiasma frequency (10.0/cell) was observed in the hybrid of Pak-81 x *Ae. cylindrica* acc 50-42. Hybrids of Pak-81 with two other accessions (50-43 and 21-87) showed significantly high chiasma frequency (12.0/cell) as compared with accession 50-42. Hybrids of three wheat cultivars (Pak-81, LU-26, and Shalimar-88) with one accession of *Ae. cylindrica* (38-53) also produced different chiasma frequencies, with the maximum (16.0/cell) being observed in LU-26 x *Ae. cylindrica* acc 38-53.

All the F_1 hybrid combinations except Shalimar-88 x *Ae. cylindrica* acc 39-55 produced backcross seeds which ranged between 1 and 15 seeds per combination. LU-26 showed excellent performance as a backcross parent, producing 57 BC_1 seeds as compared with 16 in the case of Pak-81 (Table 3). The F_1 hybrids of Shalimar-88 and *Ae. cylindrica* acc 34-35 did not produce seeds with either of the backcross parents.

Of the 73 BC_1 seeds, progenies of 25 seeds belonging to eight different hybrid combinations produced selfed seeds, the number ranging between 1 and 200 seeds per combination (Table 4). Progenies of six BC_1 plants did not produce selfed seeds, but produced BC_2 seeds upon second backcrossing (1-149 seeds per BC_1 progeny). Of the 261 seeds, only 6 produced BC_2 selfed seeds, with 4-150 seeds per BC_2 combination (Table 4).

Considerable reduction coupled with delay in germination was observed in all the combinations at four salinity levels. At EC 15 dS/m and in some of the combinations at EC 20 dS/m, germination started after the third day and was complete within 24 hours. In some of the combinations at EC 20 and in all but one combination at EC 25 dS/m, germination started after 8 days and was complete within 10 days (data not presented). Reduction in seed germination was observed in all the test material at the three salinity levels. At EC 20 and 25 ds/m, reduction in germination was higher in BC_1 than in BC_2 derivatives (Figure 1a). In LU-26, reduction in germination was the lowest at all salinity levels. In all the BC_2 and in one of the BC_1 derivatives, germination was higher as compared with salt-sensitive wheat cultivar Pak-81 at all salinity levels.

Like seed germination, reduction in seedling fresh weight was also observed. It was significantly higher ($P < 0.05$) in hybrid derivatives than in wheat cultivars LU-26 and Pak-81, particularly at EC 20 and 25 dS/m. Among the different hybrid derivatives, seedling fresh weight was higher in BC_2 than in BC_1 derivatives (Figure 1b).

Effects of salinity on root length and shoot length were also observed in all the test material (Figures 2a and 2b). Between EC 15 and 20 dS/m, differences in the root and shoot length of all the test material were highly significant ($P < 0.01$), while between EC 20 and 25 dS/m, significant differences ($P < 0.05$) were observed only for reduction in root length (Figure 2b). Root length and shoot length of wheat cultivar Pak-81 (salt-sensitive parent) were higher than in wheat cultivar LU-26 (salt-tolerant parent) at all salinity levels.

Considerable differences were observed in the concentrations of Na and K in all the combinations (Table 5). Na concentration in salinized plants ranged between 15.00 (Pak-81; salt-sensitive wheat parent) and 2.75 mg/g dry weight (LU-26; salt-tolerant wheat parent). In all the hybrid combinations, Na concentration was in between that of the two parents and ranged between 3.5 and 13.25 mg/g dry weight. In three hybrid combinations: [4(37-100), 2(1-200) 4(4-200)], K concentration in salinized plants was significantly higher as compared with the nonsalanized control. The K/Na ratio was higher in the plants growing under nonsaline conditions than in those growing under the saline conditions. However, in one hybrid combination [4(37-100)] growing under saline conditions, the K/Na ratio was higher than in wheat cultivar LU-26. Generally, in all the hybrid derivatives and in salt-tolerant wheat cultivar LU-26 growing under saline conditions, the concentration of Na was low and of K high, while in salt-sensitive wheat cultivar Pak-81, the concentration of Na was high and of K low in salinized plants (Table 5).

Discussion

In our earlier attempt at transferring salt-tolerance gene(s) from salt-tolerant accession D of *Ae. cylindrica*, we found considerable crossability variations in different hybrid combinations (Farooq *et al.*, 1990a). In the present study, crossability values for different wheat cultivars were of the same order except for Shalimar-88, where crossability response was similar to that of LU-26. Similarly, crossability variations observed in different accessions of *Ae. cylindrica* confirmed the earlier findings of Sharma and Gill (1983) and Farooq *et al.* (1989a) suggesting the presence of some crossability genes on *Aegilops* species that affected their crossability with wheat varieties. In the present study, efforts were made to synchronize the flowering time of *Ae. cylindrica* and LU-26. Neverthless, crossability response was low, which negates our earlier hypothesis (Farooq *et al.*, 1989b) that the poor crossability performance of LU-26 with accessions of Ae. cylindrica was due to nonsynchronization of flowering of LU-26 (early flowering) and *Ae. cylindrica* (late flowering). Thus there are some other factors operating in these crosses that have not yet been identified.

Considerably high chiasma frequency was observed at meiotic metaphase-I in all the F_1 hybrids, which indicated pairing between D genome chromosomes of *Ae. cylindrica* and of wheat. As reported earlier (Farooq *et al.*, 1992), this pairing signifies the transfer of salt-tolerance gene(s) to wheat through the exchange of genetic material, which is a stable transfer as compared with the transfer of gene(s) achieved with amphiploids and/or addition lines. As

expected, the hybrid of LU-26 with accession 38-53 produced maximum chiasmata/cell, which confirmed our earlier report (Farooq *et al.*, 1990b) on the presence of additional pairing promotor gene(s) on LU-26 that have not yet been identified. Differences in chiasma frequency observed in hybrids of one wheat variety with different accessions indicated that transfer of salt-tolerance gene(s) to cultivated wheats can be enhanced if selections are made from alien species that can produce maximum chiasmata.

Backcrossing is a routine procedure of advancing intergeneric hybridization. In the present study, the frequency of backcross seed production and of selfing in BC_1 seeds was low, which indicated variation in the chromosome number of BC_1 combinations.

Reduced and delayed seed germination coupled with decreased seedling growth due to salinity stress has been reported in many crop species (Abel and Mackenzie, 1964; Akber and Yabuno, 1974; Puntamkar *et al.*, 1970; Norylin and Epstein, 1984; Mozafar and Goodin, 1986; and Azmi and Alam, 1990). In the present study also, reductions in seed germination, root and shoot length, and seedling fresh weight were observed in all the test material. The higher values of seed germination and seedling fresh weight of LU-26 as compared with BC_1 and BC_2 derivatives indicated that the newly produced salt-tolerant material may not have higher salt-tolerance than the tolerance level already existing in LU-26, while lower values of root and shoot length of LU-26 as compared with BC_1 and BC_2 derivatives indicated higher level of tolerance of the hybrid derivatives as compared with LU-26. As reported earlier (Kingsbury and Epstein, 1984), salt-tolerance response at early growth stages

is not an indication of the same response at later growth stages therefore, it is possible that the salinity response of the test material observed at seedling stages may not remain the same at later growth stages. Nevertheless, the results did show a variability in salt-tolerance that needs to be examined at later growth stages and under higher salinity levels.

In the present study, as mentioned earlier, three hybrid combinations growing in saline medium exhibited significantly higher concentrations of K as compared with the nonsaline control. This is a rare observation and indicated the presence of K-Na discrimination phenomenon in these genotypes. Since Na is known to compete with potassium for the common absorption site, high potassium uptake can result only if the inherent mechanism of the test material can discriminate between K and Na and allow preferential uptake of the former. As reported by Gorham (1990), the K-Na discrimination factor operates only at low salinities, while in the present study, this factor seems to have been operational at EC 15 dS/m, which is not very low for wheat. It would be good for breeding programs if the expression of K-Na discrimination operating at seedling stages could be detected during the screening of the same material at later growth stages, as selection for salt-tolerant plants would then be possible at seedling stages using the K-Na discrimination factor as a marker.

Significance of the Study:

This study indicated two findings that are important for plant breeding programs aimed at improveming polygenically controlled traits such as salt tolerance.

First, the study establised the potential of a wide hybridization approach for improving of salt-tolerance. This approach can be used sucessfully in breeding programs, particularly when intervarietal crosses fail to improve the prevailing stress situation. Since salt-tolerance is a polygenic trait, and the genes are scattered on different chromosomes, identification and transfer of all the genes to wheat is difficult. In *Ae. cylindrica*, the salt tolerance seems to be controlled by major gene(s) which can be transferred to wheat through crossing over. In countries such as Pakistan where the salinity problem is increasing at a rapid pace, the production of salt-tolerant material through wide hybridization will be of twofold significance: it can be tested in the field for selection of salt-tolerant varieties, and it can also be used to derive salt-tolerant germplasm, which is desperately needed in breeding programs.

The second important finding of this study is the inheritance and expression of the K-Na discrimination factor in hybrid derivatives. This finding is of immense importance in practical breeding as it helps avoid the absorption of extra Na in plants, a major cause of yield reduction under saline environments.

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References

Abel, G. H and A. J. Mackenzie. 1964. Salt-tolerance of Soybean varieties during germination and later growth. Crop Sci. 4: 157-160.

Akbar, M. J. Yabuno. 1974. Breeding for saline resistane varieties of rice II. Comparative performance of some rice varieties to salinity during early development stages. Jpn. J. Breed. 24:176-181.

Annonymous. 1954. Diagnosis and improvement of saline and alkali soils. USDA Hand Book No: 60. USDA, USA.

Annonymous. 1993. Annual Report. Wheat Research Institute, Faisalabad, Pakistan.

Azmi, A. R. and S. M. Alam. 1990. Effect of salt stress on germination, growth, leaf anatomy and mineral element composition of wheat cultivars. Acta. Physiol. Plantarum. 3: 215-224.

Dewey D. R. 1960. Salt tolerance of twenty five strains of Agropyron. Agron., 52: 631-635.

Dvorak, J., R. Kathleen and S. Mendlinger. 1985. Transfer of salt tolerance from Elytrigia pontica to wheat by the addition of an incomplete Elytrigia genome. Crop. Sci. 25: 306-309.

Farooq, S., Aslam, Z., Niazi, M. L. K., and Shah, T. M. 1988. Salt tolerance potential of wild resources of tribe Triticeae-I. Screening of perennial genera. Pak. J. Sci. Ind. Res. 31: 506-511.

Farooq, S., M. L. K. Niazi, N. Iqbal and T. M. Shah. 1989a. Salt tolerance potential of wild resourses of the tribe *Triticeae*-II. Screening of species of the genus Aegilops. Plant and Soil, 119: 255-260.

Farooq, S., N. Iqbal and T. M. Shah. 1989b. Intergeneric hybridization for wheat improvement-I. Influence of maternal and paternal genotypes on hybrid production. Cereal Res. Commun. 17:17-22.

Farooq, S., T. M. Shah and N. Iqbal. 1990a. Variation in crossability among intergeneric hybrids of wheat and salt tolerant accessions of three *Aegilops* species. Cereal Res. commun. 18: 335- 338.

Farooq, S., N. Iqbal and T. M. Shah. 1990b. Intergeneric hybridization for wheat improvement-III. Genetic variation in *Triticum* species affecting homoeologus chromosome pairing. Cereal Res. Commun. 18: 233-235.

Farooq, S., N. Iqbal and M. Asghar and T. M. Shah. 1992. Intergeneric hybridization for wheat improvement-VI. Production of salt tolerant germplasm through crossing wheat (*T. aestivum*) with *Aegilops cylindrica* and its significance in practical agriculture. J. Genet. Breed. 46: 125-132.

Farooq, S., T. M. Shah and M. Asghar. (1993) Intergeneric hybridization for wheat improvement-VII. Transfer in hexaploid wheat of salt tolerance gene(s) from *Thinopyrum scirpeum*. J. Genet. Breed. 47, 191-198.

Farooq, S., N. Iqbal, M. Asghar, M. Arif and T. M. Shah. 1993. *Aegilops cylindrica*: a potential donor of salt tolerance gene(s) to hexaploid wheat. Poster Abstract Seventeenth Internal Congress of Genetic. August 15-21, 1993., Birmingham, U.K.

Foster, B. P., J. Gorham and T. E. Miller. 1987. Salt tolerance of two wheat-Agropyron junceum disomic addition lines. Genome. 30:559- 564.

Gorham, J. 1990. Salt tolerance in Triticeae: K/Na discrimination in *Aegilops* species. J. Exp. Bot. 41: 615-621.

Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Sta. Uni. Calif Circular No. 347.

Kingsbury, R. W. and E. Epstein. 1984. Selection for salt resistant spring wheat. Crop Sci. 24:310-315.

Mc.Guire, P.E. and J. Dovorak. 1981. High salt tolerance potential in wheat grasses. Crop Sci. 21: 702-705.

Mozafar, A. and J. R. Goodin. 1986. High salt tolerance of two differently tolerant wheat genotypes during germination and early seedling growth. Plant and Soil. 96: 303-316.

Norylin, J. D. and E. Epstein. 1984. Variability in salt tolerance of four triticale lines at germination and emergence. Crop Sci. 24: 1090-1092.

Puntamker, S. S., D. C. Sharma and S. P. Sethi. 1970. Effect of common salts of sodium and calcium on the germination of different wheat varieties (*Triticum aestivum* L.). Ind. J. Plant Physiol. 13: 233-239.

Qureshi, R. H., M. Salim, Z. Aslam and G. R. Sandhu. 1977. An improved gravel culture technique for salt tolerance studies on plants. Pak. J. Agric. Sci. 14, 11-18.

Qureshi, R. H. 1985. Selection of crop varieties and plant species suitable for salt afected areas of Pakistan. In: Proc. Nat. Workshop on Biosaline Research in Pakistan, University of Agriculture, Faisalabad, Pakistan, pp. 28-42.

Qureshi, R. H., R. Ahmad, M. Ilyas and Z. Aslam, 1980. Screening of wheat (*Triticum aestivum* L.) for salt tolerance. Pak. J. Agri. Sci., 17:19-25.

Rana, R. S. 1986. Genetic diversity for salt stress resistance of wheat in India. Rachis. 5: 32-37.

Sayed, H. I. 1985. Diversity of salt tolerance in germplasm collection of wheat (*Triticum* spp.). Theor. Appl. Genet. 69: 651- 657.

Sharma, H. C. and B. S. Gill. 1983. Current status of wide hybridization in wheat. Euphytica, 32: 17-31.

Story, R., R. D. Gorham and K. W. Shepherd. 1985. Modification in salinity response of wheat by the genome of *Elytrigia elongata*. Plant and Soil. 83: 327-330.

Table 1. Production of F_1 hybrids between diferent wheat varieties and various salt tolerant accessions of *Aegilops culindrica*.

Male Parent	Florets pollinated			Seed Set		
Aegilops cylindrica/ accession	Pak-81	LU-26	Shalimar	Pak-81	LU-26	Shalimar
Aegilops crlindrica 50-42	396	20	0	138(35.7)	0	0
Aegilops crlindrica 50-43	150	20	0	27(18.0)	0	0
Aegilops crlindrica 38-53	254	24	58	34(13.4)	1(4.2)	1(1.7)
Aegilops cylindrica 17-59	272	0	0	18(6.6)	0	0
Aegilops cylindrica 42-61	0	34	16	0	0	1(6.3)
Aegilops crlindrica 34-35	0	0	22	0	0	1(4.5)
Aegilops cylindrica G	97	0	0	51(52.6)	0	0
Aegilops crlindrica 21-87	118	56	0	2(1.7)	0	0

Figures in parenthesis are % seed set.

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Table 2. Mean meiotic chromosomal associations at metaphase-I of wheat cultivar Pak-81, LU-26 and Shalimar-88 with different salt tolerant accessions of *Ae.cylindrica*.

F1 Hybrid combinations	No.of plant analyzed	No.of cells analyzed	Mean meiotic association					χ̄/Cell
			I	II	(II)	III	IV	
Pak 81 x *Ae.cylindrica* 50-42	5	500	21.00	4.00	3.00	0	0	10.00a
Pak 81 x *Ae.cylindrica* 50-43	3	450	19.02	4.01	3.98	0	0	11.97b
Pak 81 x *Ae.cylindrica* 21-87	8	600	18.00	4.00	3.00	1.0	0	12.00b
Pak 81 x *Ae.cylindrica* 38-53	5	300	20.00	3.5	4.00	0	0	11.5ab
Shalimar 88 x *Ae.cylindrica* 38-53	6	400	20.00	4.5	3.00	0	0	10.5a
LU-26 x *Ae.cylindrica* 38-53		500	15.00	3.97	6.03	0	0	16.03c

Figures followed by the same letter are not significantly different from each other at 5% level of significance according to DMRT.

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Table 3 Production of backcross seeds in F1 hybrid combinations of different wheat varieties with various accessions of *Ae.cylindrica*.

Female Parent (F1 combination)	Florets pollinated with		Seed Set in	
	LU-26	Pak-81	LU-26	Pak-81
Pak.81 x *Ae.cylindrica* G.	830	184	15(1.8)	5(2.7)
Pak.81 x *Ae.cylindrica* 50-42	1580	780	14(0.9)	2(2.1)
Pak.81 x *Ae.cylindrica* 50-43	188	132	1(0.5)	0
Pak.81 x *Ae.cylindrica* 38-53	298	142	2(0.7)	1(0.7)
Pak.81 x *Ae.cylindrica* 34-35	334	100	3(0.4)	2(2.0)
Shalimar-88 x *Ae.cyl*: 38-53	168	40	15(8.9)	0
Shalimar-88 x *Ae.cyl*: 34-35	64	100	0	0
LU-26 x *Ae.cylindrica* 50-43	186	186	1(0.5)	5(2.7)
LU-26 x *Ae.cylindrica* 38-53	302	34	6(2.0)	1(2.9)

Figures in parenthesis are % seed set.

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Table 4. Frequency of production of BC_1 selfed seeds, BC_2 seeds and BC_2 selfed seeds in different hybrid combination of three cultivars with various salt tolerant accessions of *Ae.cylindrica*.

Hybrid combinations with pedigree	BC1		BC2			
	No.of combinations	Range of selfed seeds/ combination	No.of combinations	Range of seed/ BC1 plant	No.of seed	Range of selfed seeds/ BC2 combination.
Shalimar 88 /*Ae.cylindrica*//LU 26 38-53	7	(4-65)				
pak 81 /*Ae.cylindrica* //Pak 81 50-42	5	(6-150)				
Pak 81 /*Ae.cylindrica* G //LU 26	4	(37-100)				
Pak 81 /*Ae.cylindrica* //LU-26 21-87	2	(1-200)				
Pak 81 /*Ae.cylindrica* //Pak 81 38-53	1	(37)				
Pak 81 /*Ae.cylindrica* //Pak 81 50-40	1	(1)				
LU-26 /*Ae.cylindrica* //Pak 81 38-53	2	(2-6)				
LU-26 /*Ae.cylindrica* //LU-26 38-53	3	(7-67)				
LU-26 /*Ae.cylindrica* //LU-26[42] 38-53			1	(21)		
LU-26 /*Ae.cylindrica* //Pak 81/3/LU-26 50-43			2	(1-16)		
Pak 81 /*Ae.cylindrica* G //Pak 81/3/LU-26			2	(7-67)		
Pak 81 /*Ae.cylindrica* //LU-26/3/Pak 81 38-53			1	(149)		
Pak 81 /*Ae.cyl*: G// Pak 81/3/LU-26						(4-100)
Pak 81 /*Ae.cyl*: G// LU-26 /3/Pak 81						(17)
LU-26 /*Ae.cyl*: 38-53//Pak 81/3/LU-26						(150)

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Table 5. Comparative mean values of concentration and content of Na and K and their ratio in different hybrid derivatives combinations after two weeks growth in saline medium of EC 15 dS/m and non saline control.

pedigree designation	Na conc: mg/g dry wt.			K conc: mg/g dry wt.			K/Na			Na content % of plant dry wt.			K content % of plant dry wt.		
	S	N.S	Mean	S	N.S	Mean	S	N.S	mean	S	N.S	mean	S	S.N	mean
5(6-15)	13.75	0.13	6.94^{d}	9.00	14.62	11.81^{a}	0.65	31.11	15.88^{b}	1.37	.05	0.71^{d}	0.9	1.46	1.18
4(37-100)	4.25	0.37	2.31^{b}	21.87	12.25	17.6^{d}	5.14	33.11	19.13^{cd}	0.42	0.37	0.39^{b}	2.15	1.22	1.68
2(1-200)	7.87	0.32	4.1^{c}	17.50	12.50	15.00^{c}	2.22	39.6	20.91^{d}	0.78	0.32	0.55^{c}	1.74	1.25	1.49
...-100)	7.50	0.45	3.98^{c}	23.5	14.75	19.13^{e}	3.13	32.78	17.96^{c}	0.65	0.45	0.53^{c}	2.08	1.48	1.78
1(500)	3.50	0.50	2.00^{b}	13.00	11.00	12.00^{b}	3.7	22.00	12.85^{a}	0.35	0.05	0.43^{b}	1.30	1.10	1.2^{b}
Pak 81	15.00	0.37	7.68^{e}	12.50	10.00	11.25^{a}	0.83	27.35	14.09^{ab}	1.52	0.04	0.78^{d}	1.24	1.01	1.13^{a}
LU-26	2.75	0.32	1.54^{a}	13.25	12.00	12.60^{b}	4.82	37.5	21.16^{d}	.23	.03	0.03^{a}	1.31	0.57	0.94^{a}
Mean	7.00b	0.35^{a}		15.80^{b}	12.44^{a}		2.33^{a}	31.92^{b}		0.37^{b}	0.19^{a}		1.53^{b}	1.16^{a}	

S = Saline, NS = Non saline.

Figures followed by the same letters in each column and row are not significantly different from each other at 5% level of significance according to Duncan's Multiple Range Test.

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Fig. 1a Mean seed germination at three salinity levels (% of control in non-saline)

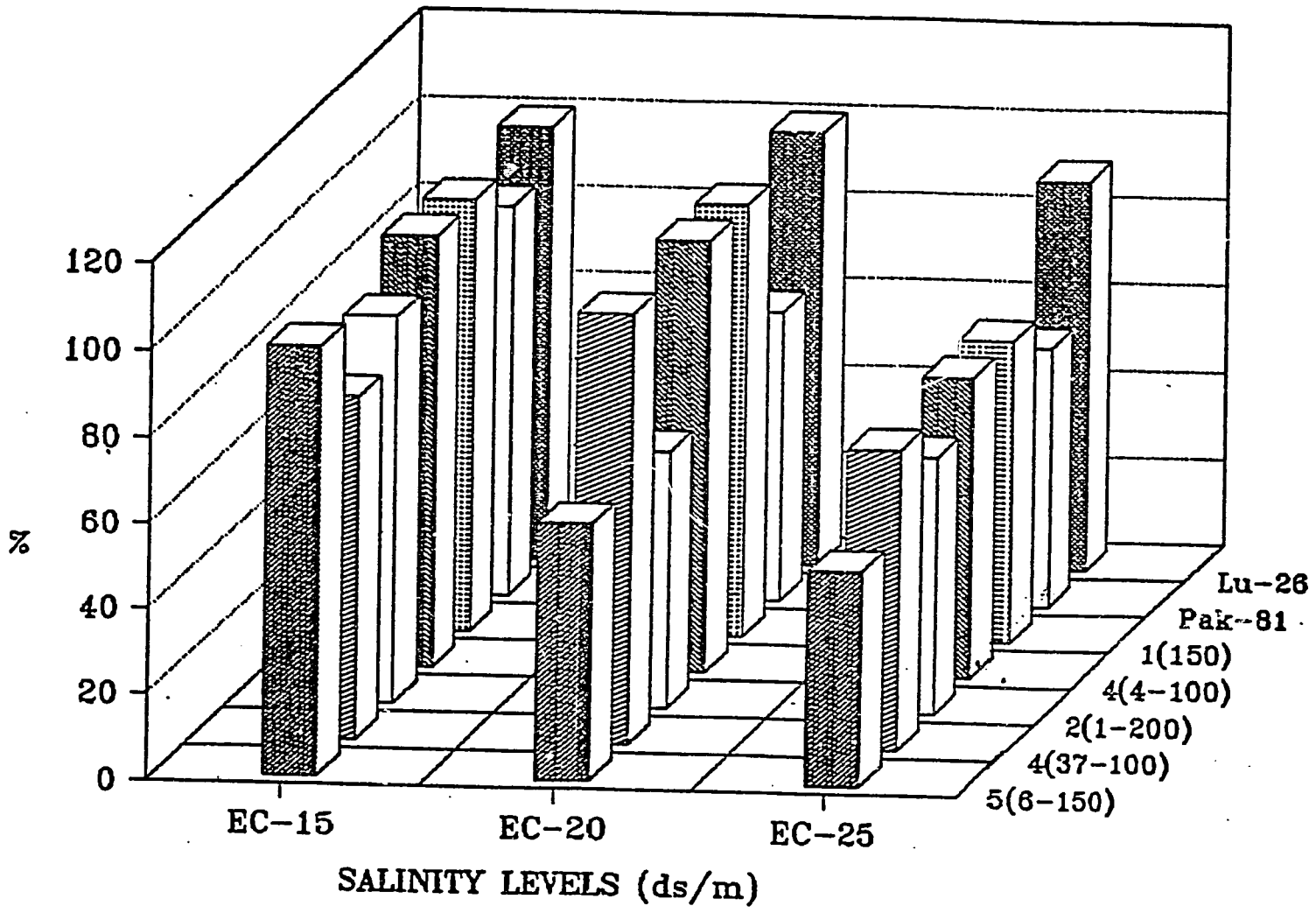


Fig. 1b Plant fresh weight
at three salinity levels
(% of control in non-saline)

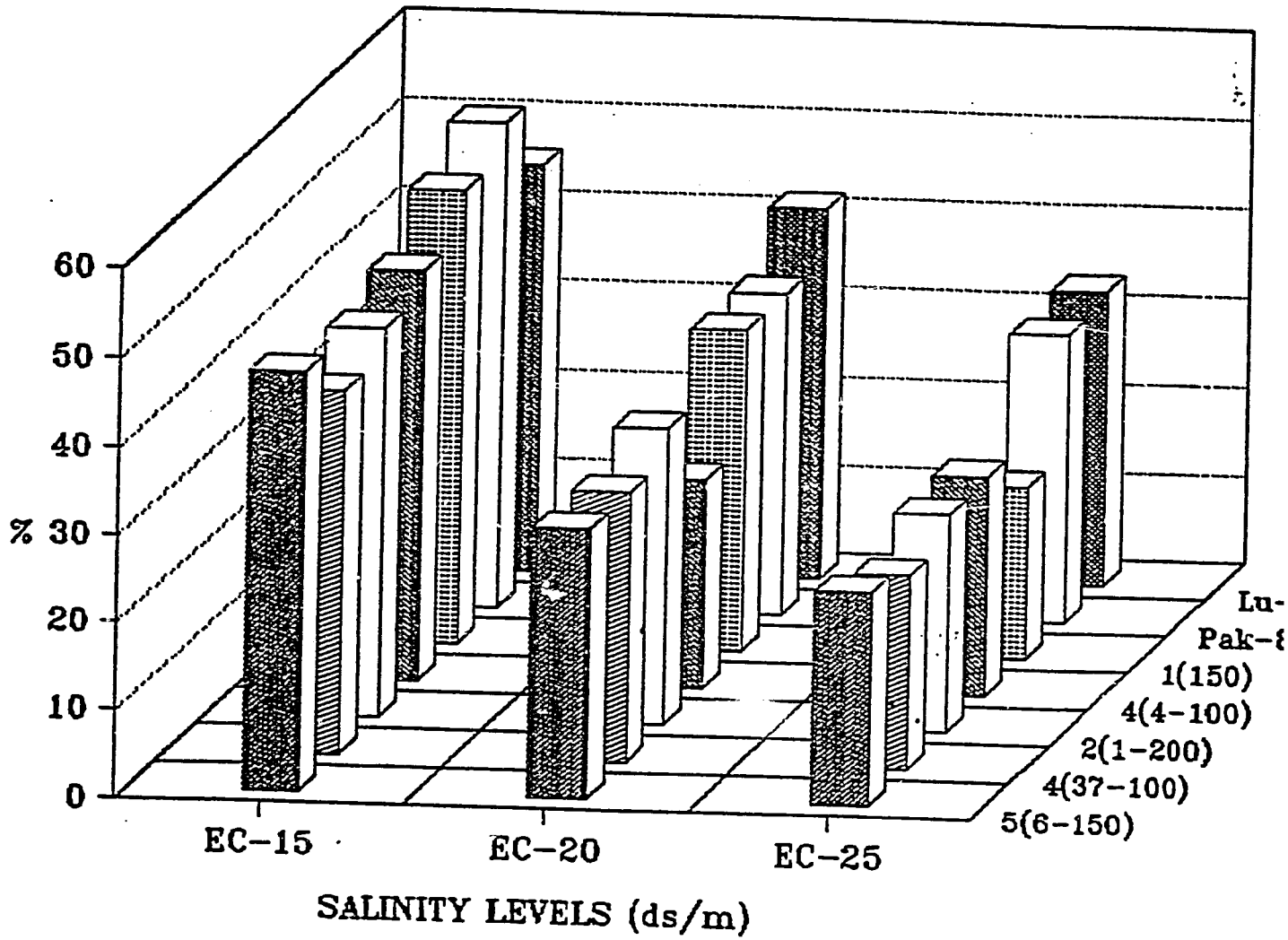


Fig. 2a Mean shoot length at three salinity levels (% of control in non-saline)

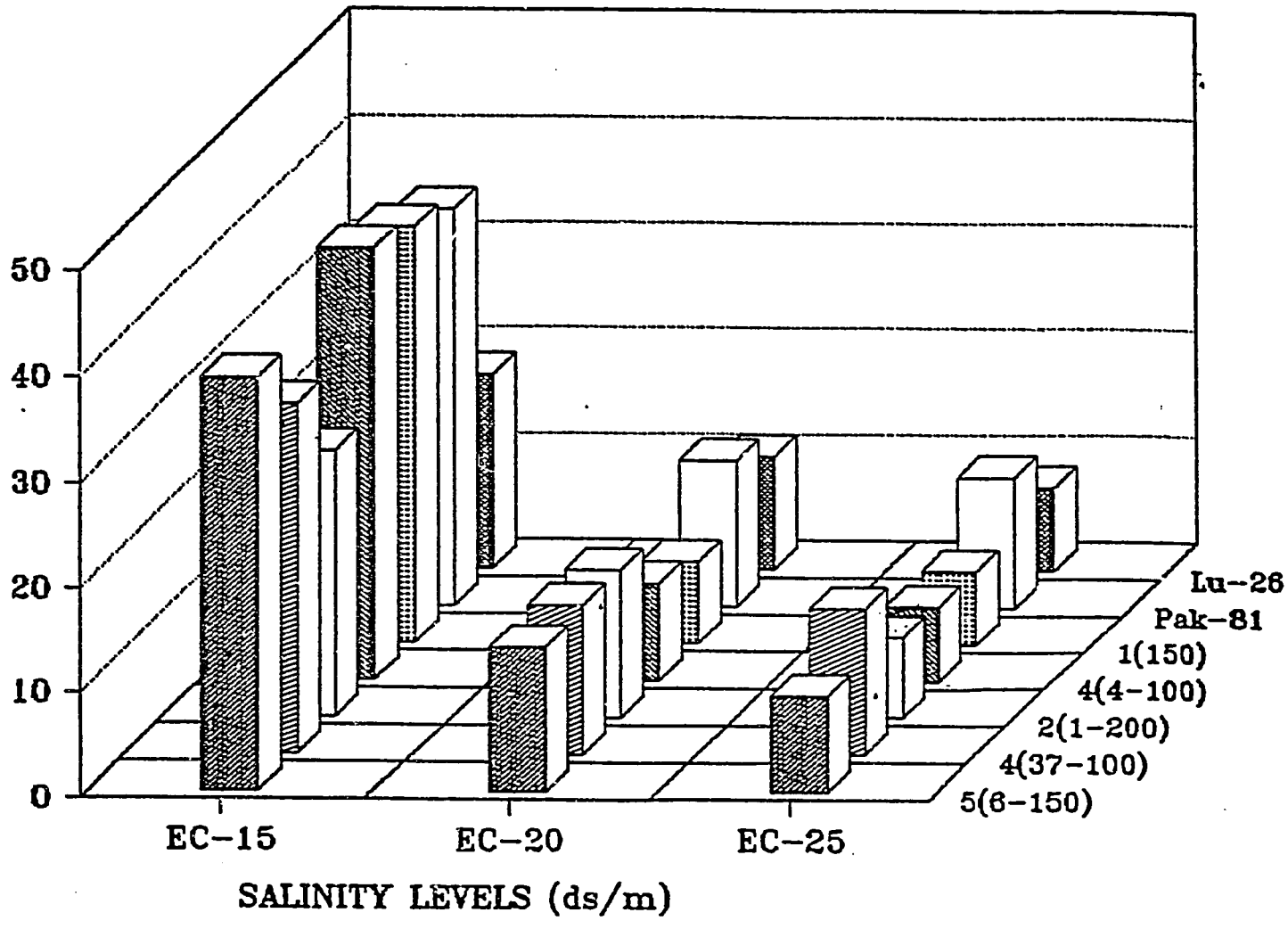
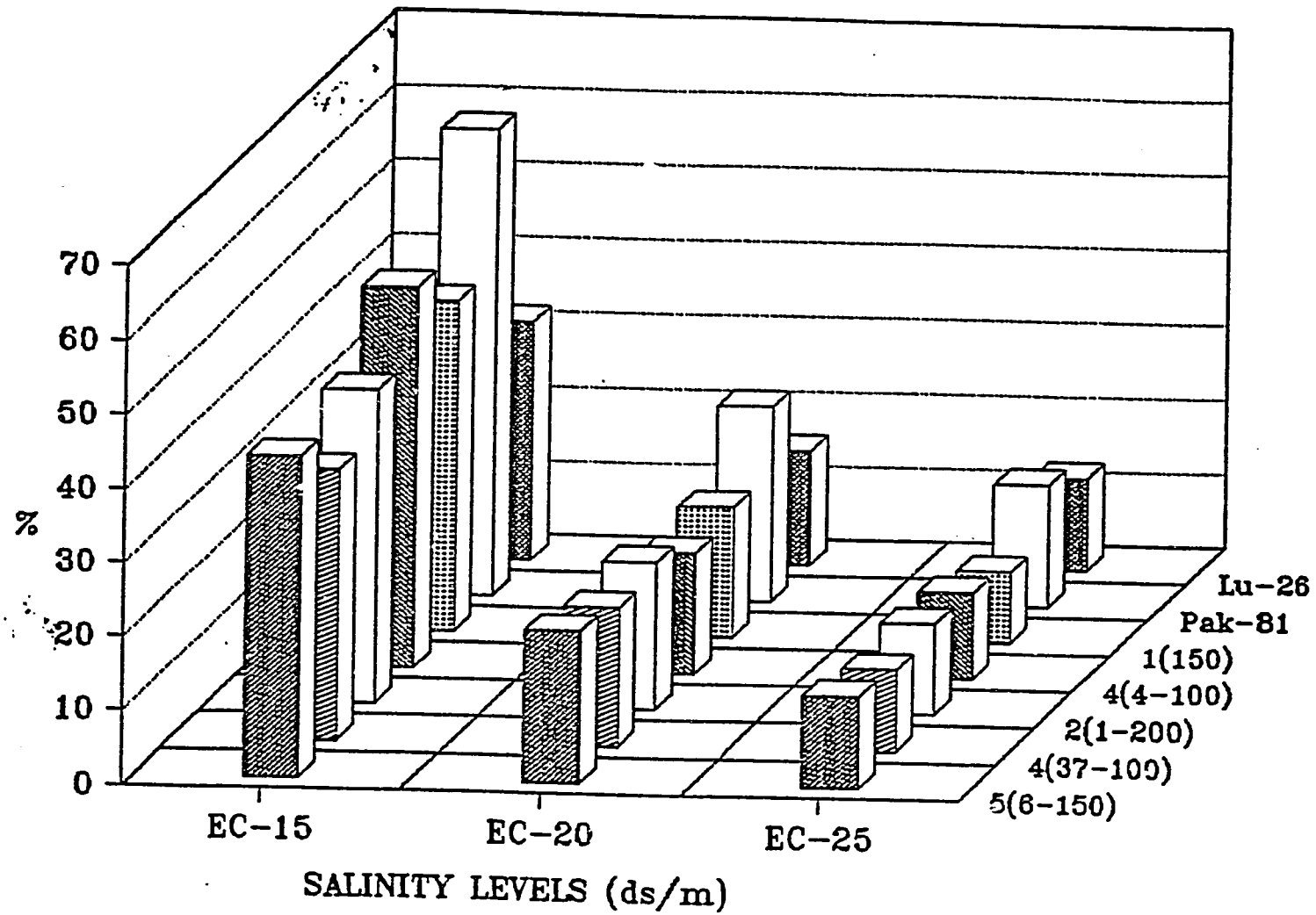


Fig. 2b Mean root length at three salinity levels (% of control in non-saline)



Production of salt-tolerant wheat germplasm through crossing cultivated wheat with *Aegilops cylindrica* -II. Field evaluation of salt-tolerant germplasm.

S. Farooq, M. Asghar, N. Iqbal, A. A. Zaidi and T. M. Shah.
Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Keywords: Salt tolerance, *Aegilops cylindrica*, *Triticum aestivum*, Saline soil, Saline irrigation, Potassium-sodium discrimination.

ABSTRACT

The performance of salt-tolerant wheat germplasm produced through crossing wheat with *Aegilops cylindrica* as well as that of three cultivated wheat varieties, was tested in three sets of experiments under saline fields of EC 6-10 dS/m. In set 1, all the germplasm was sown by direct seeding . In set 2, 2-week-old seedlings were transplanted. Sets 1 and 2 were given irrigation with saline water of EC 15 dS/m made in full-strength Hoagland nutrient solution. In set 3, a nonsaline plot was sown by direct seeding and was irrigated with nutrient solution only. This plot was kept as the control. Data were recorded at maturity, while K and Na concentrations were calculated at seedling, tillering and maturity stages.

There was not much difference in the yield components of the salt-tolerant wheat lines grown in the saline and nonsaline fields, except for a reduction in the mean grain yield, which ranged between 11 and 12% less than that of the nonsaline control. The

cultivated wheat varieties, on the other hand, showed significant reduction in grain yield, which ranged between 19 and 20% less than that of the nonsaline control. Similarly, differences in sowing and transplanting methods were generally nonsignificant in the wheat lines, as well as in the cultivated wheat varieties. One of the wheat lines tested in the present study showed an 18% increase in spike weight over LU-26. Spike length in three lines and grains per spike in six lines showed an increase over LU-26, (increase ranged from 13 to 23% and 14 and 37%, respectively). Weight of 100 grains was low in all the lines as compared with LU-26. Two lines (359 and 1076) produced significantly higher yields as compared with LU-26 under saline conditions. The values for K and K/Na ratio with the transplanting method, particularly at the heading stage, were higher in all the wheat lines as compared with the cultivated wheat varieties; however, no correlation could be established between high K/Na ratio and higher yield. The overall performance of the germplasm under saline fields proved better than that of salt-tolerant wheat cultivar LU-26. It is anticipated that after 2 to 3 years of testing and selection under saline fields, a replacement for LU-26 can be selected.

Introduction

Soil salinity affects different plant species differently, depending upon the environmental conditions (Greenway and Munns, 1980; Mass, 1986; Cheesman, 1988; Lauchli and Epstein, 1990). It is one of the major causes of low crop productivity in arid and semi-arid regions of the world (Pal *et al.*, 1984; Rabie *et al.*, 1985). The low productivity is generally attributed to reduced plant growth, an immediate response of plants exposed to salinity. There are, however, quantitative differences in the degree of plant response under salinity stress. The decrease in yield under saline as compared with nonsaline soil varies among species (Rush and Epstein, 1976), among cultivars (Abel and Mackenzie, 1964), and within varieties (Flower and Yeo, 1981). In our wide hybridization program, we have developed salt-tolerant wheat germplasm (Farooq *et al.*, 1992) using salt-tolerance genes from one of the salt-tolerant accessions of *Aegilops cylindrica* (Farooq *et al.* 1989). To test the comparative salt-tolerance levels of different lines of this germplasm, preliminary field trials were conducted at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, using saline and nonsaline fields, sowing and transplanting methods, and saline and nonsaline irrigation. This paper presents results on yield and yield component obtained in these experiments.

Materials and methods

Materials tested in the present study comprised nine salt-tolerant wheat lines (41, 359, 751, 879, 880, 883, 886, 1073, and 1076), three cultivated wheat varieties (Pak-81, Inqalab, and Pasban), and salt-tolerant wheat cultivar LU-26 as the check. The wheat lines were developed by crossing hexaploid wheat cultivar Pak-81 and LU-26 with salt-tolerant accession D of *Ae. cylindrica* (Farooq *et al.*, 1992). Four saline plots of 4 x 3.75 m were selected from a saline area situated at the campus of the Nuclear Institute for Agriculture and Biology (NIAB). The soil of these plots was 2:1 type, sandy loam, series Faisalabad (Annonymous, 1989). Electrical conductivity (EC), soil pH, and saturation percentage (SP) were determined (by the methods given in Handbook 60) for 24 samples per plot collected randomly. The EC, pH, and SP of the samples ranged from 6 to 10 dS/m, 7 to 7.6 and 34.9 to 41.81%, respectively. The experiments started in November 1992, when temperature ranges were between 26-28 ^{0}C/15-20 ^{0}C (day/night), relative humidity was above 55%, and daylight was about 10 hours.

In set one, all the germplasm was sown by direct seeding in a randomized complete block design with three replications (18 plants per replication). In set 2, 2-week-old seedlings of the same germplasm were transplanted. Both the sets were given irrigation with saline water of EC 15 dS/m, made by mixing $NaSO_4$, $MgCl_2$, $CaCl_2$ and NaCl (Qureshi *et al.*, 1977) in the ratio of 10:1:5:4 in Hoagland nutrient (Hoagland and Arnon, 1950). A total of 10 irrigations each of 40 liters per plot, were given from sowing (and/or trasplanting) to maturity. In set 3, a nonsaline (control) plot of the same size

was sown by direct seeding. This plot was given irrigations of the same number and volume, but with nutrient solution only.

Plants were allowed to grow in the three plots until maturity. Data with respect to number of tillers per plant, spikes per plant, spike length, spike weight, grains per spike, 100-grain weight, and grain yield were recorded at maturity. Concentrations of potassium and sodium were determined at the seedling, tillering (nearly boot), and matuirty stages. For this analysis, plant material at the different stages (seedling, tillering, and maturity) was harvested, briefly washed in three changes of distilled water to remove surface contamination, dried at 70 ^{0}C for 72 hours and ground to form a powder. The powder was than digested with concentrated nitric acid (HNO_3) and the digest was analyzed flamephotometerically (Anonymous, 1954). Data were analyzed statistically using Duncan's Multipal Range Test. At some points, the standard error of the means was calculated.

Results

Table 1 shows mean values of different yield components of all the salt-tolérant wheat lines, cultivated wheat varieties, and salt-tolerant wheat cultivar LU-26 when they were grown under saline and nonsaline (control) fields. There was no significant difference in overall performance of any of the yield determinants of wheat germplasm growing under the saline and nonsaline fields, except reduction in mean grain yield was observed under saline fields which ranged from 11 to 12% below that of the nonsaline control. On the other hand, cultivated wheat varieties under saline conditions exhibited considerable reduction in most of the yield components,

including grain yield, which ranged between 19 and 20% beow that of the nonsaline control. Similarly, considerable yield losses were observed in LU-26 under saline environment as compared with the nonsaline control. Generally, the reduction in wheat yield under saline conditions was less in the salt-tolerant wheat germplasm as compared with the cultivated wheat varieties and salt-tolerant wheat cultivar LU-26 (Table 1).

Significant ($p < 0.05$) differences were observed in all the yield components of the wheat lines and wheat varieties. (Table 2). There was no significant difference in most of the yield components and grain yield of wheat lines 885, 886, and 751 and wheat cultivar LU-26. In one line (880), spike weight increased by 18% over LU-26 (Figure 1). Grains per spike increased in six lines (41, 359, 751, 879, 883 and 1076) while grain yield increased significantly ($p < 0.05$) in two lines (Figure 2).

Comparision of the sowing and transplanting methods reveals nonsignificant differences in most of the yield components, except 100-grain weight, which was reduced in some of the wheat lines in the sowing method (Table 3). No significant difference in grain yields was observed except a small increase in yield in some of the wheat lines that were transplanted, as indicated by an average yield that was 103.4% that of the nonsaline control. Similarly, for all the wheat varieties, performance on all the yield components was better with the transplanting than with the sowing method. The response of LU-26 was the best, as indicated by its average grain yield with the transplanting method, which ranged from 112 to 141%

that of the nonsaline control and was significantly higher than the yield obtained with the sowing method.

Significant ($p < 0.05$) differnces were observed in the mean values of Na and K concentrations in all the wheat lines and at the three growth stages (Table 4). At the seedling stage, Na concentration was higher with the sowing method while K concentration and K/Na ratio were higher with the transplanting method. At the heading stage, there was no difference in Na concentration, but the K concentration was significantly ($P < 0.05$) higher with the transplanting than with the sowing method, while the K/Na ratio was similer with both methods. At maturity, as in the seedling stage, the K concentration and the K/Na ratio were higher with the transplanting than with the sowing method. It is interesting to note that Na concentration in all the plants grown under saline fields was low and K concentration was high at all the growth stages, irrespective of the cultivation method.

Discussion

The present study was conducted to assess the comparative salt tolerance potential of wheat lines produced through wide hybridization, the comparative performance of the sowing and transplanting methods and possible effects of cultivation methods on organic ion concentrations in plants.

As already mentioned, salinity stress reduces the rate of growth of whole plants as well as of specific shoot organs (Aspinall, 1986), with the degree of growth reduction depending on the salt-tolerance level of the germplasm (Mass and Hoffman, 1977). In the present study, the reduction in grain yield of wheat

germplasm was 12-13% under the saline fields and was low as compared with the reduction in grain yield (20-21%) observed in the cultivated wheat varieties and salt-tolerant cultivar LU-26. It is therefore logical to infer that wheat germplasm tested in the present study exhibited tolerance to salinity at a level not available in the cultivated wheat varieties.

Reduction in grain yield of wheat under salinity stress has been reported frequently (Rabie *et al.* 1985; Azmi and Alam, 1990), and is attributed mainly to reduction in number of tillers, spikelets per spike, kernels per spike, and kernel weight. In the present study also, yield components were affected by salinity, but there was some compensation in the form of increases in spike length, number of grains per spike, and spike weight, which resulted in increased yield in two of the wheat lines (359 and 1076).

The general method of sowing being used by farmers for cultivation of wheat in saline soil in Pakistan is broadcasting of presoaked seeds in the saline field having 7-10 cm of water on its surface. This practice helps in leaching salts down below the surface, allowing the presoaked seeds to establish and germinate under a comparatively less saline environment. The drawback of this method could be a reduction in the germination of wheat seeds under standing water. To avoid this drawback, the transplanting method was adopted to cultivate the saline soil with 2-week-old seedlings, followed by irrigation. The first irrigation of wheat is normally given after 15-20 days of germination, depending upon the moisture content of the soil (Annonymous, 1993). Therefore, irrigation at transplanting time was a necessity of that growth stage, as well as

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an aid to get the seedlings established in the saline soil. Although there was no significant difference in yield and yield components for the material tested by the sowing and transplanting methods, a reduction in number of spikes and 100-grain weight was observed with the sowing method, which was probably one of the reasons for the lower grain yield obtained under the sowing as compared with the transplanting method. A significant increase in the average yield of LU-26 with the transplanting method also indicated that if saline fields are transplanted, the reduction in yield caused by salinity can be managed to some extent. However, the feasibility of the transplanting method cannot be determined unless comparative trials of transplanting, direct sowing, and broadcasting of presoaked seed on highly saline lands are conducted simaltaneously. These experiments are in progress.

To understand other reason(s) for the increased yield under the transplanting method, analysis of Na and K at three growth stages was conducted. Theoretically, Na concentration should be high in the plants directly sown in the saline soil as compared with the transplanted plants. Since high uptake of Na, particularly when the growth habit changes from the vegetative to generative stage, can cause growth depression and yield reduction in wheat plants (Azmi and Alam, 1990), this could be one of the factors in the comparatively low yield of the plant sown directly. However, no significant difference in Na concentration between plants under the two methods of cultivation was detected at either the heading or the maturity stage, indicating that reason(s) other than Na

concentration might be responsible for the comparatively higher yield observed with the transplanting method.

Unlike Na, K concentration and K/Na ratio were very high in the transplanted plants, indicating, that in some of the wheat genotypes, the K-Na discrimination factor is operative, particularly under the transplanting method. According to Gorham (1990), gene(s) for the K-Na discrimination factor is (are) located on the long arm of chromosome 4D, and that express(es) only under low salinities (Gorham *et al.*, 1987). Since the present germplasm was developed by crossing wheat with *Ae. cylindrica* (CCDD), it is possible that gene(s) for K-Na discrimination present on the D genome of *Ae. cylindrica* may have transferred to the wheat, and that their cumulative effect was expressed under the transplanting method. To get an in-depth understanding of K-Na discrimination and its significance in practical breeding, particularly under different cultivation methods, more experiments are required, especially under fields of low and high salinity. Such experiments are in progress.

Conclusions

The germplasm tested for salt tolerance under the present study was developed by crossing hexaploid wheat with salt-tolerant accession D of *Aegilops cylindrica*, with a view to increasing variability for salt tolerance in hexaploid wheat. During the present field trials, which represent the first field testing of this material, a few findings emerged very clearly. First, the overall performance of the germplasm under the saline fields proved better than that of the salt tolerant wheat cultivar LU-26. Second some of the lines produced slightly higher yields than LU-26, but

two of the lines produced significantly higher yields than LU-26. In most of the wheat lines, yield reduction was largely compensated by increases in spike length and number of grains per spike. Third, the material was tested in the field using the sowing and transplanting methods, but no significant difference was observed in the performance of the wheat lines, wheat varieties, or wheat cultivar LU-26 under these two cultivation methods. Finally, high values for concentration and K/Na ratio were observed in the wheat lines that were transplanted in the field, but no correlation could be established between high yield and high K/Na ratio.

The significance of these findings lies in the fact that in Pakistan, at present, only wheat cultivar LU-26 is available for cultivation in saline soils, and it has now become susceptible to rust diseases. Therefore, it is not being recommended for general cultivation. Given this situation, some of the lines tested in the present study could easily replace LU-26 after 2-3 years of testing and selection. Since in most of the lines, reduction in grain yield is compensated by increases in spike length and grains per spike, this material may prove better than LU-26 both as a cultivar for saline lands and as a germplasm for breeding program aimed at improving yield components of wheat varieties. This material has also been proved resistant against most of the prevalent rust diseases at the seedling stage (data not presented); screeing for the adult plant stage is in progress in collaboration with Ayub Agricultural Research Institute. It is hoped that some of the lines will prove to be resistant to all the prevalent diseases. Such material will be of immense importance in the future when wheat

cultivation is extended into areas characterized by different types of stresses.

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References

Abel, G. H., and A. J. Mackenzie. 1964. Salt tolerance of Soya bean varieties (*Glycine max*. L. Merrill) during germination and later growth. Crop. Sci. 4: 157-160.

Annonymous, 1989. Chemical properties of soil. In: Hussain T and S. Mohammad (ed) Soil Science P.5. University of Agriculture Press, Faisalabad, Pakistan.

Annonymous, 1993. Annual Report. Directorate of Wheat Research, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

Annonymous, 1954. Diagnosis and improvement of of saline and alkali soils. USDA Hand Book No: 60. USDA, USA.

Aspinall, D. 1986. Metabolic effect of water and salinity stress in relation to expantion of the leaf surface. Aust. J. Plant Physiol. 13: 59-73.

Azmi, A. R. and S. M. Alam. 1990. Effect of salt stress on germination, growth, leaf anatomy and mineral elements composition of wheat cultivars. Acta. Physiol. Plantarum 12: 215-234.

Cheesman, J. M. 1988. Mechanism of salinity tolerance in plants. Plant Physiol. 87: 547-550.

Farooq, S., M. L. K. Niazi, N. Iqbal and T. M. Shah. 1989. Salt tolerance potential of wild resources of tribe Triticeae-II.

Screening of species of the genus *Aegilops*. Plant and Soil. 119: 255-260.

Farooq, S., N. Iqbal, M. Asghar and T. M. Shah. 1992. Intergeneric hybridization for wheat improvement VI. Production of salt tolerant germplasm through crossing wheat (*Triticum aestivum* L.) with *Aegilops cylindrica* and its significance in practical agriculture. J. Genet. and Breed. 46: 125-132.

Flowers, T. J. and A. R. Yeo. 1981. Variability in the resistance of sodium chloride salinity with rice (*Oryza sativa* L.) varieties. New Phytol. 88: 363-373.

Gorham, J., C. Hardy, G. R. Wyn Jones; L. R. Jopa and C. N. Law. 1987. Chromosomal location of K/Na discrimination character in the D. genome of wheat. Theor. Appl. Genet. 74:584-588.

Gorham, J. 1990. Salt tolerance in *Triticeae*: K/ Na discrimination in *Aegilops* species. J. Exp. Bot. 41: 615-621.

Greenway, H. and R. Munns. 1990. Mechanism of salt tolerance in non halophytes. Annu. Rev. Plant Physiol. 31: 149-190.

Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. Agric. Exp. Sta. Univ. Calif., Circular No. 347.

Luchli, A. and E. Epstein. 1990. Plant response to saline and sodic conditions. In: K. K. Tanji (ed) Agricultural salinity Reports on Engineering practices No. 71. Amer. Soc. Civil Eng; New york. N. Y.

Mass, E.V. 1986. Salt tolerance of Plants. Applied Agricultural Research 1: 12-26.

Mass E. V. and G. H. Hoffman. 1977. Crop salt tolerance assessment. J. Irrigation Driange Div. Proc. Amer. Soc. Civil Eng. 103: 115-134.

Pal, B., C. Singh and H. Singh. 1984. Barley yield under saline water cultivations. Plant and Soil. 81: 221-228.

Qureshi, R.H., M. Saleem, Z. Aslam, G.R. Sandhu. 1977. An improved gravel culture technique for salt tolerance studies on plants. Pak. J. Agric. Sci. 14: 11-18.

Rabie, R. K., M. K. Matter, A. M. K. Khanis and M. M. Mostaga. 1985. Effect of salinity and moisture cotent of soil on growth, nutrient uptake and yield of wheat plant. Soil Sci. and Plant Nutr. 31: 545.

Rush, D. W. and E. Epstein. 1976. Genotypic responses to salinity differences between sensitive and salt tolerance genotypes of tomato. Plant Physiol. 57: 162-166.

Table 1. Mean values and range of different agronomic characteristics of wheat germplasm developed through hybridization, 3 cultivated varieties and salt tolerant wheat cultivar LU-26 after growing under saline field of ECe 6-10 dS/m and non saline control.

Agronomic characteristics.	Salt tolerant germplasm		Wheat varieties		LU 126	
	Saline	Non-saline	Saline	Non-saline	Saline	Non-saline
No. of tillers	6.3 ±0.3	7.5±0.3	7.3 ±0.5	8.3±0.4	8.5±0.6	10.0 ±0.2
	(5-8)	(6-9)	(6-8)	(7-10)	(8-10)	(8-10)
No. of spikes/plant	6.0 ±0.2	7.0 ±0.3	7.4 ±0.2	8.3±0.4	7.3 ±0.1	10.0 ±0.3
	(6-7)	(5-8)	(6-8)	(7-10)	(7-9)	(8-10)
Spike length (cm)	16.0 ±0.7	15.3 ±0.5	15.7 ±0.4	15.0 ±0.5	15.2 ±1.0	15.0 ±1.0
	(13-18)	(12-18)	(15-17)	(14-18)	(14-17)	(15-18)
Grains/spike	58.3 ±1.9	45.0±1.7	56.1 ±3.4	60.0 ±2.0	46.1 ±2.2	48.0 ±1.7
	(50-65)	(43-60)	(46-62)	(59-63)	(43-51)	(46-53)
100 grain weight (gm)	3.5 ±0.1	3.5 ±0.2	3.6 ±0.3	3.7 ±0.4	4.3 ±0.2	4.4 ±0.3
	(2.9-4.1)	(2.9-4.2)	(3.1-4.3)	(2.9-4.2))	(3.5-4.5)	(4.0-4.7)
Grain yield/plant (gm)	13.9 ±0.6	14.8 ±1.3	13.9 ±0.6	16.9 ±1.6	13.7 ±1.4	15.8 ±1.8
	(12-17)	(13-19)	(12.4-15.1)	(15.2-18.9)	(12-17)	(15-18)
Grain yield(% of non saline control)	(87.8-88.9)		(80.1-81.4)		(80-94)	

Figures in parenthesis indicate range of different parameters.

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Table 2. Comparative agronomic performance of different salt tolerant wheat lines developed through hybridization and salt tolerant wheat cultivar LU-26 after growing under saline field of ECe 6-10 dS/m.

Entries	No. of tillers	No. of spike	Spike weight (gm)	Spike length (cm)	Grain per spike	100 grain weight (gm)	Grain yield (gm)
41	8.00^{bc}	7.67^{b}	2.00^{b}	17.50^{c}	61.22^{bc}	3.59^{ab}	15.31^{bc}
359	7.00^{ab}	7.00^{b}	2.14^{bc}	13.00^{a}	65.74^{c}	3.56^{ab}	16.76^{c}
1	7.56^{b}	7.33^{b}	1.98^{b}	17.00^{c}	65.33^{c}	3.56^{ab}	14.51^{b}
879	7.00^{ab}	6.67^{ab}	2.10^{bc}	17.00^{c}	54.55^{b}	3.24^{ab}	14.00^{ab}
880	6.00^{a}	5.33^{a}	2.25^{c}	16.5^{bc}	51.83^{ab}	4.07^{bc}	11.99^{a}
885	7.00^{ab}	6.83^{ab}	1.97^{ab}	17.00^{c}	61.00^{bc}	3.42^{ab}	13.23^{ab}
886	9.00^{c}	7.67^{b}	1.54^{a}	15.00^{b}	53.33^{ab}	2.87^{a}	12.54^{ab}
1073	7.00^{ab}	6.50^{ab}	1.76^{ab}	13.00^{a}	50.00^{ab}	3.72^{bc}	12.09^{ab}
1076	8.00^{bc}	7.67^{b}	2.16^{bc}	18.5^{d}	65.11^{c}	3.65^{b}	16.57^{c}
LU-26	8.00^{bc}	7.33^{b}	1.98^{b}	15.00^{b}	48.00^{a}	4.48^{c}	13.86^{ab}

In each column, figures followed by the same letters are not significantly different from each other at 5% level of significance according to Duncan's Multiple Range Test.

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Table 3. Mean values and range of different agronomic characteristics of wheat germplasm developed through hybridization 3 cultivated varieties, and salt tolerant wheat cultivars LU-26 after sowing and transplanting under saline field of ECe 6-10 dS/m.

Agronomic characteristics.	wheat lines		Wheat varieties		LU-126	
	Sowing	Transplanting	Sowing	Transplanting	Sowing	Transplanting
No. of tillers	6.3 ±0.3	6.5±0.1	7.2 ±0.5	7.5±0.7	7.7 ±1.0	6.5±0.7
	(5.0-8.0)	(6.0-7.0)	(6.5-8.5)	(6.5-9.5)	(6.0-10.0)	(5.0-8.0)
No. of spikes/plant	6.7 ±0.2	7.4 ±0.4	7.5 ±0.2	8.0 ±0.5	6.3 ±0.5	8.5±0.7
	(5.8-7.5)	(5.5-8.3)	(7.0-8.2)	(7.2-9.5)	(5.0-7.0)	(7.0-10.0)
Spike length (cm)	16.0 ±0.6	14.8 ±1.4	15.7 ±0.4	17.4 ±1.0	15.3 ±0.5	16.0 ±1.7
	(13.0-18.5)	(14.0-17.5)	(15.0-17.0)	(16.0-20.0)	(14.0-16.0)	(13.0-19.0)
Grains/spike	58.3 ±0.1	56.9 ±2.7	56.1 ±3.4	55.7 ±1.5	47.2 ±0.1	52.1 ±4.0
	(50.0-85.7)	(42.8-64.4)	(46.1-61.8)	(53.3-59.1)	(45.0-49.0)	(45.1-59.4)
100 grain weight (gm)	3.5 ±0.1	3.7 ±0.1	3.6 ±0.3	3.7 ±0.2	3.9 ±0.3	4.0 ±0.4
	(2.9-4.1)	(3.5-4.1)	(3.1-4.3)	(3.4-4.1)	(3.3-4.5)	(3.3-4.7)
Grain yield/plant (gm)	13.9 ±0.6	14.3 ±0.7	13.9 ±0.6	14.6 ±1.5	13.7 ±0.1	17.3 ±1.3
	(11.5-16.6)	(11.4-17.1)	(12.4-15.1)	(11.7-17.3)	(13.5-13.7)	(15.1-19.6)
Yield/% of non saline	(99.0 -103.4) Final		(94.0-114.0)		(112.0-141.3)	

Figures in parenthesis indicate range of different parameters.

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Table 4. Ion concentration (mg/g dry weight) in shoots of salt tolerant wheat germplasm sown and transplanted in saline field of ECe 6-10 dS/m.

Parameter		Seedling	Heading	Matuirty
Na Concentration mg/g dry weight	S	11.8 ±0.9 (7-15.53)	1.3 ±0.2 (1.0-1.8)	2.0 ±0.2 (1.4-2.3)
	T	0.7 ±0.1 (0.5-1.1)	1.5 ±0.1 (1.2-1.9)	1.6 ±0.1 (1.1-1.7)
K Concentration mg/g dry weight	S	4.7 ±0.8 (2.6-6.8)	68.5 ±5.1 (55-82.5)	7.2 ±1.1 (5.0-8.8)
	T	5.3 ±0.3 (4.2-6.2)	77.8 ±6.3 (61.2-90)	7.9 ±1.1 (5.5-11.2)
K/Na	S	0.4 ±0.3 (0.1-1.5)	53.9 ±5.7 (42.2-54.0)	3.8 ± 0.4 (2.5-5.9)
	T	7.6 ±0.7 (5.6-9.5)	51.5 ±2.84 (44.8-58.3)	5.1 ± 0.7 (3.3-6.6)

S = Sowing, T = Transplanting,
Figures in parenthesis indicate range of different parameter.

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Fig. 1 Comparative performance of salt tolerant wheat germplasm under saline field of EC 6–10 ds/m. All values are % of salt tolerant wheat check (Lu-26)

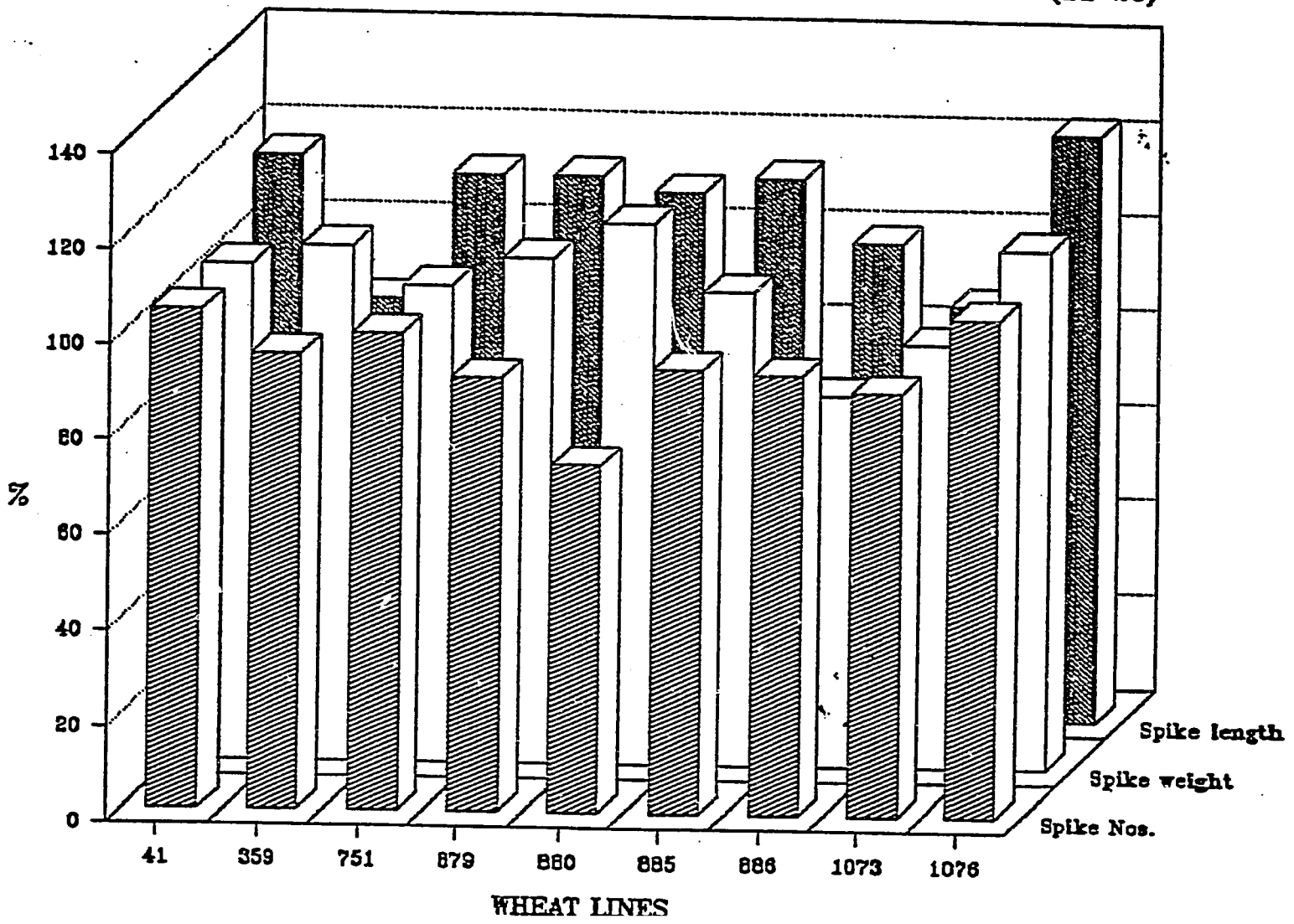
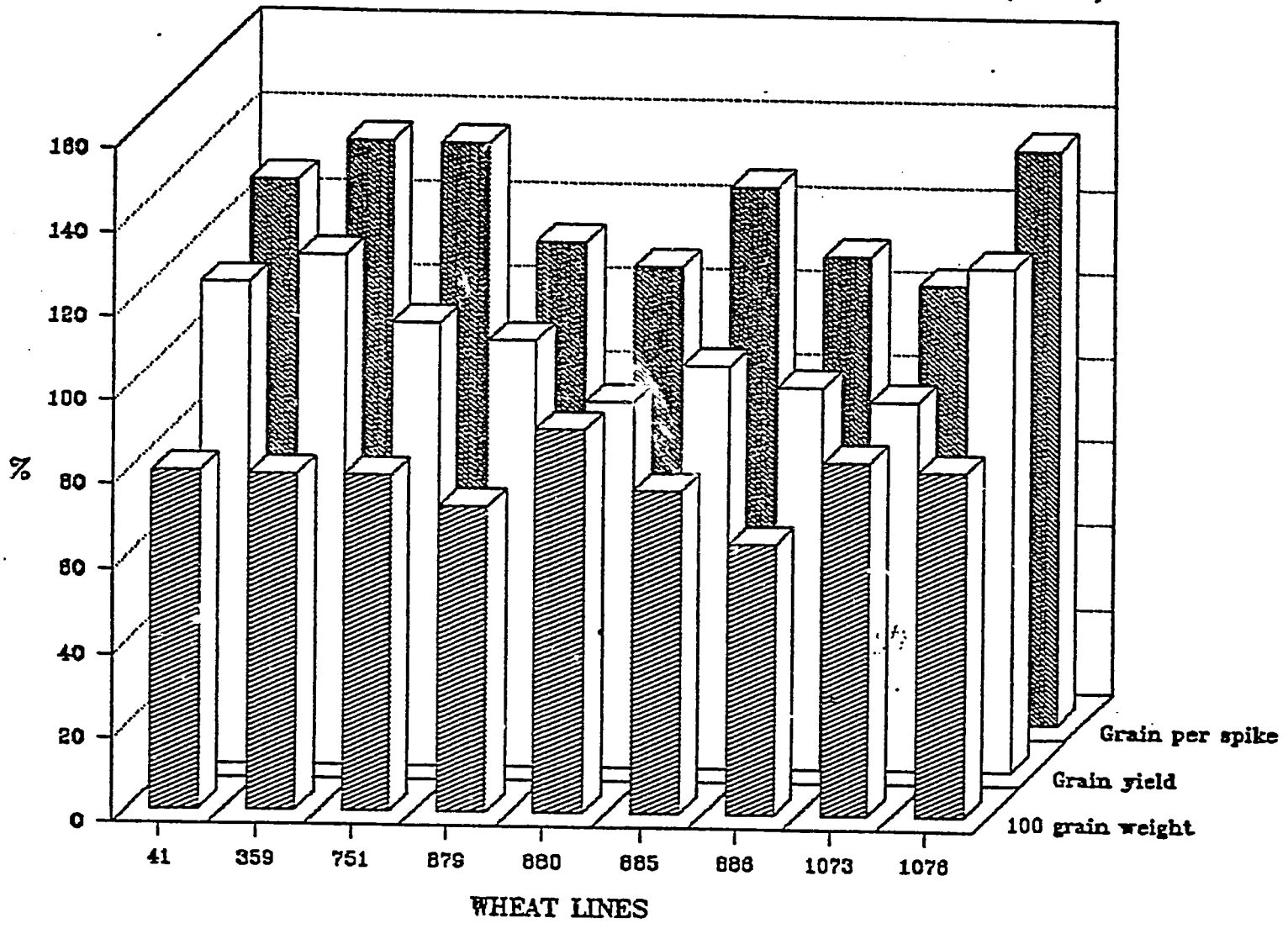


Fig. 2 Comparative performance of salt tolerant wheat germplasm under saline field of EC 6-10 ds/m. All values are % of salt tolerant wheat check (Lu-26)



Identification of different wheat genotypes through Polymorphism based on Random Amplified Polymorphic DNA (RAPDs)

S. Farooq, T. M. Shah, E. Askari, A. A. Zaidi and N. Iqbal

Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Summary: Polymerase Chain Reaction was utilized to detect polymorphism in ten different salt tolerant wheat introgression lines (produced through wide hybridization), the cultivated wheat varieties and salt tolerant accession D of *Aegilops cylindrica*. DNAs extracted from fresh leaves by CTAB method were amplified using 25 ul reaction volume in Perkin Elmer Thermalcycler and randomly sequenced 10 mer synthetic Primers. Based on the amplification reactions, all the primers were divided into four categories. Category A primers reacted with all the wheat lines while category B with none. Category C primers reacted with 10-14 lines while category D primers reacted with 3-7 wheat lines only. Of the 18 reactive primers, 11% detected polymorphism in all the test material. The level of polymorphism was low and ranged between 1.2 to 22.8%. Most of the primers produced monomorphic but very intense bands while other types of bands included intense bands, easily visible and less visible bands. Of the 9 salt tolerant introgression wheat lines, 6 were identified on the basis of polymorphic bands. Our study indicated possibilities of using RAPD markers to detect specific variation in the genome which can be used for varietal finger printing.

Key words. RAPD markers. DNA polymorphism, PCR, Primers, *Triticum aestivum, Aegilops cylindrica*.

Introduction

Identification of different genotypes of crop species and varieties is important, particularly when new crop varieties are to be released, newly produced germplasm is to be registered, different accessions of wild species are to be characterized, and /or purity of the germplasm is to be determined. For a long time, such identification has been based on phenotypic differentiation or morphological traits such as ear morphology, awning, and hairiness of neck or peduncle (Worland *et al* 1984). However, since there are many varieties with awns and hairy peduncle, identification of two similar varieties is often difficult. Similary, the introgression lines, which are sometime different from parent by only few base pair sequences are difficult to differentiate from each other on morphological or phenotypic bases. To overcome such difficulties, biochemical markers, such as isozymes, were developed and are being used successfully for the last two decades (Arus *et al.*, 1982, Bassiri, 1976; Stageman, 1984; Gupta and Robbelen, 1986; Ladizinsky and Hymowitz, 1979; Vangham and Denford, 1986). Since the frequency of polymorphism detected by biochemical markers in different crop species was low, therefore molecular markers such as Restriction Fragment Length Polymorphism (RFLP) were developed and are being used widely for mapping of plant genomes such as maize (Weber and Helentjaris, 1989), tomato (Tanksley _*et al.*, 1992), rice (McCouch *et al.*, 1988), potato (Gebhardt *et al.*, 1989), lettuce (Landry *et al.*, 1987), Arabidosis (Reiter *et al.*, 1992), Conifer (Carlson *et al.*, 1991), barley (Graner *et al.*, 1991) wheat (Anderson *et al.*, 1992), peanut (Kochert *et al.*, 1991), *Brassica rapa* (Song *et al.*,

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1991) and Cupbea (Webb *et al.*, 1992).

Recently a new class of molecular markers (Rafalski *et al.*, 1991) known as Random Amplified Polymorphic DNA (RAPDs) were developed, which, unlike RFLP markers do not require large amount of DNA, restriction digestion, southern blotting, and radioactive labelling.

In our wide hybridization programme, we crossed wheat cultivar Pak-81 with salt-tolerant accessions of *Aegilops cylindrica* (Farooq *et al.* 1992) and produced different salt tolerant introgression wheat lines which have plant height, ear morphology, heading date and grains per spike of wheat cultivar Pak-81 and LU-26 and salt tolerance of donor species *Ae. cylindrica*. Our objective was to display visible differences simaltaneously in Pak-81, LU-26, *Ae. cylindrica* and salt tolerant introgression lines. In the present attempt, we have used RAPD markers to differentiate these lines from parents and from each other. Some of these results are presented in this report.

Material and Methods

Material

Ten different wheat lines produced through wide hybridization, two cultivated wheat varieties (Pak.81 and LU-26) used as female parent for production of F_1 hybrid and backcross derivatives and accession D of *Ae. cylindrica,* salt tolerant wheat cultivar "Pasban" and tetraploid wheat cultivar "Durum" were used in RAPD assay (Table-1) Twenty synthetic 10 mer primers belonging to series-S, obtained from Operon Technologies (Alameda, California) were used for amplification. Taq Polymerase together with 10x PCR

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buffer, $Mgcl_2$ and dNTPs were purchased from Perkin Elmer Cetus. Perkin Elmer DNA Thermal Cycler was used for DNA amplification.

DNA extraction

Healthy seeds of the above mentioned material were germinated on moist filter paper in petri plates. Seedlings were raised in growth chamber at 20°C ± 2°C. Two to three week old seedlings were used for DNA extraction following the CTAB method of Rogers and Bendich (1988), replacing dry ice with liquid nitrogen. DNA concentration and its quality were determined on a Spectronic-21 spectrophotometer at wave length of 260 and 280 nm. From this measurement, a working solution of 5ng/ul DNA was prepared. Each dilution was then repeatedly re-checked on the Spectrophotometer till the exact concentration (5ng/ul) was obtained.

DNA amplification

The reaction volume (25ul) containing 2.5 ul (1x) PCR buffer, 2.5 ul (2.5 mM) $Mgcl_2$, 2.5 ul (0.2 mM each) dNTPs, 0.2 ul (one unit) Taq Polymerase, 0.5 ul (0.4um) 10 mer primer, 5ul (5ng/ul) genomic DNA and 11.8 ul water over laid with one drop of mineral oil was amplified according to the procedure of Wiliams *et al.* (1990), using step cycle file programmed for 40 cycles. After amplification, one drop of 5x RAPD dye (bromophenol blue mixed with 10% glycerol, 0.1 M EDTA and 2% SDS) was added to the reaction mixture. Only 12 ul of this reaction mixture was loaded on 2.0 % agarose gel submerged in TAE buffer. Lambda DNA digested with Hind III was used as a molecular size marker. Samples were electrophoresed for aproximately 2 hours at 100 volts. After electrophoresis, the gels were rinsed twice with distilled water,

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stained in 1ug/ul ethidium bromide for 20 minutes followed by two rinses in distilled water. The amplified products were viewed under U.V. fluoresence and photographed using the Stratagene Eagle Eye still video system. All the reactions were repeated twice.Those reactions where any specific primer failed to produce amplification products were repeated three times, in order to confirm the non reactivity of the primers.

Scoring of data

Data were scored from three good quality photographs produced for three replicates of each amplification reaction product. Bands were scored from top of the gel (band No.1) to the bottom. The left lane of the gel was considered as Lane-I. The bands produced by one primer in all the wheat lines used in this study were scored under the heading of "Total bands produced by that primer". All the characteristic bands produced by one primer that are repeatedly present and/or absent in only some of the lines, were scored as polymorphic bands. Reproducibility of the reaction was confirmed when a specific band repeatedly appeared in a specific region. All the bands less than 500 bp except those which are clearly visible were sometimes difficult to score and were omitted. Based on the presence and absence of amplification products, all the primers were divided into four categories. Category A comprised 7 different primers which produced amplification products in all the wheat lines while category B comprised two primers which failed to produce any amplification product in any of the wheat lines. Category C comprised 5 primers each of which produced amplication products in 67-93% of the wheat lines, while category D comprised

6 primers each producing amplification products in only 13-60% of the wheat lines, respectively.

Results

Table-2 shows details of amplification products produced by 20 different primers in all the wheat lines used in the study. All the primers, except S-02 and S-15, produced amplification products that ranged between 2-119 amplification products per primer. Among the 18 primers 7 primers (8, 9, 11, 12, 16, 17 and 19) termed as category A and produced amplification products in all the wheat lines, 6 primers (1, 4, 5, 7, 18 and 20) termed as category C and produced amplification products in most of the lines while 5 primers (3, 6, 10, 13, 14) termed as category D and produced amplification products in some of the lines. Of all the primers, (61%) detected, polymorphic bands which ranged between 1 (S-07) to 9 (S-09) bands per primer. The level of polymorphism was low and ranged between 1.2 (primer S-07) to 22.8% (S-01). Most of the scorable bands (96%) produced in all the test material were monomorphic and in all the reactions they appeared between 2000 to 500 base pair region except in reaction with primer S-9 where less than 500 base pair band was produced.

The amplification products, produced by category A primers were divided into four classes (Table-3). All the primers, except S-19, amplified products of varying frequencies which appeared as high intensity bands, intense bands, easily visible bands and less visible bands. Generally, in all the amplified products, frequency of highly intense and visible bands was higher than very intense and less visible bands.

The amplification products obtained with some of the primers are given in Figs. 1, 2, & 3.

Fig. 1, shows a set of 2 and 3 bands respectively in wheat line 880 and 883 (lane 4 and 5) immediately below the very intense band in the middle. These bands are not present in wheat cultivar LU-26, and hence differentiate wheat line 880 from 883 and both of them from LU-26. Similarly, there is a set of bands located above the very intense bands in wheat line 886 which is also present in *Ae. cylindrica* (lane 13) and absent in LU-26 and hence differentiate 886 from the other wheat lines.

Fig. 2 presents amplification products obtained with primer OPS-9. In this figure most of the amplification products are different from each other. Wheat line 41 and 359 produced identical profile which is different from the profile of wheat line 880 by the presence of band number 2 in this line. Wheat line 880 and 883 could be differentiated on the basis of differences in size of the two bands each present in the bottom of both the lines and presence of first band in 883 which is missing in 880. In wheat lines 885 and 886, difference in size of very intense band in the middle is evident. Similary, presence of two bands at the top of both the lines differentiated both of them from LU-26. In wheat lines 1073 and 1076, variation in size of the last two bands is clear. In Durum and *Ae. cylindrica* last band is missing while in Pasban, very intense band in the bottom is missing. All the lines can therefore be easily separated from each other and from the parents. This is the only primer (S-09) which produced at least one polymorphic band (Lane 12) which is less than 500 base pair size.

Fig. 3 presents amplification products obtained with primer OPS-12. In this profile, wheat line 41 can be differentiated from wheat lines 359 and 879 on the basis of absence of a band in line 359. Likewise, wheat line 880 can be differentiated from 883 by the presence of band number 1 which is also present in wheat lines 885 and 886 but missing in wheat lines 359, 880, 1073, 1076 and LU-26 and seperate these lines from rest of the lines.

Discussion

Since the discovery of RAPD markers (Williams *et al.*, 1990; Welsh and McClellend, 1990), they have been utilized extensively to accelerate the construction of linkage maps. Their large scale utilization in tomato (Martin *et al.*, 1991; Klein-Lankhorst *et al.*, 1991); lettuce (Michelmore *et al.*, 1991); Brassica, broccoli and Cauliflower (Quirose *et al.*, 1991; Hu and Quirose, 1991) have also successfully demonstrated their effectiveness in the identification of strains and varieties through genomic finger printing. In the present study, we have successfully utilized the technique of Polymerase Chain Reaction for the generation of RAPD markers in different introgression lines of wheat produced through wide hybridization. The polymorphism thus detected was largely based on the presence and/or absence of a characteristic band and in some cases variation in the size of the bands and was used for identification of different genotypes.

In the three earlier attempts on utilization of RAPD markers in wheat, polymorphism was either detected by specifically sequenced primers (D' Ovidio *et al.*, 1990; Weining and Langridge,

1991) or through randomly sequenced two Operon primers of series A and B (Devos and Gale, 1992). In the present study , 20 Operon S-series primers were used to detect polymorphism in wheat varieties as well as in wheat introgression lines. Of the 20 primers, 11 detected polymorphism based on bands absent in both the parents and polymorphic bands coming from the doner parent *Ae. cylindrica*. The source of polymorphism detected in the present study could be due to the changes in or beween the priming sites due to the introgression of foreign DNA or it could be due to the introgression of whole segment of *Ae. cylindrica* DNA. Category A primers detected polymorphism in all the wheat lines, nevertheless, the level of polymorphism was generaly very low for all the primers. Such observation has been reported earlier by Devos and Gale (1991) which, as explained by these authors could be due to the fact that most of the amplified products in wheat originate from repeated DNA sequences which are abundant in most cereal (Flavell *et al.*, 1974).

In the present study, 18 out of 20 primers reacted with all the wheat genotypes and 11 of them detected polymorphism. This differential behaviour of the primers indicated that for a meaning full RAPD analysis, selection of proper primer is very important which could possibly be made either after testing large number of randomly selected primers or by selecting primers of known sequences.

All the primers which reacted with wheat, amplified 4 different types of bands of which the frequency of visible bands was very high. Such classification in banding profile has recently

been reported by Heun and Helentjaris (1993) in corn. In their study , frequency of quantitative polymorphism which is based on the variation in intensity of the bands is very high. We have also observed such variations which although can be scientifically justified are not the basis of genotypic identification made in the present attempt. In the present study, of the 9 introgression lines, 6 were easily identified on the basis of polymorphic bands. Since, introgression lines generally look similar, it is very difficult to differentiate among them. The present study clearly evidenced that RAPD analysis is perhaps one of the techniques which can be effectively utilized to detect such variation.

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References

Anderson J, Ogihara Y, Sorrells ME, Tanksley SD (1992) Development of a Chromosome Arm Map for wheat based on RFLP markers. Theor Appl Genet 83: 1035-1043

Arus P, Tanksley SD, Orton TJ, Jones RA (1982) Electrophoretic variation as tool for determining seed purity and for breeding hybrid varieties of *Brassica oleraceae*. Euphytica 31: 417-428

Bassiri A (1976) Barley cultivar identification by use of isozyme electrophoretic patterns. Can J Plant Sci 56: 1-6

Carlson JE, Tulsieram LK, Glanbitz JC, Lutvwk, Kauffeldt C, Rutledge R (1991) Segregation of random amplified DNA marker in F1 progeny of conifers. Theor App Genet 83: 194-200

Devey ME, Jermstad KD, Tauer CG, Neale DB (1991) Inheritance of

RFLP loci in a loblolly pine tree - Generation Pedigree. Theor App Genet 83: 238-242

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in Wheat. Theor App Genet 84: 567-572

D'Ovidio R, Tanzarella OA, Porceddu E (1990) Rapid and efficient detection of genetic polymorphism in wheat through amplification by polymerase chain reaction. Plant Mol Biol 15: 169-171

Farooq S, Iqbal N, Asghar M, Shah TM (1992) Intergeneric hybridization for wheat improvement VI. Production of salt tolerant germplasm through crossing wheat (*Triticum aestivum* L.) with *Aegilops cylindrica* and its significance in practical agriculture. J Genet Breed 46: 125-132

Flavell RB, Bennett MD, Smith JB, Smith DB (1974) Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochem Genet 12: 257-269

Gebhardt C, Ritter E, Debener T, Schachtschnabel U, Walkemeier B, Uhring H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78: 65-75

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of Barley. Theor Appl Genet 83: 250-256

Gupta SK, Robbelen G (1986) Identification of rapeseed (*Brassica napus*) cultivars by electrophoresis. Plant Breeding 96: 363-370

Heun M, Helentjaris T (1993) Inheritance of RAPDs in F1 hybrids of corn. Theor Appl Genet 85: 961-968

Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Report 10: 505-511

Kangfu Yu, Pauls KP (1992) Optimization of the PCR Programme for RAPD analysis. Nucleic Acid Research 20: 2606

Klein - Lankhorst RM, Vermunt A, Weide R, Liharska T, Zabel P (1991) Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor Appl Genet 83: 108-114

Kochert GT, Halward, Branch WD, Simpson CE (1991) RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor Appl Genet 81: 565-570

Ladizinsky G, Hymowitz T (1979) Seed protein electrophoresis in taxonomic and evolutionary studies. Theor Appl Genet 54: 145-151

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987) A genetic map for lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116: 331-337

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a pseudomonas resistance gene in tomato by using random primers and near isogeneic lines. Proc Natl Acad Sci USA 88: 2336-2340

McCouch SR, Korchert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76: 815-829

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant

analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828-9832

Quirose CF, Hu J, This P, Chevre AM, Delseny M (1991) Development and chromosomal localization of genome - specific markers by polymerase chain reaction in Brassica. Theor Appl Genet 82: 627-632

Rafalski JA, Tingey SV, Williams JGK (1991) RAPD markers - a new technology for genetic mapping and plant breeding. Ag Biotech News Inf 3: 645-648

Reiter RS, Williams JGK, Feldman KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89: 1477-1481

Rogers SO, Bendich AJ (1988) Extraction of DNA from plant tissues. Plant Mol Biol Manual A6: 1-10

Song KM, Suzuki JY, Slocum MK, Williams PH, Osborn TC (1991) A linkage map of *Brassica rapa* (*Syn. compestris*) based on Restriction Fragment Length Polymorphism loci. Theor Appl Genet 82: 296-304

Stagemann H (1984) Retrospect on 25 years of cultivar identification by protein pattern and prospects for the future. Seed Sci & Technol 15: 20-31

Tanksley SD, Ganal MW, Prinee JP, De Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giouanonni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder

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MS, Wing RA, Wu W, Young ND (1992) High density Molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141-1160

Vaughan JG, Denford KE (1968) An acrylamide gel electrophoretic study of the seed protein of Brassica and Sinapis species with special reference to their taxonomic nature. J Exp Bot 19: 724-732

Webb DM, Knapp SJ, Tagliam LA (1992) Restriction Fragment Length Polymorphism and Allozyme linkage map of Cupbea lanceolata. Theor Appl Genet 83: 528-532

Weber D, Helentjaris T (1989) Mapping RFLP loci in maize using B-A translocations. Genetics 121: 583-590

Weining S, Langridge P (1991) Identification and mapping of polymorphism in cereals based on polymerase chain reaction. Theor Appl Genet 82: 209-216

Welsh J, McClelland M (1990) Finger printing genome using PCR with arbitrary primers. Nucleic Acids Res 18: 7213-7218

Williams JGK, Kubelik AE, Levak KJ, Rafalski JA, Tingey SC (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531-6535

Worland AJ, Gale MD, Law CN (1984) Wheat Genetics. In: Lupton FGH (ed) Wheat Breeding: its scientific basis. Chapman and Hall London, pp 129-166

Table.1. Detail of germplasm used in the study

S.No.	Plant designation number	Parentage
1.	41	LU-26 / *Ae. cylindrica* D. // Pak.81
2.	359	LU-26 / *Ae. cylindrica* D. // Pak.81
3.	879	LU-26 / *Ae. cylindrica* D. // LU-26
4.	880	LU-26 / *Ae. cylindrica* D. // LU-26
5.	83	Durum / *Ae. cylindrica* D. // Pak.81
6.	885	LU-26 / *Ae. cylindrica* D. // Pak.81
7.	886	LU-26 / *Ae. cylindrica* D. // Pak.81
8.	1073	LU-26 / *Ae. cylindrica* D. // LU-26
9.	1076	LU-26 / *Ae. cylindrica* D. // Pak.81
10.	LU-26	(salt tolerant wheat parent)
11.	Durum	(salt sensitive tetraploid wheat)
12.	Pasban	(salt tolerant cultivated wheat)
13.	*Ae. cylindrica*	Accession D
14.	Pak.81	(salt sensitive cultivated wheat)
15.	751	LU-26 / *Ae. cylindrica* D. // Pak.81

Further detail of germplasm in Farooq *et al.* (1992)

Table 2. Detail of total bands and poymorphic bands produced by 20, S-series primers and their distribution in salt tolerant wheat germplasm.

Primer	Total scorable bands produced in the test material	Poly-morphic bands	Distribution of Polymorphic bands in the test matrial														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S-01	22	5(22.8)	5	+	+	+	+	+	+	+	+	0	0	0	0	0	0
S-02	00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S-03	04	0	0	0	0	0	0	+	0	0	0	+	0	0	0	0	0
S-04	119	7(5.9)	+	+	+	+	+	2	+	1	+	+	1	1	2	0	0
S-05	33	0	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+
S-06	04	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0
S-07	87	1(1.2)	+	+	+	+	+	+	+	+	+	+	+	+	1	+	0
S-08	67	7(6.5)	+	+	+	1	1	1	1	1	+	+	1	+	1	+	+
S-09	62	9(14.5)	+	+	+	+	2	1	1	2	1	1	+	1	+	+	+
S-10	55	2(3.6)	0	0	0	0	0	+	0	1	0	+	0	+	1	0	+
S-11	115	3(2.6)	+	+	+	+	+	+	+	+	+	+	1	1	1	+	+
S-12	65	5(7.7)	+	+	+	1	1	+	+	2	+	+	+	+	1	+	+
S-13	41	0	0	+	+	0	+	+	0	+	0	+	+	0	0	0	0
S-14	62	2(3.2)	+	+	0	0	+	0	0	+	0	+	0	+	2	0	0
S-15	00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S-16	30	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-17	101	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S-18	80	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0
S-19	83	1(1.2)	+	+	+	+	+	+	+	+	+	+	+	+	1	+	+
S-20	48	3(6.3)	+	+	+	0	+	+	1	+	0	0	1	1	+	+	+

+ = Monomorphic bands 0 = No bands
Figures in parenthesis are % Polymorphic bands

Table 3. Details of different types of bands produced by 18 S-series 10 mer primers in salt tolerant wheat germplasm.

Catagory of Primer	Primer designation and sequence		Total bands	% Frequency of different bands produced by each primer			
		5' to 3'		*	+	(+)	(-)
A	S-08	TTCAGGGTGG	108	1.9	7.4	35.2	55.6
	S-09	TCCTGGTCCC	62	1.6	24.2	38.7	35.5
	S-11	AGTCGGGTGG	115	0.9	2.6	61.7	34.8
	S-12	CTGGGTGAGT	65	13.9	4.6	40.0	41.5
	S-16	AGGGGGTTCC	30	7.0	10.0	23.0	40.0
	S-17	TGGGGACCAC	011	1.0	3.0	67.0	19.0
	S-19	GAGTCAGCAG	83	-	36.0	17.0	47.0
C	S-01	CTACTGCGCT	22	-	4.5	59.1	36.4
	S-04	CACCCCCTTG	119	2.6	15.1	47.1	25.2
	S-05	TTTGGGGCCT	33	-	-	100.0	-
	S-07	TCCGATGCTG	87	-	-	65.5	34.5
	S-18	CTGGCGAACT	80	7.0	5.0	45.0	43.0
	S-20	TCTGGACGGA	48	-	4.2	60.4	35.4
D	S-03	CAGAGGTCCC	4	50.0	-	-	50.0
	S-06	GATACCTCGG	4	-	-	50.0	50.0
	S-10	ACCGTTCCAG	55	3.6	12.7	43.6	40.1
	S-13	GTCGTTCCTG	41	-	4.9	39.0	56.1
	S-14	AAAGGGGTCC	62	-	-	45.1	54.9

*= Very intense bands += Intense bands (+)= Visible bands
(-)= Less visible bands

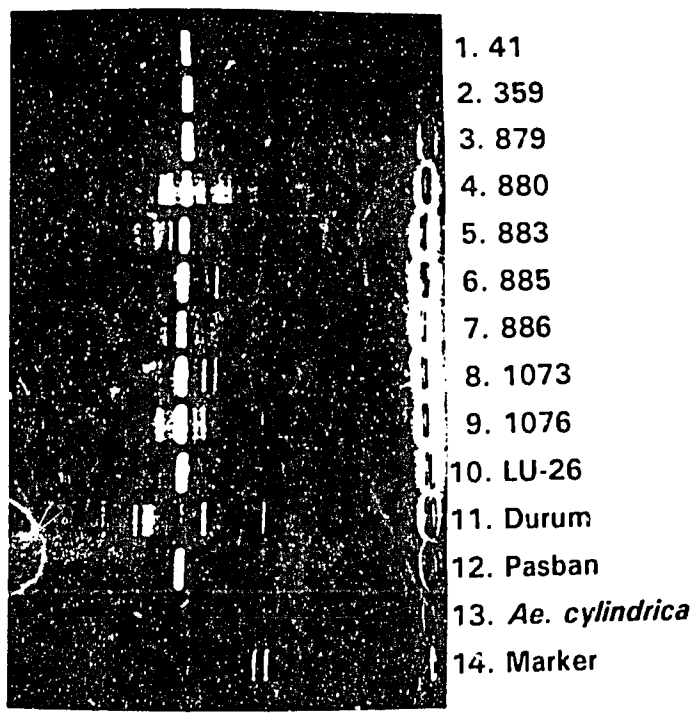

Fig. 1. Amplification products produced by primer OPS- 8. Wheat lines 880, 883 (lane 4 and 5), 886 (lane 7) and LU-26 (lane 10) are clearly distinguishable.

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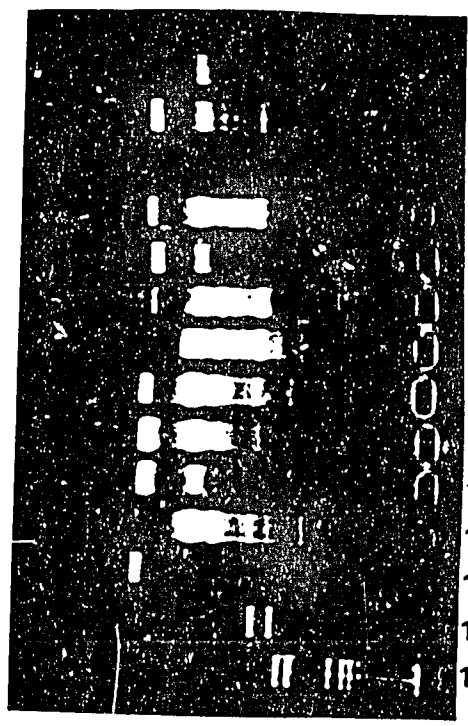

1. 41
2. 359
3. 879
4. 880
5. 883
6. 885
7. 886
8. 1073
9. 1076
10. LU-26
11. Durum
12. Pasban
13. *Ae. cylindrica*
14. Marker

Fig. 2. Polymorphism produced by primer OPS-9. Most of the lines can easily be separated from each other and from parents.

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1. 41
2. 359
3. 879
4. 880
5. 883
6. 885
7. 886
8. 1073
9. 1076
10. LU-26
11. Durum
12. Pasban
13. *Ae. cylindrica*
14. Marker

Fig. 3. Amplification products obtained with primer OPS-12. Polymorphism can be detected in wheat lines 41, 359, 879, 880, 1073, 1076, and LU-26.

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BREEDING SUNFLOWER FOR SALT TOLERANCE:
Physiogenetic mechanism of salt tolerance in sunflower (*Helianthus annuus* L.) [1]

Medhet Kamil Hussain and Obaid Ur-Rehman [2]

[1] Contribution of Dep. of Plant Breeding and Genetics, University of Agriculture, Faisalabad(Pakistan) in collaboration with National Academy of Sciences/ BOSTID, Washington D.C., U.S.A.

[2] Associate Professor and Ph.D. Research Fellow, Department of Plant Breeding and Genetics, Univ. of Agri. Faisalabad (Pakistan).

ABSTRACT

Three experiments were conducted to evaluate 15 sunflower (*Helianthus annuus* L.) genotypes against three salinity levels. Initially, the genotypes were grown unde saline conditions and were classified as being salt-tolerant or salt-sensitive depending on their seed yield. Next, the concentration of chloride, sodium and potassium ions in the leaves of each genotype were measured at both the seedling and flower initiation stages. The differences between salt-tolerant and salt-sensitive lines for ions concentration were then compared. At a moderate salinity level (EC_e 5 dSm^{-1}), tolerant sunflower lines decreased sodium and thus had a better potassium-to-sodium ratio at the seedling stage. The tolerant lines also decreased sodium, increased potassium, and kept a higher potassium to sodium ratio in leaves at flower-initiation. At higher salinity (EC_e 10 dSm^{-1}), the salt-tolerant lines showed a decrease in sodium concentration and an increase in potassium, and thus maintained a higher potassium-to-sodium ratio at the seedling and flower-initiation stages. A decrease in sodium concentration and a higher potassium to sodium ratio was common at both growth stages in salt-tolerant cultivars, and this was found to be the physiological mechanism for salt tolerance in sunflower. These traits were also found to be highly heritable at both stages of growth, and thus in future breeding programs can be used as guidposts to salt-tolerance.

Index Words

Sunflower, physiogenetic mechanism, salt tolerance.

INTRODUCTION

Salinity is one of the most severest soil problems confronting irrigated agriculture throughout the world. It has been estimated that salinity seriously limits crop production on 20 million hectares of the world's cultivated land (El-Ashry et el., 1985). Pakistan has an arid/semi-arid subtropical climate and shares this problem with many other countries of the world. The magnitude of the problem in Pakistan

can be seen in the fact that about 6.3 million hectares, including about 18.4% of the country's canal-commanded area, is salt-affected (Anonymous, 1989). Another 1.20 million ha of salt-affected area exists in patches (Chaudhri et al., 1978).

Soil is the major source of mineral nutrients for plants. Although seeds contain considerable quantities of mineral nutrients, uptake from the soil starts shortly after germination and soon contributes most of the ions entering the roots and shoots. Some toxic ions are also required for stimulating the growth of various species especially halophytes (Storey and Wyn Jones, 1979) but the range between beneficial and toxic concentrations is very narrow for most plants. The toxicity in glycophytes under saline conditions has been attributed to "ion excess" (Yeo and Flowers, 1984). Growth may be limited also by metabolic limitations caused by ion toxicity due to the accumulation of Na^+ and Cl^- used for osmotic adjustment. The cell may be energy-limited because of the diversion of metabolic carbon into biosynthesis processes required for maintenance of salinity tolerance (Yeo, 1983); such a situation may result in substantial partitioning of carbon away from growth processes.

Salinity of the root medium caused by NaCl interferes with the absorption and translocation of K^+ and Ca^{++} by plants (Kuiper, 1984). Both K^+ and Ca^{++} are required in the growth medium to maintain the selectivity and integrity of cell membranes. Calcium is also needed for selective transport of ions such as K^+ across the membrane (Wyn Jones and Lunt, 1967). Also in saline environments, the accumulation of Cl^- parallels that of Na^+ (Yeo and Flowers, 1985), and chloride-induced NO_3 deficiency has been shown to be responsible for growth retardation in plants exposed to high levels of NaCl salinity (Torres and Bigham, 1973). Higher concentrations of Na^+ and Cl^- in plant tissues have reduced the growth and

yield of many crops, and reductions in the growth and yield of sunflower under saline conditions have also been reported (Heikal et al., 1980, Girdhar, 1988; Ur-Rehman and Hussain, 1992; Hussain and Ur-Rehman, 1992). In sunflower, seed yield has been shown to start decreasing beyond EC_e[1] 2.5 dSm^{-1} and to reach to 30% losses at EC_e 11.3 dSm^{-1} (Anonymous, 1992), but 49.21% seed yield losses at EC_e 10 dSm^{-1} have also been reported (Hussain and Ur-Rehman, 1992).

In barley, sensitive varieties tend to accumulate high amounts of Na^+ and Cl^- in younger leaves, while tolerant varieties accumulate these ions in older leaves. The tolerant plants are able to retranslocate K^+ more efficiently from older to developing leaves, exchanging them with Na^+ and thus maintaining favorable K^+/Na^+ ratios in younger leaves and stems (Boegmans and Stassart, 1987). It has been reported that Na^+ and Cl^- accumulation increased in sunflower leaves with increasing salinity (Cheng, 1984), and similar findings have been reported for the seedling stage (Ur-Rehman and Hussain, 1993). Na^+ accumulation in sunflower leaves at flower initiation has been shown to decrease at moderate salinity levels, and then increased at higher salinity levels as compared with non saline conditions (Hussain and Ur-Rehman, 1993). Potassium accumulation in mature sunflower has been found to increase at moderate salinity (Heikal et al.,1980; Hussain and Ur-Rehman, 1993) but to decrease at higher salinity levels as compared with non saline conditions (Heikal, 1977; Hussian and Ur-Rehman, 1993); however, it decreased consistently with each increment in salinity level at the seedling stage (Ur-Rehman and Hussain, 1993).

The objective of the present research was to determine the physiological behavior of ion accumulation at both the seedling and

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[1] Electrical conductivity of saturation extract at 25 °C.

flower initiation stages and its genetic basis in salt-tolerant and -sensitive sunflower lines in order to formulate selection criteria for salt tolerance in sunflower.

MATERIALS AND METHODS

The following three experiments were carried out.

Experiment 1: Identification of salt-tolerant and salt-sensitive lines

A preliminary experiment was conducted to identify salt-tolerant and -sensitive sunflower lines on the basis of seed yield in order to study further the physiogenetic mechanism for salt tolerance in sunflower (*Helianthus annuus* L.). The seed yield under saline conditions was taken as the measure of the relative salt tolerance of the different lines. Of the 15 sunflower inbreds, 5 of the lines tested (GIMSUN-4, -76, -198, -476, and -671) had been previously developed from open pollinated Russian and Romanian cultivars; the other 10 (GIMSUN-157, -232, -343, -403, -603, -740, -764, -790, -802, and -856) had been developed from segregating populations of commercial hybrids from the United States, after six successive years of selfing. These lines were evaluated for salt tolerance using a randomized complete block design with three replications under two salinity levels. There were 5 pots each of 3 replications of all combinations of 15 lines at 2 salinity levels making 450 pots in the experiment. The seeds were planted in polyethylene-lined clay pots, having an internal diameter and depth of 26 cm. Each pot was filled with 8 kg. of air dried and sieved potting mixture composed of sand and soil in a ratio 3:1.

Two basal salinity levels i.e., EC_e 2 (control), and 10 dSm^{-1} were created using a mixture of salts, NaCl, Na_2SO_4, $CaCl_2$, and $MgSO_4$ in the ratio of 5:9:5:1, respectively. Irrigation water (EC 0.29 dSm^{-1}) was

applied throughout the experiment whenever required. Nitrogen and phosphorous fertilizers were added in amounts equivalent to 86 and 62 kg ha^{-1}, respectively (Chaudhry, 1985). The phosphorous fertilizer was applied at the time of planting, the nitrogenous fertilizer was applied half at planting and half at flowering. Data were recorded on seed yield per plant at physiological maturity.

Experiment 2: Ion concentrations at the seedling stage

The 15 sunflower inbreds included in the first experiment were evaluated for ion accumulation at the seedling stage in the second experiment. This experiment was conducted in polyethylene bags (25 x 13 cm) filled with 1.25 kg of the same media used in the first experiment. Three basal salinity levels --EC_e 2(control), 5, and 10 dSm^{-1}-- were developed using procedures similar to those in the first experiment. All other experimental procedures were the same as in the first experiment. There were 3 plants each of 3 replications of all combinations of 15 lines at 3 salinity levels making 405 plants in the experiment. After 21 days following sowing, the two topmost fully expanded leaves were collected from each plant, washed in distilled water, blotted dry, and stored in a deep freezer. A single channel emission flame photometer designed for the routine determination of sodium (Na^+), and Potassium (K^+), and digital chloridometer designed to determine the chloride (Cl^-) ion concentration of a solution were used to determine Na^+, K^+, and Cl^- concentrations by tissue sap analysis; the K^+/Na^+ ratio was calculated mathematically.

Experiment 3. Ion concentrations at the flower initiation stage

The third experiment was conducted to determine ion accumulation in

sunflower leaves at flower initiation of same fifteen lines used in the first two experiments. The sunflower was seeded in polyethylene lined clay pots of same size as those used in the first experiment. Three basal salinity levels --EC_e 2 (control), 5, and 10 dSm^{-1}-- were developed using procedure similar to those in the first experiment. All other experimental procedures were the same as in the first experiment. There were 8 pots each of 3 replications of all combinations of 15 lines at 3 salinity levels making 1080 pots in the experiment. The top most fully expanded leaf from three plants each per genotype, replication and salinity level was collected following similar procedure as used in second experiment. Na^+, K^+ and Cl^- accumulation was determined by tissue sap analysis using similar procedures as used in second experiment. The other five plants of each genotype per replication and salinity level were harvested at physiological maturity and data were recorded on seed yield per plant.

Statistical analysis.

The data were analyzed using analysis of variance (Steel and Torrie, 1980). Estimates of phenotypic and genotypic coefficients of variability and broad sense heritability (as a ratio of genetic variance to phenotypic variance) were calculated using the procedure of Lothrop et al. (1985). The average performance of the salt-tolerant and -sensitive group of inbred lines, along with the overall average of the 15 lines studied for various ion accumulations at the seedling and flower initiation stages, was plotted against salinity levels and presented graphically.

RESULTS AND DISCUSSION

Experiment 1. Identification of salt-tolerant and -sensitive lines.

Significant differences were found among inbred lines, salinity

levels, and inbred-salinity interaction. Soil salinity reduced seed yield with each increment of salinity, but responses of the different genotypes varied. Compared to the control, 3 of the lines GIMSUN-603, -764, and -856, showed less than a 30% reduction in yield underr saline conditions (EC_e 10 dSm^{-1}) and were designated as being "salt-tolerant". Three of the lines GIMSUN-198, -157, and -802, showed more than a 60% reduction in seed yield under the same saline conditions and were designated as "salt-sensitive".

The cumulative behavior of lines belonging to the salt-tolerant and -sensitive groups was studied for ion accumulation at the seedling and flower initiation stages.

Experiment 2. Ion concentrations at the seedling stage

The results exhibited significant ($P < 0.01$) differences among salinity levels, genotypes, and genotype-salinity interaction for Cl^-, Na^+, and K^+ concentrations and K^+/Na^+ ratio in sunflower leaves at the seedling stage.

The Cl^- concentration in the seedling leaves increased with an increase in salinity in both the salt-tolerant and -sensitive groups (Figure 1). There was only very slight differences between the lines within each group.

The Na^+ concentration showed highly significant differences between the groups as the salinity level increased (Figure 2). The mean Na^+ concentrations in the salt-sensitive lines increased with each increment in salinity. The Na^+ concentration in seedling leaves of the salt-tolerant lines, on the other hand, decreased significantly as salinity inceased from EC_e 2- to 5- to 10 dSm^{-1}. The curve for tolerant lines intersected the sensitive-lines curve at EC_e 5 dSm^{-1}.

The K^+ concentration in the salt-tolerant lines decreased significantly at moderate salinity, but showed an increase at the higher salinity level (Figure 3). The sunflower lines that were sensitive to salinity increased slightly in K^+ concentration from EC_e 2 to EC_e 5 dSm^{-1} but increased significantly at EC_e 10 dSm^{-1}. However, K^+ was much higher in tolerant than in sensitive lines at the EC_e 2 dSm^{-1}.

The ratio of the K^+ to Na^+ in the seedling leaves was quite instructive (Figure 4). The K^+/Na^+ ratio of the salt tolerant lines decreased with an increase in salinity from the EC_e 2 to the EC_e 5 dSm^{-1}, with a small (nonsignificant) decrease at EC_e 10 dSm^{-1}. By contrast, this ratio in the salt-sensitive lines increased from EC_e 2 to EC_e 5 dSm^{-1}, then was essentially unchanged to EC_e 10 dSm^{-1}.

In general, sunflower appears to have the ability to increase K^+ to counter the toxic effects of Na^+. This was particularly the case in the salt-tolerant lines but even the salt-sensitive lines increased K^+ to combat salinity (Figure 3). Although the sensitive group showed an increase in K^+/Na^+ ratio with an increase in salinity, the ratio was still lower than in the tolerant group (Eigure 4).

Table 1 indicates that standard deviation values for Na^+ and K^+ concentration and K^+/Na^+ ratio in leaves at the seedling stage in sunflower lines were greater than those for Cl^- concentration. Maximum variation was observed in these traits, which is also evident from their respective range values. Smaller standard deviation values for Cl^- concentration suggest low variability in this trait. Higher values for genotypic coefficients of variation for all ion concentration show that maximum variation was controlled by genetic factors. A larger value of broad sense heritability indicates the highly heritable nature of various ion concentrations in sunflower leaves at the seedling stage.

Experiment 3. Ion concentrations at flower initiation stage

Ion concentration in sunflower leaves at flower initiation showed significant ($P < 0.01$) differences among salinity levels for all the parameters. Differences among lines were found significant at 0.01 probability level for Na^+ concentration and K^+/Na^+ ratio, significant at 0.05 probability level for K^+ concentration, and nonsignificant for Cl^- concentration. The genotype-salinity interaction was found nonsignificant for all the parameters studied.

Differences were also found significant among salinity levels, genotypes, and genotype-salinity interaction for seed yield per plant. Identification of the salt-tolerant and -sensitive lines in the first experiment was confirmed again in the mature plant experiment, with a slight variation in seed yield or in loss of seed yield under saline conditions, but the inbred lines remained in their respective groups on the basis of their ability to tolerate salinity (Table 2).

The behavior of same groups of salt-tolerant and -sensitive lines was studied for ion concentration in leaves at flower initiation stage. The average Cl^- concentration in leaves at flower initiation increased with an increase in salinity (Figure 5). The salt-tolerant and -sensitive lines followed the same pattern. The average Na^+ concentration decreased at moderate salinity, then increased at higher salinity levels (Figure. 6). The tolerant lines decreased in Na^+ much more than the sensitive lines. The average K^+ concentration (Figure. 7) among the sensitive lines increased slightly at moderate salinity, and then decreased at higher salinity levels. However, the tolerant group maintained K^+ and did not show any significant decrease. Thus K^+ concentration in leaves of the tolerant lines was higher at EC_e 10 dSm^{-1} than was the case for sensitive lines. The behavior of Na^+ and K^+ concentration is clearer when the

cumulative effect of potassium and sodium ions is studied as the K^+/Na^+ ratio. The tolerant and sensitive groups increased K^+/Na^+ ratio at moderate salinity, then decreased at higher salinity levels (Figure 8), but the K^+/Na^+ ratio in leaves of salt-tolerant lines was much higher at moderate and higher levels of salinity than in the salt-sensitive lines.

Table 3 indicates that standard deviation values for Na^+ and K^+ concentration and K^+/Na^+ ratio in leaves of sunflower genotypes at flower initiation are greater than for Cl^- concentration. Maximum variation was observed in these traits, which is also evident from their respective range values. The smaller standard deviation value for Cl^- concentration suggests low variability in this trait. Higher values for genotypic coefficients of variation for sodium ion concentration suggest that maximum variation was controlled by genetic factors. The estimates of broad sense heritability are lower than those at the seedling stage, and only Na^+ showed quite comparable $h^2_{B.S.}$ estimates for the two stages.

CONCLUSIONS

Snflower has been classified as moderately salt-tolerant by various researchers (Karami, 1974; Heikal et al., 1980). If we look at overall ion concentration at moderate salinity, it becomes clear that sunflower leaves showed decreased sodium (Figure 2) and a higher K^+/Na^+ ratio (Figure 3) at the seedling stage, and decreased sodium (Figure 6), increased K^+ (Figure 7), and kept a higher K^+/Na^+ ratio (Figure 8) at the flower initiation stage. Sunflower was evaluated on the same criteria at higher salinity levels. The salt-tolerant lines, as identified earlier by their ability to produce higher seed yield under saline conditions, showed a greater decrease in sodium (Figure 2) and a higher K^+/Na^+ ratio (Figure 3) at the seedling stage, and in addition to these, maintained

higher potassium levels at flower initiation.

Decreased Na^+ concentration and a higher K^+/Na^+ ratio were common at both seedling and flower initiation stages in the salt-tolerant lines, and were found to act as the physiological mechanism for salt tolerance in sunflower. It is evident from these experiments that lower Na^+ concentration and a higher K^+/Na^+ ratio in younger sunflower leaves could be used as criteria for selecting salt-tolerant sunflower lines.

Broad sense heritability estimates are valid when homozygous lines are studied (Singh, 1986). On the basis of high broad sense heritability estimates for Na^+ and K^+/Na^+ ratio presented in Table 1 and 3, it is suggested that selection can be based on physiological criteria for salt tolerance in sunflower. Thus salt-tolerant lines selected on the basis of lower Na^+ concentration and a higher K^+/Na^+ ratio can be used in future breeding programs.

It is interesting to note along with the decrease in Na^+ concentration in leaves of salt-tolerant sunflower lines under elevated salinity levels there might be a synthesis of other compatible osmotica, such as proline and glycine that build up osmotic pressure in sunflower leaves. The results of the present work suggest the need for more detailed studies on this aspect.

MANAGEMENT IMPLICATIONS:

Soil salinity is a global problem. It is adversely affecting the economy of Pakistan by deteriorating the soils and negating the country,s aspirations for food self-sufficiency. Under the prevalent conditions, soil salinization is on the increase; salt balance studies in the Indus Plain indicate a net addition of 0.98 to 2.47 tons of salt ha^{-1} yearly through various sources. Each year, about 0.2 to 0.4% of the Pakistan's

total arable land is being put out of cultivation because of salinity and waterlogging (Qureshi, 1978). The physiological criteria of selection suggested by the present study can be used to identify salt-tolerant sunflower lines. A decrease in Na^+ accumulation and a higher K^+/Na^+ ratio in young growing leaves can also be used in future breeding programs for the identification and selection of salt tolerance in sunflower.

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REFERENCES

1. Anonymous. 1989. Reconnaissance soil survey report. Soil survey of Pakistan, Lahore, Pakistan.
2. Anonymous. 1992. Crops and soils, NDSU extension service bulletin No.1, May 1, 1992, North Dakota State University, Fargo, ND.
3. Bogemans, J., and J.M. Stassart. 1987. Ion segregation in different plant parts within different barley cultivars under salt stress. Dordrecht, Netherland; Martimus Nijhoff. p.239-246. (Pl.Br.Absts. 58(4):3096; 1988.).
4. Chaudhry, A.R. 1985. Sunflower cultivation in Pakistan-retrospects and prospects. In: Proc. Natl. Seminar on Oilseed Res. and Develop. in Pakistan, 7-9 May, NARC, Islamabad, Pakistan. P. 69-88.

5. Chaudhri, M.B., M.A. Mian, and M. Rafiq. 1978. Nature and magnitude of salinity and drainage problems in relation to agriculture development in Pakistan. Pak. J. Forestry 28(2): 70-72

6. Cheng, S.F. 1984. Effects of salinity, fertility, and water on the production and nutrients uptake. Diss. Abst. Inter. 44(12): 3600-3601 (Crop Physiol. 12(4): 1465; 1986).

7. El-Ashry, M.T., J.V. Shilfaarde, and S. Schiffman. 1985. Salinity pollution from irrigated agriculture. J. Soil and Water Conserv. 40: 48-52.

8. Girdhar, I.K. 1988. Effect of soil and water salinity on growth, leaf expansion and yield of sunflower (*Helianthus annuus* L.). P. 332-338. In: Proc. Inter. Symp. on Solonetz Soils. Yugoslavia, June 15-20. (Soil and Fert. 52(12): 14306; 1989).

9. Heikal, M.D.D. 1977. Physiological studies on salinity. 6-changes in the water content and mineral composition of some plants over a range of salinity stresses. Plant Soil 48: 223-233.

10. Heikal, M.M., A.M. Ahmad, and A. Shadad. 1980. Changes in dry weight and mineral composition of some oil producing plants over a range of salinity stresses. Biologia Plantanum 22(1): 25-33.

11. Hussain, M.K. and O. Ur-Rehman. 1992. Breeding sunflower for salt tolerance: Genetic variability for yield and yield components for salt tolerance in sunflower (*Helianthus annuus* L.). In: Proc. All Pak. Sci. Conf. 16-21 May, Khanspur, Pakistan. p.112-115.

12. Hussain, M.K., and O. Ur-Rehman. 1993. Breeding sunflower for salt tolerance: Physiological basis for salt tolerance in sunflower (*Helianthus annuus* L.). Helia, 16(18): 77-84.

13. Karami, E. 1974. Emergence of nine varieties of sunflower (*Helianthus annuus* L.) in salinized soil cultures. J. Agri. Sci. Camb.83:359-362.

14. Kuiper, P.J.C. 1984. Functioning of plant cell membrane under saline conditions: Membrane lipid compositions and ATPases. In: R.G. Staples,and G.H. Toenniessen (Eds.), Salinity tolerance in plants; Strategies for crop improvement . Jhon Wiley and Sons; New York. p.77-91.

15. Lothrop, J.E., R.E. Atkins, O.S. Smith. 1985. Variability for yield and yield components in IAPIR grain sorghum random mating population. I.Mean variance components and heritabilities. Crop Sci. 25: 235-240.

16. Qureshi, S.A. 1978.Soil salinity and salt tolerance in plants. In: Proc. seminar/workshop on membrane biophysics and development of salt tolerance in plants, Faisalabad, Pakistan, March 11-21. p. 3-6.

17. Singh, B.D. 1986. Plant Breeding. Kalyani publishers, New Dehli, India. p.94.

18. Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics. McGraw Hill Book Co., New York.

19. Storey, R., and R.G. Wyn Jones. 1979. Responses of *Atriplex spongiosa* and *Suaeda monoica* to salinity. Plant Physiol. 63:156-162.

20. Torres, C.B., and F.T. Bingham. 1973. Salt tolerance of Mexican wheat. I. Effect of NO3 and NaCl on mineral nutrition, growth and grain production of four wheats. Soil Sci. Soc. Amer. Proc. 37: 711-715.

21. Ur-Rehman, O., and M.K. Hussain. 1992. Breeding sunflower for salt tolerance: Effect of salinity on growth and development of cultivated sunflower (*Helianthus annuus* L.). In: Proc. All Pak. Sci. Conf. 16-21 May, Khanspur, Pakistan. p. 118-123.

22. Wyn Jones, R.G., and O.R. Lunt. 1967. The function of calcium in plants. Bot. Rev. 33: 407-426.

23. Yeo, A.R. 1983. Salinity resistance: Physiology and prices. Physiol. Plant. 58: 214-222.

24. Yeo, A.R., and T.J. Flowers. 1984. Mechanism of salinity resistance in rice and their role as physiological criteria in plant breeding. In: R.G. Staples, and G.H. Toenniessen (Eds.). Salinity tolerance in plants; Strategies for crop improvement. Jhon Wiley and Sons. New York.p. 151-170.

25. Yeo, A.R., and T.J. Flowers. 1985. The absence of an effect the Na+/Ca++ ratio on sodium chloride uptake by rice (*Oriza sativa* L.). New Phytol. 99:81-90.

Table.1 Mean, Range, Standard deviation (S.D), Phenotypic coefficient of variation (P.C.V.), Genotypic coefficient of variation (G.C.V.), and Heritability ($h^2_{B.S.}$) estimates for different ion concentrations in sunflower leaves at seedling stage.

TRAIT	MEAN	RANGE	S.D.	P.C.V.	G.C.V.	$h^2_{B.S.}$
Cl^-	63.23	13.33-101.00	17.12	27.08	26.79	0.98
Na^+	651.27	338.6-2008.4	265.91	71.54	70.39	0.97
K^+	20754.43	52365.9-92263.6	16708.86	80.51	80.17	0.99
K^+/Na^+	40.34	6.61-187.03	35.25	87.38	85.57	0.96

Table. 2 Mean seed yield per plant (g) of representative salt-tolerant and -sensitive lines and overall average at different salinity levels, with percent decrease (-) over control in parentheses.

INBRED LINES	SALINITY LEVELS		
	EC_e 2 dSm^{-1} (cont.)	EC_e 5 dSm^{-1}	EC_e 10 dSm^{-1}
SALT-TOLERANT			
1. GIMSUN-603	6.28 c!	5.56(-11.46) d	6.15(-02.07) c
2. GIMSUN-856	8.35 a	6.85(-17.96) c	6.27(-24.91) c
3. GIMSUN-764	6.56 c	5.22(-20.43) d	4.68(-28.66) c
Average	A # 7.06	B 5.88(-16.71)	C 5.70(-19.26)
SALT-SENSITIVE			
1. GIMSUN-198	7.96 b	3.48(-56.28) f	1.63(-79.52) h
2. GIMSUN-157	5.35 d	4.32(-19.25) e	1.85(-65.42) h
3. GIMSUN-802	7.12 b	4.58(-35.67) e	2.43(-65.87) g
Average	A 6.81	B 4.13(-39.35)	C 1.97(-71.07)
Average of 15 inbreds in Expt.2	A 6.38	B 4.94(-22.57)	C 3.15(-50.63)

! Means sharing similar letters are nonsignificantly different.
Capital letters show statistical differences horizontally.

Table. 3 Mean, Range, Standard deviation (S.D), Phenotypic coefficient of variation (P.C.V.), Genotypic coefficient of variation (G.C.V.), and Heritability ($h^2_{B.S.}$) estimates for different ion concentrations in sunflower leaves at flower initiation.

TRAIT	MEAN	RANGE	S.D.	P.C.V.	G.C.V.	$h^2_{B.S.}$
Cl^-	73.80	32.33-116.00	15.41	20.88	11.06	0.28
Na^+	124.90	49.50-214.87	55.39	44.35	39.59	0.80
K^+	7274.6	5946.6-9978.27	931.32	12.80	9.12	0.51
K^+/Na^+	69.30	33.14-134.49	25.67	37.05	27.99	0.57

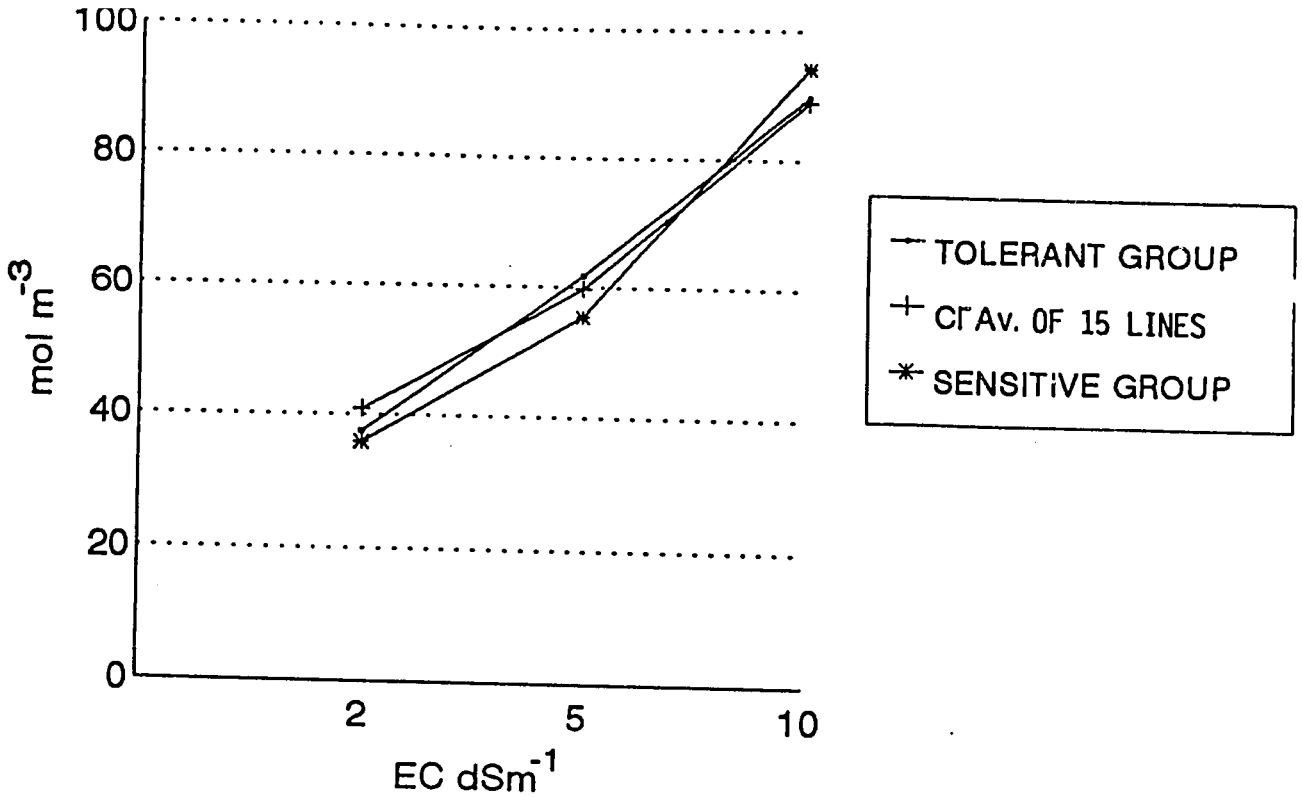

FIG: 1 Cl^- (mol m^{-3}) CONCENTRATION IN SUNFLOWER LEAVES AT SEEDLING STAGE

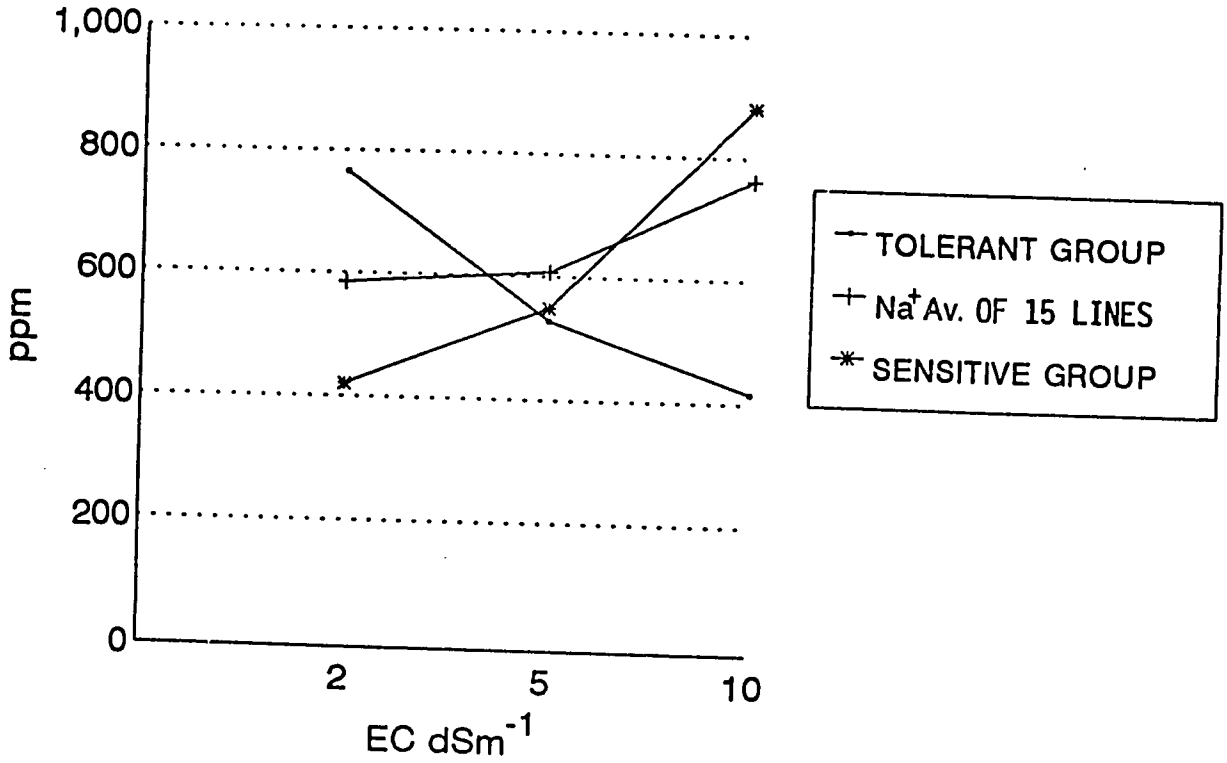

FIG: 2 Na^+ (ppm) CONCENTRATION IN SUNFLOWER LEAVES AT SEEDLING STAGE

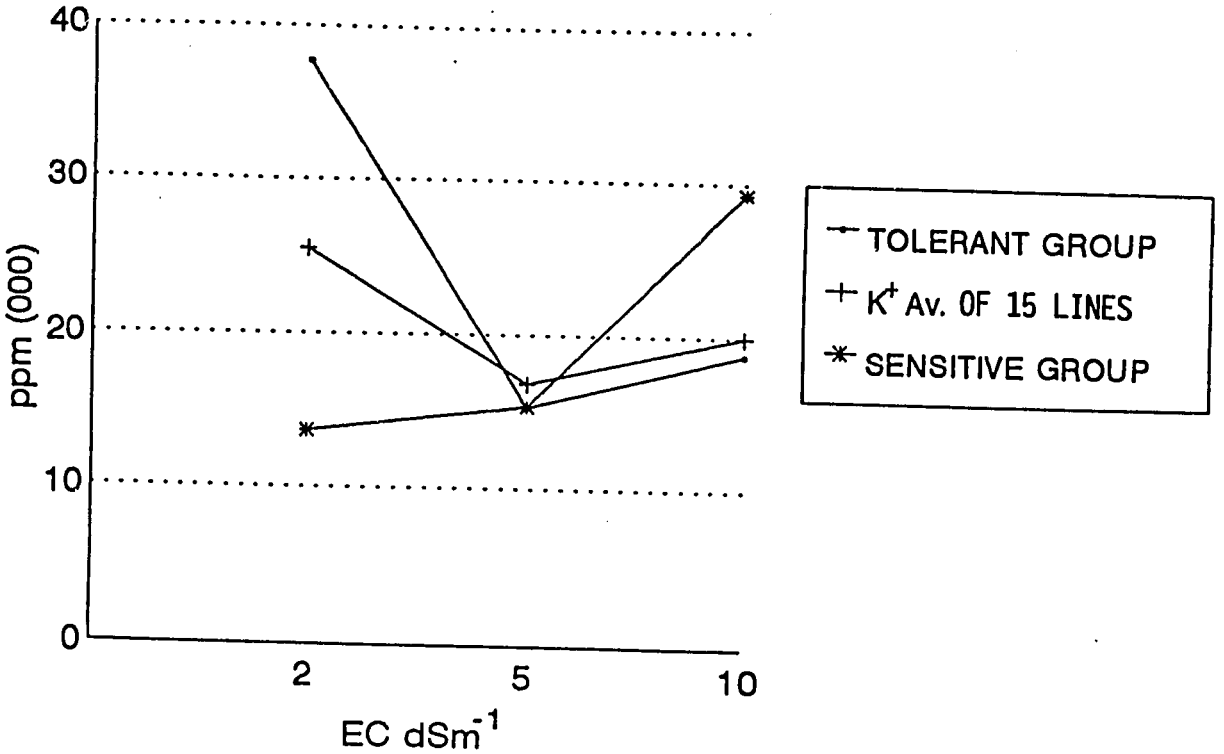

FIG: 3 K^+ (ppm) CONCENTRATION IN SUNFLOWER LEAVES AT SEEDLING STAGE

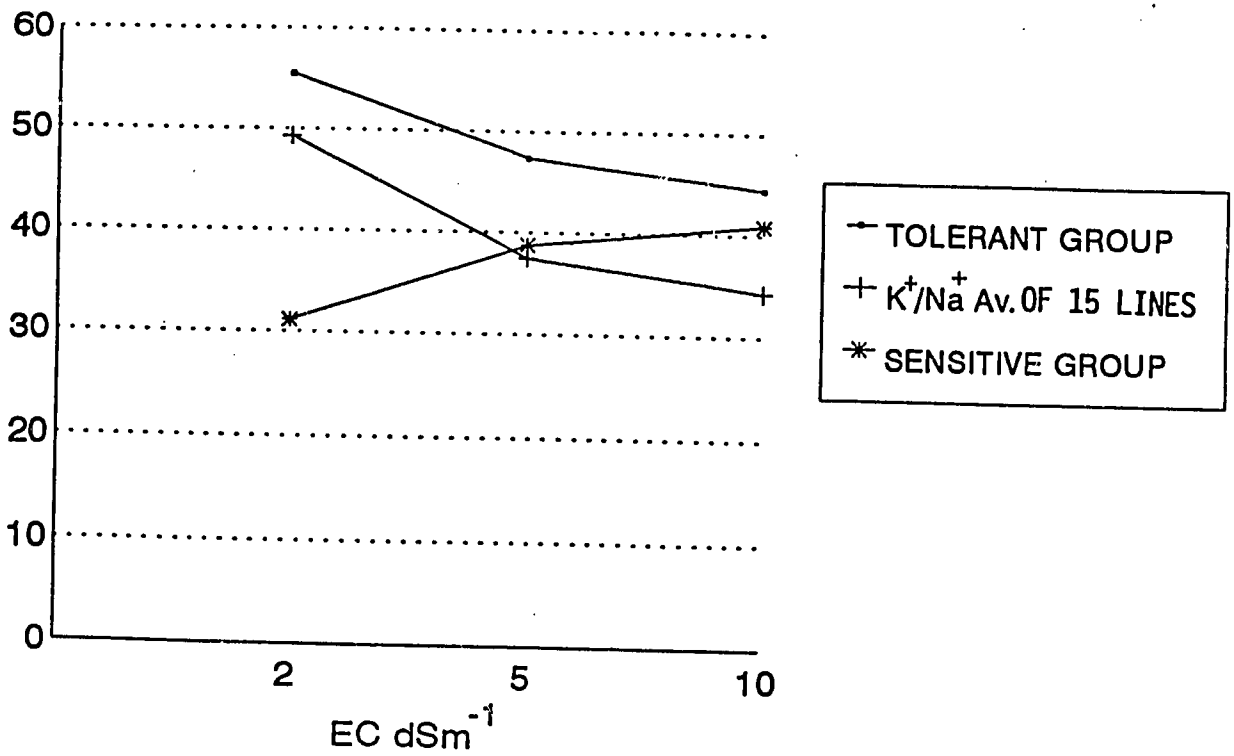

FIG: 4 K^+/Na^+ RATIO IN SUNFLOWER LEAVES AT SEEDLING STAGE

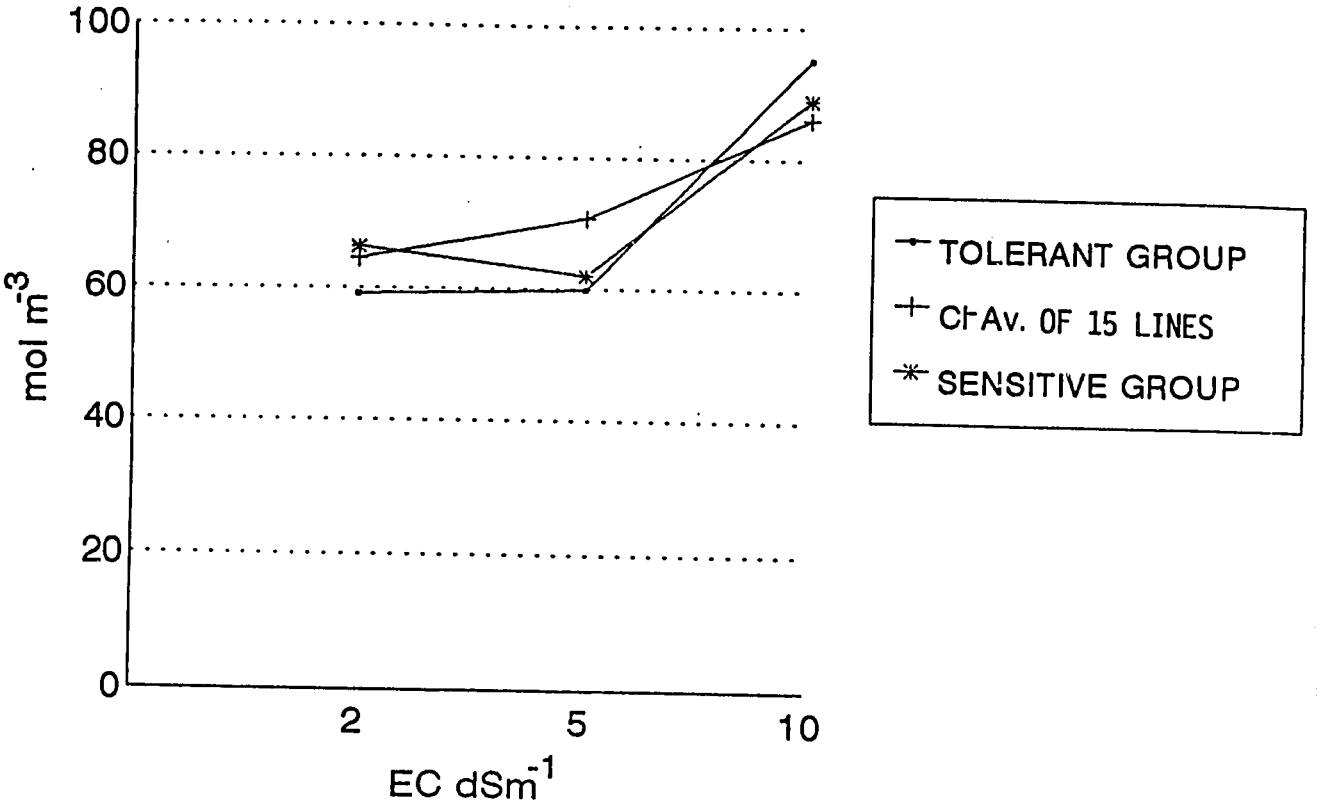

FIG:5 Cl^- (mol m^{-3}) CONCENTRATION IN SUNFLOWER LEAVES AT FLOWER INITIATION

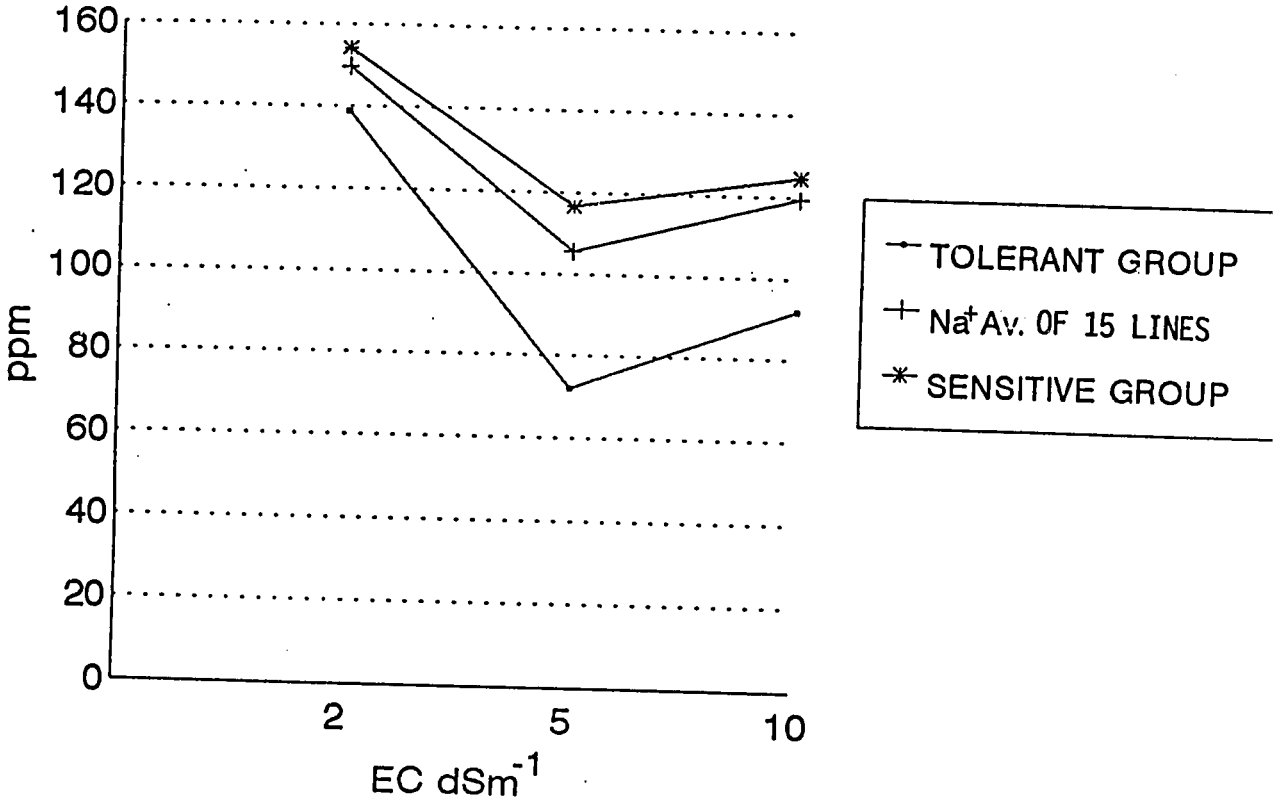

FIG:6 Na^+ (ppm) CONCENTRATION IN SUNFLOWER LEAVES AT FLOWER INITIATION

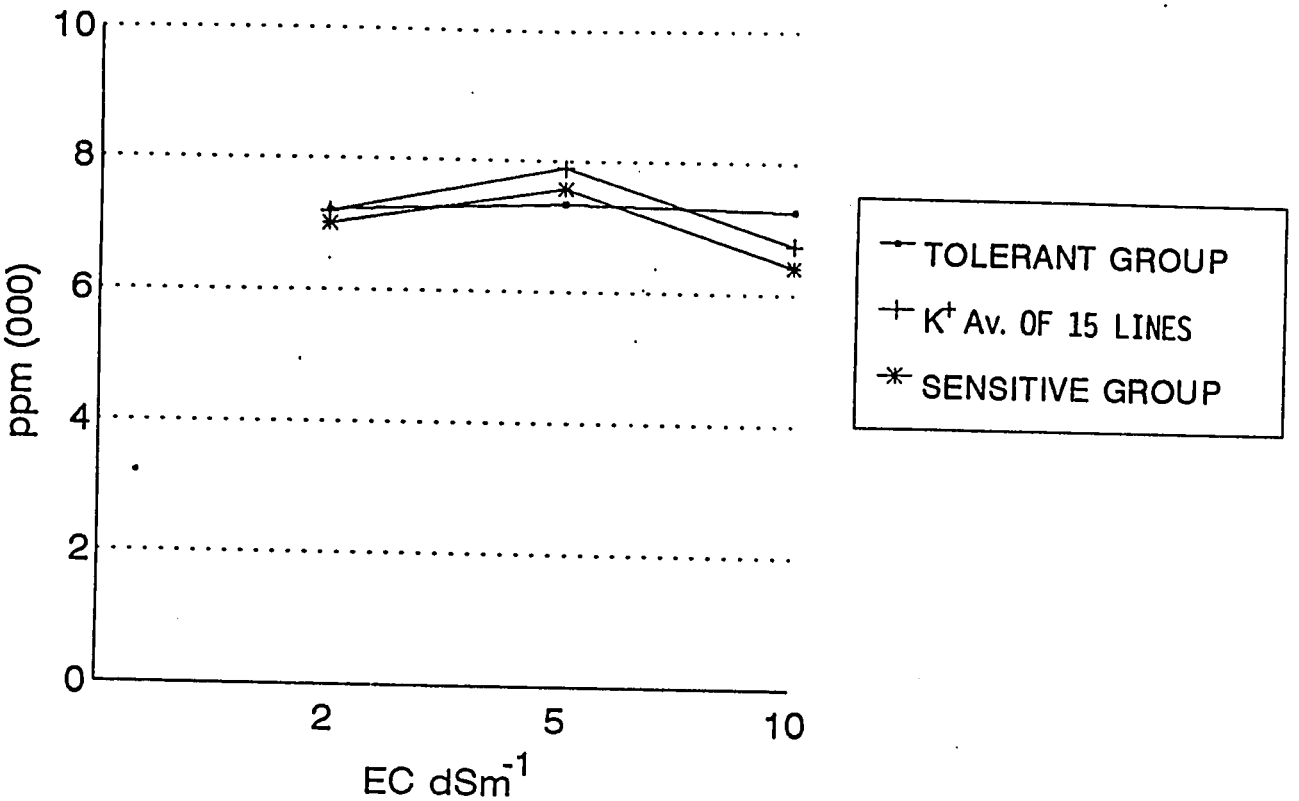

FIG:7 K^+ (ppm) CONCENTRATION IN SUNFLOWER LEAVES AT FLOWER INITIATION

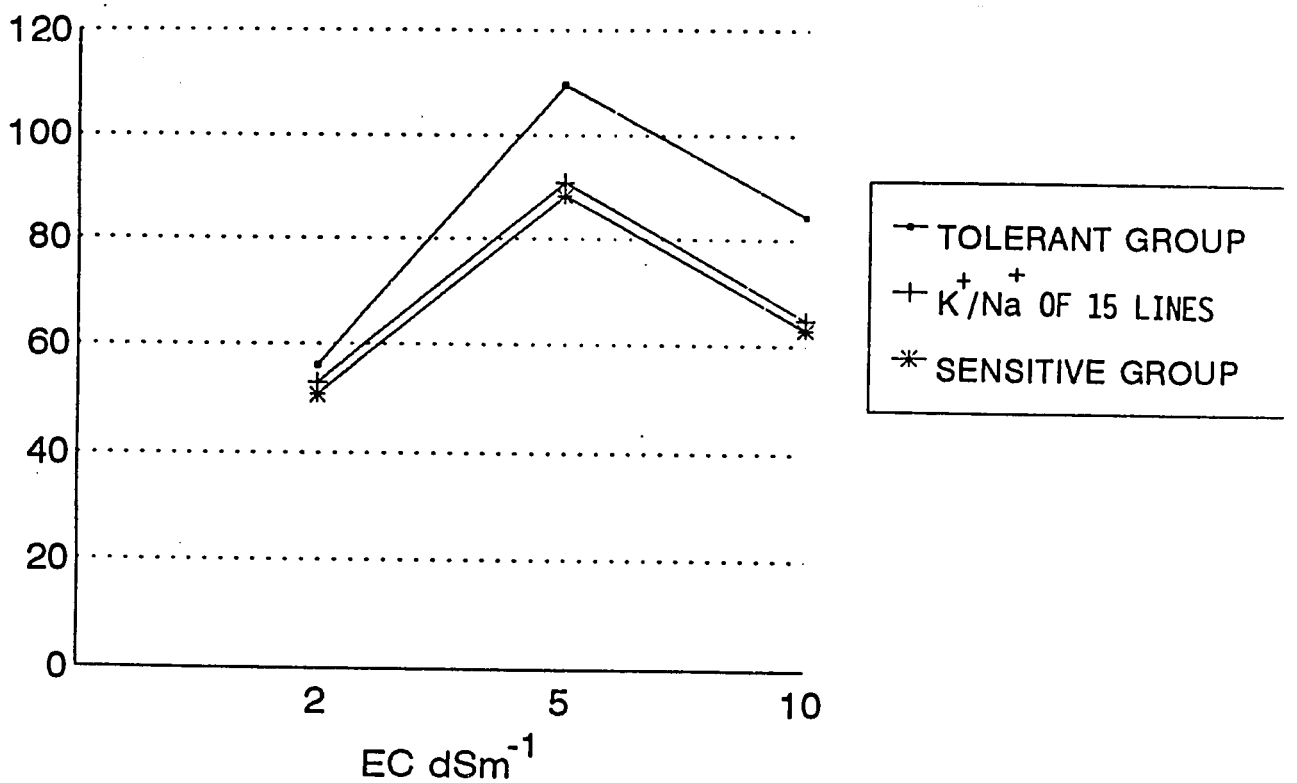

FIG:8 K^+/Na^+ CONCENTRATION IN SUNFLOWER LEAVES AT FLOWER INITIA

BREEDING SUNFLOWER FOR SALT TOLERANCE:
Association of seedling growth and mature plant traits for salt tolerance in cultivated sunflower (*Helianthus annuus* L.) [1]

Medhet Kamil Hussain and Obaid-Ur-Rehman [2]

ABSTRACT

The relationships of different seedling growth parameters to yield and yield components were studied in cultivated sunflower under nonsaline (EC_e 2 dSm^{-1}) and saline conditions (EC_e 10 dSm^{-1}). Inbred lines having shallow roots with low fresh weight and greater shoot length at the seedling stage produced higher seed yield under nonsaline conditions. Seedling parameters showed very complex associations with seed yield under saline conditions. It was rather difficult to select high-yielding lines at the seedling stage under saline conditions, but oil yield could be increased indirectly through more seed weight, greater head diameter, and high oil content by selecting lines with shallow roots having more dry weight, and longer shoots having more fresh and dry weight. Selection of inbred lines having longer shoots with more fresh and dry weight under nonsaline conditions appeared to be effective for achieving high seed-yielding lines for saline soils.

Key Words: *Helianthus annuus* L., correlations, seedling growth, seed yield, saline conditions.

INTRODUCTION

Soil salinity is a common problem in irrigated production areas of the world. It is seriously affecting the economy of Pakistan by limiting crop productivity to a large extent over a vast area. All phases of plant growth, from germination to maturity, are affected by the environment in which the plant grows. Saline soils contain a sufficient amount of salts to impair the growth of plants (Ponnamperuma and Bandyopadhya, 1980). Salt tolerance is the ability of crops to produce an economic yield under the adverse soil conditions caused by excessive salts in the rootzone

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[1] Contribution of Dep. of Plant Breeding and Genetics, University of Agriculture, Faisalabad(Pakistan) in collaboration with National Academy of Sciences/ BOSTID, Washington D.C., U.S.A.

[2] Associate Professor and Ph.D. Research Fellow, Dep. of Plant Breeding and Genetics, University of Agriculture, Faisalabad(Pakistan).

(Bresler et al., 1982).

Sunflower seedlings have shown severe reduction in shoot growth (Charsalli and Cherif, 1979) and shoot dry matter (Cheng, 1984), while root growth was comparatively less affected (Charsalli and Cherif, 1979), however, root dry matter yield was reduced when grown in soils with high salinity levels (Heikal et al., 1980). Similarly, increasing levels of salinity have been shown to reduce 100-seed weight, head diameter (Mehmood, 1986), seed yield, and oil percentage (Girdhar, 1988). Seedling dry matter has been positively correlated with seed yield (Chaudhry and Anand, 1985), as has 100-seed weight, both at phenotypic and genotypic levels (Singh et al., 1985).

The screening of salt-tolerant lines/cultivars has been attempted by many researchers in various species on the basis of their root and shoot growth at the seedling stage (Wu, 1981; Ashraf et al., 1986, 1989; Ab-Shukor et al., 1988). The relationships of various seedling growth parameters to seed yield and yield components under saline conditions are important for the development of salt-tolerant cultivars for production under saline conditions. The present study was undertaken because this needed information is not available for sunflower. The objective was to identify seedling plant growth parameters of sunflower that could be used as selection criteria for early evaluation of different lines for salt tolerance.

MATERIALS AND METHODS

Fifteen sunflower (*Helianthus annuus* L.) lines were evaluated for salt tolerance at the seedling and mature plant stages under normal and saline conditions. The sunflower lines included inbreds GIMSUN-4, -76, -198, -476, and -671, were developed from open pollinated Russian and

Romanian cultivars, and inbreds GIMSUN-157, -232, -343, -403, -603, -740, -764, -790, -802, and -856, developed from segregating populations of commercial hybrids from the United States, after six successive years of selfing. The study was divided into two experiments for management simplicity.

Experiment 1: Seedling studies

The first experiment, on the sunflower seedlings, was laid out in a randomized complete block design with three replications and two salinity treatments. Ten seedlings of each genotype were grown in each salinity treatment and replication in polyethylene bags (25 x 13 cm) filled with 1.25 kg of an air-dried and sieved sand and soil mixture in the ratio of 3:1 to facilitate harvesting and recording of data on root growth parameters. Two basal salinity levels, EC_e[3] 2 (control) and 10 dSm^{-1}, were developed using a mixture of NaCl, Na_2SO_4, $CaCl_2$, and $MgSO_4$ in a ratio of 5:9:5:1, respectively. The fertilizer required was calculated as 86 and 62 kg ha^{-1} of nitrogen and phosphorus, respectively (Chaudhry, 1985) and applied before sowing. Irrigation water (EC[4] 0.29 dSm^{-1}) was applied throughout the experiment as required. Similar cultural and agronomic procedures were used for all entries. Seedlings were uprooted 21 days after planting, and data were recorded for the following parameters on five randomly selected seedlings:

1. Root length (cm)
2. Root fresh weight (g)
3. Root dry weight (g)
4. Shoot length (cm)
5. Shoot fresh weight (g)
6. Shoot dry weight (g)

Experiment 2. Mature plant studies

The second experiment, on the mature plants, was conducted in polyethylene-lined clay pots, 26 cm in diameter and depth, filled with

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[3] Electrical conductivity of saturation extract at 25 °C

[4] Electrical conductivity of solution at 25 °C.

8 kg of the same sand and soil mixture as that used in the seedling experiment. The experiment was laid out in a randomized complete block design with three replications under two salinity levels. There were 5 pots each of 3 replications of all combinations of 15 lines at 2 salinity levels making a total of 450 pots in the study. All other procedures were similar to the seedling experiment, except that nitrogenous fertilizer was applied in two doses, one-half before sowing and the other half at flowering. Data were recorded on the following parameters at physiological maturity:

1. Head diameter (cm)
2. 100-seed weight (g)
3. Seed yield per plant (g)
4. Oil content (%)

Statistical analysis

The data were subjected to analysis of variance and covariance (Steel and Torrie, 1980). Phenotypic and genotypic correlation coefficients were computed between seedling growth parameters (Experiment 1) and mature plant traits (Experiment 2) as proposed by Kwon and Torrie (1980). The significance of the phenotypic correlation coefficient was tested using the t-test as proposed by Steel and Torrie (1980). The standard error of the genotypic correlation coefficient was calculated using the procedure of Reeve (1955) and Robertson (1959). The estimates of genotypic correlation coefficient were considered significant if their absolute value exceeded twice their respective standard error.

RESULTS AND DISCUSSION

The results presented in Table 1 show the relationships between seedling growth parameters and mature plant traits under nonsaline conditions. These results indicate that root length at the seedling stage was negatively correlated with seed weight, seed yield, and oil content at the phenotypic level under nonsaline conditions. Root fresh weight

showed negative phenotypic and genotypic correlation with 100-seed weight, and seed yield. All other root growth parameters had a nonsignificant correlation with mature plant traits under nonsaline conditions. The results on root growth parameters show that lines having comparatively smaller roots at the seedling stage with high fresh weight produce higher seed yield under nonsaline conditions.

The results obtained on shoot growth parameters (Table 1) show that shoot length was positively correlated phenotypically with head diameter and seed yield, but genotypically with head diameter only. All other shoot growth parameters had a nonsignificant association with mature plant traits under nonsaline conditions. The results on shoot growth parameters show that lines with greater shoot length may also produce higher seed yield by increasing their head diameter.

Therefore, the results presented in Table 1 suggest that lines having comparatively small roots with low fresh weight and greater shoot length at the seedling stage may produce more seed yield under nonsaline conditions.

The results on root growth parameters under saline conditions (Table 2) show that root length was correlated negatively with head diameter and positively with oil content at the phenotypic and genotypic levels. Root length also showed a negative genotypic correlation with seed yield. Root fresh weight showed a negative phenotypic correlation with head diameter and a positive phenotypic correlation with oil content. Root fresh weight showed a negative genotypic correlation with both seed yield and oil content. Root dry weight showed positive associations with seed weight and oil content at the phenotypic level and negative associations with seed weight at the genotypic level. All other root growth parameters had nonsignificant associations with mature plants under saline conditions.

The results on root growth parameters under saline conditions reveal that lines with comparatively small roots having low fresh weight and more dry weight may produce higher seed yield.

The results obtained on shoot growth parameters under saline conditions (Table 2) show that shoot length was positively correlated with 100-seed weight at the phenotypic level and with oil content at both the phenotypic and genotypic levels. Shoot fresh weight showed a positive phenotypic correlation with both 100-seed weight and oil content. Similarly, shoot dry weight showed a positive phenotypic correlation with 100-seed weight and oil content. All other shoot growth parameters had a nonsignificant relationship with mature plant traits under saline conditions. The results on shoot growth parameters under saline conditions reveal that lines having longer shoots with more fresh and dry weight may produce higher seed yield.

The results presented in Table 2 suggest that seedling parameters have very complex associations with seed yield, and it is rather difficult to select high seed-yielding lines at the seedling stage. However, oil yield can be increased through more seed weight, larger head diameter, and higher oil content. This can be achieved by selecting lines with comparatively small roots having low fresh and high dry weight, and longer shoots having higher fresh and dry weight.

Another set of correlations between seedling growth parameters under nonsaline conditions and seed yield and its components under saline conditions was attempted to determine whether, if selection is made under normal conditions, the improvement achieved will be carried over when the later generations are transferred to saline conditions. The idea of genetic correlation provides the basis for an answer to this question (Falconer, 1989) where phenotypic correlation falls out of significance.

The results presented in Table 3 show the relationship of seedling growth parameters measured under nonsaline conditions with mature plant traits under saline conditions. The results on shoot growth parameters show that shoot length under nonsaline conditions was correlated positively with 100-seed weight under saline conditions at the genotypic level. Shoot fresh weight showed a positive correlation with head diameter and 100-seed weight at the genotypic level. Similarly, shoot dry weight showed a positive genotypic correlation with 100-seed weight and seed yield. All other shoot growth parameters had a nonsignificant association with mature plant traits. Similarly, all root growth parameters had a nonsignificant association with mature plant traits. Therefore, the results presented in Table 3 suggest that shoot growth parameters at the seedling stage under nonsaline conditions are more important than root growth parameters when selecting high seed-yielding lines for saline conditions. It is further suggested that inbred lines having longer shoots with higher fresh and dry weight may produce higher seed yield under saline conditions.

CONCLUSIONS

The results presented above suggest that high seed-yielding sunflower lines could be identified at the seedling stage. Shoot growth parameters are relatively more important than root growth parameters. It was found that lines with longer shoots at the seedling stage may produce more yield under nonsaline conditions. Seedling parameters showed no direct association with seed yield under saline conditions. However, seed yield, as well as oil yield, can be improved by increasing head diameter, 100-seed weight, and oil content through selection of lines having comparatively small roots with lower fresh and higher dry weight, and

longer shoots with higher fresh and dry weight. It is further suggested that selection of high seed-yielding lines for saline soils is also possible under nonsaline conditions by selecting lines having longer shoots with higher fresh and dry weight. These selection criteria can save considerable time in identifying salt-tolerant sunflower lines at the seedling stage. These criteria will be further evaluated in field nurseries in the future.

MANAGEMENT IMPLICATIONS

The menace of soil salinity is not confined to Pakistan, but is a global problem. Each year about 0.2 to 0.4% of the Pakistan's total arable land is being put out of cultivation because of salinity and waterlogging (Qureshi, 1978). Identification of salt-tolerant lines on the basis of economic yield production is a time-consuming process. The results of this study show that the association of seedling growth parameters with seed yield and yield components can be used to evaluate sunflower lines for salt tolerance at the seedling stage. Selection of salt-tolerant lines is possible when seedlings are grown under both saline and nonsaline conditions. The procedures suggested here can provide a time-saving tool in the early evaluation of sunflower for salt tolerance. These can enhance the screening efficiency for large numbers of germplasm for salt tolerance, so that only selected lines will be taken to physiological maturity for conformation.

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REFERENCES

1. Ab-Shukor, N.A., Q.O.N. Kay, D.P. Stevens, and D.O.F. Skibinski. 1988. Salt tolerance in natural population of *Ttrifolium repens* L. New Phytol. 109: 483-490.
2. Ashraf, M., T. McNeilly, and A.D. Bradshaw. 1986. The potential for evolution of salt (NaCl) tolerance in seven grass species. New Phytol. 103: 299-309.
3. Ashraf, M., T. McNeilly, and A.D. Bradshaw. 1989. The potential for evolution of tolerance to NaCl, $CaCl_2$, $MgCl_2$, and sea water in four grass species. New Phytol. 112: 245-254.
4. Bresler, E., B.L. McNeal, and D.L. Carter. 1982. Saline and sodic soils: Principles-Dynamics.Modelling, Springer-Verlag, New York. 235p.
5. Charsalli, M., and M. Cherif. 1979. Effect of sodium chloride on the plant growth and lipid content of sunflower (*Helianthus annuus* L.). Physiologic Vegetale. 17(2): 215-229. (Soil and Fert. Absts. 44(2) 1666; 1981).
6. Chaudhry, A.R. 1985. Sunflower cultivation in Pakistan-retrospect and prospects. In: Proc. Natl. Seminar on Oilseed Res. and Develop. in Pakistan, 7-9 May, NARC, Islamabad, pakistan. p. 69-88.
7. Chaudhry, S.K., and I. J. Anand.1985. Influence of various characters on yield of sunflower. Ind. J. Oilseed Res. 2(1): 78-85. (Pl.Br.Abst. 57(4): 3141; 1987).
8. Cheng, S.F. 1984. Effect of salinity, fertility, and water on the production and nutrients uptake of sunflower (*Helianthus annuus* L.). Effect on seed yield, oil concentration, oil and dry matter yield. Soils and Fert. Taiwan. p. 7-26. (Soil and Fert. Absts. 48(6): 6489; 1985).

9. Falconer, D. S. 1989. Introduction to quantitative Genetics. Longman Scientific and Technical, Essex, England. p. 322.

10. Girdhar, I.K. 1988. Effect of soil and water salinity on growth leaf expansion and yield of sunflower (*Helianthus annuus* L.). In: Proc. Inter. Symp. on Solonetz Soils. Yugoslavia, June 15-20 (Soil and Fert. Absts. 52(12): 16306; 1989).

11. Heikal, M. M, A.M. Ahmad, and A. Shaddad. 1980. Changes in dry weight and mineral composition of some oil producing plants over a range of salinity stresses. Biologia Plantarum 22(1): 25-33.

12. Hussain, M. K., O. Ur-Rehman. 1992. Breeding sunflower for salt tolerance: Genetic variability for yield and yield components for salt tolerance in sunflower (*Helianthus annuus* L.). In: Proc. All Pak. Sci. Conf. 16-21 May, Khanspur, Pakistan. p. 112-115.

13. Kwon, S.H. and J.H. Torrie. 1964. Heritability and interrelationship among traits of soybean populations. Crop Sci. 4: 196-189.

14. Mehmood, S. 1986. Effect of salinity (NaCl) on germination, growth yield and chemical composition of sunflower (*Helianthus annuus* L.) Cv "Shams". Ph.D. Thesis, Dept. of Botany, Univ. of Agric., Faisalabad (Pakistan).

15. Ponnamperuma, F. N., and A.K. Bandyopadhya. 1979. Soil salinity as constraint on food production in the humid tropics. In: Proc. Symp. Soil related Const. Food Prod. Tropics, June 4-8, Int. Rice Res. Inst. LosBanon, (Phillipines). P. 203-216.

16. Qureshi, S.A. 1978. Soil salinity and salt tolerance in plants. In: Proc. seminar/workshop on membrane biophysics and development of salt tolerance in plants, Faisalabad, Pakistan, March 11-21. p. 3-6.

17. Reeve, E.C.R. 1955. The variance of genetic correlation coefficients. Biometrics 11: 357-374.

18. Robertson, A. 1959. The sampling variance of genetic correlation. Biometrics 15: 469-485.

19. Singh, J.V., T.p. Yadava, and R. P. S. Kharb. 1985. Correlation and path-coefficient analysis in sunflower. Ind. J. Agric. Sci. 55(4): 243-246 (Pl. Br. Abst. 55(11): 8873; 1985).

20. Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co.New York.

21. Wu, L. 1981. The potential for evolution of salinity tolerance in *Agrostis stolonifera* L. and *Agrostis tenvis* Sibth. New Phytol. 80: 471-486.

Table.1 Phenotypic and Genotypic correlation coefficients between seedling and seed yield parameters under nonsaline conditions in sunflower.

SEEDLING PARAMETER		SEED YIELD PARAMETERS			
		Head Diameter (cm)	Seed Weight (g)	Seed Yield (g)	Oil Content (%)
Root Length	P !	-0.276^{NS}	-0.530^{**}	-0.616^{**}	-0.297^{*}
	G #	-0.294^{NS}	-1.010^{NS}	-1.352^{NS}	$+0.479^{NS}$
Root Fresh Weight	P	$+0.011^{NS}$	-0.449^{**}	-0.482^{**}	$+0.083^{NS}$
	G	-0.025^{NS}	-0.939^{*}	-1.059^{*}	$+0.084^{NS}$
Root Dry Weight	P	$+0.207^{NS}$	$+0.139^{NS}$	-0.248^{NS}	-0.140^{NS}
	G	$+0.306^{NS}$	$+0.236^{NS}$	-0.309^{NS}	-0.148^{NS}
Shoot Length	P	$+0.315^{*}$	$+0.174^{NS}$	$+0.297^{*}$	$+0.032^{NS}$
	G	$+0.867^{*}$	$+0.471^{NS}$	$+0.649^{NS}$	$+0.099^{NS}$
Shoot Fresh Weight	P	$+0.225^{NS}$	$+0.286^{NS}$	-0.060^{NS}	$+0.166^{NS}$
	G	$+0.458^{NS}$	$+0.556^{NS}$	-0.117^{NS}	$+0.259^{NS}$
Shoot Dry Weight	P	$+0.249^{NS}$	$+0.194^{NS}$	-0.092^{NS}	-0.043^{NS}
	G	$+0.065^{NS}$	$+0.475^{NS}$	-0.109^{NS}	-0.028^{NS}

*, ** $P < 0.05$ and 0.01, respectively.
NS Nonsignificant.
! Phenotypic correlation coefficient.
Genotypic correlation coefficient.

Table.2 Phenotypic and Genotypic correlation coefficients between seedling and seed yield parameters under saline conditions in sunflower .

SEEDLING PARAMETER		SEED YIELD PARAMETERS			
		Head Diameter (cm)	Seed weight (g)	Seed Yield (g)	Oil Content (%)
Root Length	P !	-0.339*	+0.226NS	-0.253NS	+0.303*
	G #	-1.162*	-0.655NS	-1.056*	+1.023*
Root Fresh Weight	P	-0.445**	+0.010NS	-0.284NS	+0.382*
	G	-1.834NS	-0.043NS	-0.850*	-1.169*
Root Dry Weight	P	+0.017NS	+0.416*	+0.145NS	+0.715**
	G	+0.206NS	-0.826*	-0.171NS	+0.047NS
Shoot Length	P	-0.081NS	+0.385*	-0.044NS	+0.646**
	G	-0.103NS	+0.443NS	-0.044NS	+0.799* (0.279)
Shoot Fresh Weight	P	+0.019NS	+0.402*	-0.043NS	+0.308*
	G	-0.142NS	+0.582NS	-0.034NS	+0.448NS
Shoot Dry Weight	P	-0.184NS	+0.418*	-0.027NS	+0.403*
	G	-0.166NS	+0.538NS	-0.044NS	+0.536NS

*, ** P < 0.05 and 0.01, respectively.
NS Nonsignificant.
! Phenotypic correlation coefficient.
Genotypic correlation coefficient.

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Table.3 Phenotypic and Genotypic correlation coefficients between seedling parameters measured under nonsaline conditions and seed yield parameters under saline conditions in sunflower.

SEEDLING PARAMETER		SEED YIELD PARAMETERS			
		Head Diameter (cm)	Seed Weight (g)	Seed Yield (g)	Oil Content (%)
Root Length	P !	-0.105^{NS}	+0.005^{NS}	-0.237^{NS}	-0.332*
	G #	-0.230^{NS}	+0.009^{NS}	-0.488^{NS}	-0.646^{NS}
Root Fresh Weight	P	-0.578**	+0.069^{NS}	-0.136^{NS}	-0.373*
	G	+0.295^{NS}	+0.119^{NS}	-0.239^{NS}	-0.669^{NS}
Root Dry Weight	P	+0.148^{NS}	+0.379*	-0.729**	+0.029^{NS}
	G	+0.166^{NS}	+0.489^{NS}	-0.066^{NS}	-0.619^{NS}
Shoot Length	P	+0.410*	+0.525**	+0.311*	-0.063^{NS}
	G	+0.645^{NS}	+0.882*	+0.765^{NS}	+0.053^{NS}
Shoot Fresh Weight	P	+0.269^{NS}	+0.657**	+0.353*	+0.273^{NS}
	G	+0.938*	+0.959*	+0.389^{NS}	+0.316^{NS}
Shoot Dry Weight	P	+0.275^{NS}	+0.649**	+0.283^{NS}	+0.128^{NS}
	G	+0.568^{NS}	+0.927*	+1.209*	+0.120^{NS}

*, ** P < 0.05 and 0.01, respectively.
NS Non-significant.
! Phenotypic correlation coefficient.
Genotypic correlation coefficient.

DEVELOPMENT OF WHEAT AND BARLEY VARIETIES FOR ROTATION WITH RICE IN MOISTURE DEFICIENT AND SALINE SOILS OF SINDH

Karam Khan Kaleri

A B S T R A C T

Wheat/Barley cropping pattern with Rice under residual moisture of rice may be efficient and potential with the development of drought tolerant materials tested from multilocations, because moisture limitations predominantly affect per unit area production. In Pakistan yields of cereals in these areas are low due to non evaluated heritability of the trait and its relationship to yield consistency in performance of new varieties which play decisive role in their adoption by farmers. Yield is indicator of drought resistance and greater progress should be made for selecting all the attributes specially related to moisture drought tolerance.

Our criteria of selecting progenies under drought locations demonstrate that target potential yields can be achieved through emperical approaches of varietal evaluation. Among three trials of wheat having thirty different entries each; only NARC-9 (125 maturity days) showed efficiency of tolerance in drought stress by recording 1790 Kg/ha grain yield in southern rice-wheat cropping pattern of Sindh (Thatta); whereas in northern part, Dokri; 90-C-043 (118 maturity days) cultivar under environmental stresses out yielded by producing 1259 Kg/ha.

Well established Barley cultivar ROHO/MAZURKA-ICB77-0169-4AP-5AP-1AP-W1218/EK/APM (120 maturity days) equally out yielded (1305.67 Kg/ha) and HARMA-02/ 11012-2/ MZQ/ 3/MA6002/ VOLLA/ WW6319/ CBB4-0422-9AA-OAP in Thatta among all the cultivars tested in various locations. B-88059 (107 maturity days); and AZARI-82 (105 maturity days) equally produced a maximum grain yield of 1166.67 Kg/ha in moisture limited areas of Dokri.

INTRODUCTION

In Pakistan wheat, is usually grown under irrigated conditions or in a drought stressed situation in a rice-wheat rotation. Compared to irrigated wheat, the yield potential of wheat grown in drought stress is low due to limited availability

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* Director, Wheat Research Institute Sakrand, Sindh (Pakistan).

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of breeding materials tolerant to drought stress, salinity and related environmental stresses. In Sindh, Pakistan, salinity is the main reason for reduction in economic yields and saline soils are rapidly increasing due to improper cultural practices and unsuitable cropping patterns. In most agricultural ecosystems, salinity is not at a steady state and the dynamics of the crops soil system favor the increase of salinity with time. Some questions arise whether breeding alone can offer a long term solution to farming in saline environment (Blum, 1991). In areas where exchangeable Na percentage (ESP) and PH are at critical level, cropping patterns of rice - wheat or rice - barley with developed and well established cultivars some extent may ameliorate the soil toward production.

Drought is a major production constraint on approximately 37% or 4 million ha of wheat in developing countries (Rajaram and Nelson, 1982). Effects of droughts are usually severe. It not only cuts off water needed for normal metabolism, but also decreases the supply of nutrients from the soil. Problems of drought stress can sometimes be overcome through crop management practices and use of drought resistant varieties. Resistant cultivars can possess a capacity for maintaining a higher leaf water potentials, [Keim and Kronstad (1981), Sojka, et al. (1981)] a greater capacity for osmotic adjustment [Sojka, et al. (1981), Blum, et al. (1981)]. Resistant cultivars are known and can be used as yardsticks by which tolerance can be measured in germplasm . Wide adaptability is desirable trait and it has been suggested that drought stress tolerance in a cultivar may be expressed as stability in

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performance over various environments [Blum (1979), MacDowall (1973) and Quisenberyy (1982)]. Under limited soil moisture it may be necessary to look at alternative production systems to maintain economic viability. Byerlee (1984) showed that drilling wheat directly into rice stubble after harvest eliminated time for land preparation and improved crop stand establishment. The objective of this study was to evaluate cultivars and lines of wheat and barley for use in rotation with rice under drought and salinity stressed conditions.

MATERIALS AND METHODS

Field experiments were conducted during the 1992-93 rice growing season at two different agro-ecological locations in Sindh. Sites are in the North and South of the province, about 350 kilometers apart. Thatta, the Southern site; has an arid-tropical type of climate. It is constantly dry but due to coastal effect the summers are not too hot. The Northern site; Dokri is arid, sub-tropical continental with hot summers Table 1. For each site soil was identified as a clay loam at both locations. The Data for soil chemical analysis of EC, PH, and ESP is integrated in Table 2.

Ninety wheats and forty barleys were tested across both locations. Cultivars were grouped and grown in groups of thirty and twenty for wheat and barley respectively.

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Table 1. Meteorological data(Dec.1992 - Dec.93) on Temperature, Relative Humidity and Precipitation of Northern and Southern part of Sindh Province.

Month	Temperature C° Maximum		Minimum		Average		Relative Humidity		Precipitation	
	Dokri	Thatta	Dokri	Thatta	Dokri	Thatta	Dokri	Thatta	Dokri	Thatta
December 92	26.0	25.3	13.7	12.2	19.9	18.8	31.2	29.4	0.5	0.6
January 93	28.3	25.2	10.6	9.0	19.5	17.1	35.4	36.2	0.9	0
February	32.3	30.3	12.9	12.0	22.5	21.1	37.0	51.7	10	15
March	35.7	32.3	18.6	18.6	26.8	23.0	44.3	62.3	0	8
April	35.8	35.2	22.3	19.7	29.1	27.5	46.4	65.5	6	0
May	40.3	39.5	28.5	25.0	34.4	32.2	55.2	68.1	0	7.5
June	42.8	40.1	30.6	27.4	36.7	33.8	60.1	72.2	0	0
July	40.5	37.2	27.3	27.2	33.9	32.2	70.0	77.3	3.8	0
August	38.6	35.2	26.4	26.3	32.5	30.8	75.0	79.1	0	0
September	35.4	32.0	24.5	23.9	30.0	28.0	75.2	82.2	298.3	328.0
October	38.7	30.5	21.3	20.5	30.0	25.6	40.8	51.1	0	0
November	35.1	29.7	16.5	14.8	25.8	22.2	38.4	33.5	50.2	83
December	25.3	25.7	13.8	12.7	19.6	19.2	33.4	25.2	0	0

Table: 2. Soil chemical analysis (random) of Dokri and Thatta locations.

	Thatta	Dokri
pH	7.9	8.4
EC m.mhos/cm	0.58	1.28
ESP	9.0	8.5
SAR	12.0	10.67

Table: 3. Production practices at Thatta and Dokri location

	WHEAT		BARLEY	
	Thatta	Dokri	Thatta	Dokri
Plot size (m)	4x3	4x3	4x3	4x3
Row spacing (cm)	30	30	30	30
Seeding rate kg/ha	150	150	100	100
Seeding date				
Trial 1&2 =	Dec 20,92	Jan 9,93	Dec 20,92	Jan 7,93
Trial 3 =	Dec 20,92	Jan 1,93		
	N P K	N P K	N P K	N P K
Fertilizer	140-70-0	140-70-0	50-25-0	50-25-0

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A randomized complete block design with three replications was used in each trial. The wheat and barley crops were grown on residual moisture after rice harvest. Recommended cultural practices for crop establishment and maintenance were used. The description is presented in Table 3. The data were recorded for germination, heading, maturity and grain yield. Top five lines based on their performance for the above traits at both sites were selected.

RESULTS AND DISCUSSION

WHEAT

GERMINATION: Out of ninety entries five cultivars were selected for high germination percentage from both locations, the result reveals that most of the cultivars/lines across stress conditions performed better than checks. Among locations germination percentage in Dokri was highest. Among all cultivars NARC-10 and NARC-15 showed viability by recording equally 80 percentage germination respectively at Dokri Table 5. Same was true with check (MEHRAN-89). However AZARI-W4 and C-1/2(91-92)KAUZ at Thatta showed viability of germination up to 70 % over checks Table 4.

HEADING AND MATURITY: All the top yielded cultivars and lines showed earlier heading and maturity at Dokri in comparison to Thatta. The heading appeared averagely in 67 to 74 days and maturity fallen in the range 119 to 128 days in both the locations Table 6. The early heading tended early maturity. High mean maximum daily temperature and severe moisture stress conditions at Dokri likely caused early maturation by shortening the vegetative stage of the plant.

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Table 4. Germination(%) of the top five wheat cultivars/lines and checks at Thatta site.

Variety(V)	Top Germinators at Thatta	Germination at Dokri	V-Mean	Diff.
AZARI-W1	70.0 a	40.0 d	55.0	30.0 **
C-1/2(91-92)KAUZ	70.0 a	50.0 c	60.0	20.0 **
NARC-6	65.0 a	40.0 d	52.5	25.0 **
NARC-9	65.0 a	60.0 b	62.5	5.0 ns
NARC-15	65.0 a	80.0 a	72.5	-15.0 **
MEHRAN-89	70.0 a	80.0 a	75.0	-10.0 **
SARSABZ	30.0 b	30.0 e	30.0	0.0 ns
L-MEAN	62.1	54.3	58.2	7.8

S.E.D.	LSD(5%)	LSD(1%)	CV%
2.7	5.6	7.6	5.8

Table 5. Germination(%) of the top five wheat cultivars/lines and checks at Dokri site.

Variety(V)	Top Germinators at Dokri	Germination at Thatta	V-Mean	Diff.
NARC-10	80.0 a	50.0 b	65.0	30.0 **
NARC-15	80.0 a	65.0 a	72.5	15.0 **
NARC-7	70.0 b	55.0 b	62.5	15.0 **
NARC-17	70.0 b	40.0 c	55.0	30.0 **
NARC-23	70.0 b	35.0 cd	52.5	35.0 **
MEHRAN-89(CHECK)	80.0 a	70.0 a	75.0	10.0 **
SARASABZ(CHECK)	30.0 c	30.0 d	30.0	0.0 ns
L-MEAN	68.6	49.3	58.9	19.3

S.E.D.	LSD(5%)	LSD(1%)	CV%
3.5	7.2	9.6	7.3

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Table 6 . Grain yield (kg/ha), Heading (Days) and Maturity of the top five wheat cultivars/lines and checks as affected by various moisture stressed and saline areas of Sindh.

TOP YIELDERS AT THATTA

Variety(V)	Top Yielder at Thatta	Yield at Dokri	V-Mean	Diff.	Heading (Days) Thatta	Dokri	V-Mean	Diff	Maturity (Days) Thatta	Dokri	V-Mean	Diff.
NARC 3	1799.00 a	827.33 d	1308.67	962.67 **	74.0 a	67.0 c	70.5	7.0 **	125.0 c	120.0 ab	122.5 bc	5.0 **
NARC 26	1694.67 b	889.00 c	1246.83	715.67 **	75.0 a	65.0 c	70.0	10.0 **	130.0 ab	120.0 ab	125.0 ab	10.0 **
C-11/3(92-93)V-L1	1543.33 c	926.00 b	1234.67	617.33 **	74.0 a	71.0 ab	72.5	3.0 ns	125.0 c	117.0 b	121.0 c	8.0 **
NARC 27	1543.33 c	926.00 b	1234.67	617.33 **	75.0 a	68.0 bc	71.5	7.0 **	128.0 bc	121.0 a	124.5 ab	7.0 **
NARC 1	1480.00 d	839.67 d	1159.83	640.34 **	72.0 a	65.0 c	68.5	7.0 **	130.0 ab	120.0 ab	125.0 ab	10.0 **
MEHRAN 83(CHECK)	1604.67 b	981.00 a	1292.84	623.67 **	75.0 a	73.0 a	74.0	2.0 ns	132.0 a	122.0 a	127.0 a	10.0 **
SARSABZ (CHECK)	1259.00 e	925.67 b	1092.33	333.33 **	75.0 a	65.0 c	70.0	10.0 **	129.0 ab	121.0 a	125.0 ab	8.0 **
L-MEAN	1546.43	902.10	1224.26	644.33	74.3	67.7	71.0	6.6	128.4	120.1	124.3	8.3 **

S.E.D.	LSD(5%)	LSD(1%)	CV%	S.E.D.	LSD(5%)	LSD(1%)	CV%	S.E.D.	LSD(5%)	LSD(1%)	CV%
10.78	22.08	29.78	1.2	1.6	3.0	1.8	2.7	3.6	4.9	1.6	1.4

TOP YIELDERS AT DOKRI

Variety(V)	Top Yielder at Dokri	Yield at Thatta	V-Mean	Diff.	Heading (Days) Dokri	Thatta	V-Mean	Diff	Maturity (Days) Dokri	Thatta	V-Mean	Diff.
90-C-043	1259.00 a	1358.00 b	1308.50	-99.00 **	70.0 a	75.0 a	72.5	-5.0 **	118.0 b	128.0 bc	123.0 bcd	-10.0 **
AZARI-W2	1137.67 b	987.67 f	1092.67	210.00 **	61.0 c	70.0 b	65.5	-9.0 **	120.0 ab	125.0 c	122.5 cd	-5.0 **
90-C-007	1172.67 c	1049.33 e	1111.00	123.34 **	70.0 a	75.0 a	72.5	-5.0 **	118.0 b	126.0 bc	122.0 d	-8.0 **
NASC-8	1172.67 c	1271.33 d	1222.00	-98.66 **	72.0 a	74.0 a	73.0	-2.0 ns	119.0 ab	128.0 bc	123.5 bcd	-9.0 **
90-C-013	1135.67 d	1296.33 c	1216.00	-160.66 **	65.0 b	73.0 ab	69.0	-8.0 **	120.0 ab	129.0 b	124.5 bc	-9.0 **
MEHRAN-83(CHECK)	1049.33 e	1382.67 a	1216.00	-333.34 **	73.0 a	75.0 a	74.0	-2.0 ns	122.0 a	132.0 a	127.0 a	-10.0 **
SARSABZ(CHECK)	925.67 f	1259.00 d	1092.33	-333.33 **	65.0 b	75.0 a	70.0	-10.0 **	121.0 ab	129.0 b	125.0 ab	-8.0 **
L-MEAN	1139.38	1229.19	1179.79	38.81	68.0	73.9	70.9	-5.9	119.7	128.1	123.9	-8.4 **

S.E.D.	LSD(5%)	LSD(1%)	CV%	S.E.D.	LSD(5%)	LSD(1%)	CV%	S.E.D.	LSD(5%)	LSD(1%)	CV%
1.83	24.23	32.69	1.9	1.4	2.9	3.9	2.0	1.9	3.0	4.1	1.7

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Yield: Cultivar locations and their interaction showed great variation in grain yield. In all the test cultivars/lines at Thatta under drought and saline stresses showed efficiency of stability by producing maximum yields, whereas same set of cultivars could not perform better at Dokri location. Among both locations in various trials NARC-9 was ranked first (1790.00 kg/ha), 77% and 70% more than checks MEHRAN-89 and SARSABZ respectively in Thatta. Many lines at both locations were superior with a great existing differences to checks in all the trials Table 6. The results reveals that most of the new lines and cultivars in relation to environmental stresses performed better than the standard cultivars check.

At Dokri site; among all screening trials the maximum grain yield was recorded in 90-C-043 (1259.00 kg/ha) over check SARSABZ and MEHRAN-89; i.e. 73 % and 83 % more than checks in test trials Table 6. The reduction in yield of SARSABZ may be due to low germination percentage Table 4 and 5.

BARLEY

Germination: The same set of cultivars/lines recorded great variation for germination and heading at various locations in both barley trials. Most of the barley cultivars did not response Thatta drought stress location but performance was satisfactory at Dokri. Germination in Dokri was satisfactory (65%) with CLIPPER (check); whereas ICBB2-0836-OSH-1AP-OAP and BEECHER recorded highest germination up to 70 percent at Dokri over the tested check Table 7 and 8.

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Table 7 . Germination of the top five barley cultivars/lines and checks at Thatta site.

Variety(V)	High germinators at Thatta	Germination at Dokri	V Mean	Diff.
ER/APM/KV/UNION ICBB3 -0548-BAP-2APP-OAP-2A P OAP-4APN-OAP	60.7 a	45.0 b	52.8	15.7 **
SANGHRI-92	60.0 a	25.0 c	42.5	35.0 **
POR/USR11776/SOTOL "S"/3/FL"S"/4/GAL/ P16384/5GLO	60.0 a	25.0 c	42.5	35.0 **
ICBB2-0836-OSH-1AP OAP	50.0 b	70.0 a	58.3	-16.7 **
MARI	50.0 b	65.0 a	57.5	-15.0 **
CLIPPER(CHECK)	60.0 a	65.0 a	62.5	-5.0 ns
L-MEAN	56.8	48.6	52.7	8.2

S.E.D.	LSD(5%)	LSD(1%)	CV%
4.0	8.2	11.2	9.3

Table 8 . Germination of the top five barley cultivars/lines and checks at Dokri site.

Variety(V)	High germinators at Dokri	Germination at Thatta	V-Mean	Diff.
ICBB2-0836-OSH 1AP-OAP	70.0 a	50.0 b	60.0	20.0 **
BEECHER	70.0 a	40.0 c	55.0	30.0 **
MARI-2	63.3 ab	50.0 b	56.7	13.3 **
N.U.B.YT-2(91-92)	60.0 b	50.0 b	55.0	10.0 *
ER/APN	60.0 b	50.0 b	55.0	10.0 *
CLIPPER(CHECK)	65.0 ab	60.0 a	62.5	5.0 ns
L-MEAN	64.7	50.0	57.4	14.7

S.E.D.	LSD(5%)	LSD(1%)	CV%
3.6	7.4	10.0	7.6

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Table 9 . Grain yield (kg/ha), Heading (Days) and Maturity of the top five barley cultivars/lines and checks as affected by various moisture stressed and saline areas of Sindh.

TOP YIELDERS AT THATTA

Variety(V)	Top Yielder at Thatta	Yield at Dokri	V-Mean	Diff.	Heading (Days) Thatta	Dokri	V-Mean	Diff	Maturity (Days) Thatta	Dokri	V-Mean	Diff.
HAPAHA-02/11012-02/11012-2/HZO/3/HA6102/VOLLA/WA6313 ICCB84-04422-3AD-0AP	1305.67 a	1111.00 ab	1208.33	194.67 *	78.0 b	70.0 bc	74.0	8.0 **	120.0 a	104.0 b	112.0	16.0 **
PONO/HAZUKA ICB 77-0163-4AP-5AP/AP-W12190/ER/AP11	1305.67 a	878.00 c	1064.33	372.67 **	71.0 c	67.0 d	69.0	4.0 **	120.0 a	103.0 a	111.5	11.0 **
B-83098	1250.67 ab	1000.00 bc	1125.34	150.67 **	80.0 b	69.0 cd	74.5	11.0 **	118.0 b	105.0 b	111.5	13.0 **
N.U.B.Y.T-2(30-31	1250.00 ab	1166.67 a	1208.34	83.33 ns	90.0 a	75.0 a	82.5	15.0 **	120.0 a	105.0 b	112.5	15.0 **
B-088059	1250.00 ab	1000.00 bc	1208.67	417.34 **	78.0 b	67.0 d	72.5	11.0 **	118.0 b	105.0 b	111.5	13.0 **
CLIFFER	556.33 b	470.67 d	518.80	96.26 ns	88.0 a	72.0 b	80.0	16.0 **	120.0 a	110.0 a	115.0	10.0 **
L-MEAN	1173.55	937.72	1046.05	269.16	80.8	70.0	75.4	10.8	119.3	106.3	112.8	13.0

S.E.D.	LSD(5%)	LSD(1%)	CV%	S.E.D.	LSD(5%)	LSD(1%)	S.E.D.	CV%	LSD(5%)	LSD(1%)	CV%
71.24	147.04	199.28	1.8	1.0	2.0	2.7	0.7	4.5	1.5	2.1	0.8

TOP YIELDERS AT DOKRI

Variety(V)	Top Yielder at Dokri	Yield at Thatta	V-Mean	Diff.	Heading (Days) Dokri	Thatta	V-Mean	Diff	Maturity (Days) Dokri	Thatta	V-Mean	Diff.
B-088059	1166.67 a	1000.33 c	1083.50	166.34 **	62.0 d	69.7 c	65.8	-7.7 **	107.0 b	125.0 a	116.0	-18.0 **
AZARI-B2	1166.67 a	1250.00 a	1208.34	-83.33 ns	75.0 a	90.0 a	82.5	-15.0 **	105.0 c	120.0 c	112.5	-15.0 **
BARBEROUSE/RHODES/GLORIA/COME	1111.03 a	1135.67 b	1123.35	-24.64 ns	71.0 b	80.0 b	75.5	-9.0 **	109.0 a	125.0 a	117.0	-16.0 **
HAPAHA-02/11012-02/11012-2/HZO/3/HA6102/VOLLA/WA6313 ICCB84-04422-3AD-0AP	1107.67 a	1305.67 a	1206.67	-198.00 **	70.0 bc	91.0 a	80.5	-21.0 **	104.0 c	120.0 c	112.0	-16.0 **
BEECHER	1003.00 a	555.66 d	819.33	527.34 **	70.0 bc	79.0 b	74.5	-9.0 **	110.0 a	122.3 b	116.2	-12.3 **
CLIPFER	589.33 b	457.33 d	523.33	132.00 *	63.0 c	67.0 d	68.0	2.0 *	109.0 a	119.0 c	114.0	-10.0 **
L-MEAN	1037.40	950.78	994.09	86.62	69.5	79.4	74.5	-9.9	107.3	121.9	114.6	-14.6

S.E.D.	LSD(5%)	LSD(1%)	CV%	S.E.D.	LSD(5%)	LSD(1%)	CV%	S.E.D.	LSD(5%)	LSD(1%)	CV%
54.23	112.05	151.86	2.3	0.9	1.8	2.4	1.7	0.3	1.8	2.4	0.8

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Heading and Maturity: Heading and maturity of top yielded entries appeared averagely 08-15 days earlier in Dokri than Thatta. All varieties showed growth duration between 104 to 127 days in both locations (Table 9). The reason for early earhead emergence and maturity in northern part (Dokri) may be severe stress conditions due to excess temperature and evapotranspiration. These stresses pushed plant toward physiological maturity.

Yield: The performance of cultivars/lines in terms of grain yield was satisfactory in Thatta location. Among locations across entries the yield differences of entries and locations were significantly different. Two entries ROHO/MAZUKA ICB 77-0169-4AP-5AP/AP-W12190/ER/APM and HARMA-02/ 11012-2/ MZQ/ 3/MA6002/ VOLLA/ WW6319/ CBB4-0422-9AA-OAP equally recorded 1305.67 kg/ha over check CLIPPER (457.33 kg/ha) at Thatta location Table 9.

The maximum yield production at Dokri location was assessed in AZARI-B2 and B-88059 lines which equally yielded 1166.67 kg/ha; 161% and 248% respectively more than check (CLIPPER) Table 9.

RECOMMENDATIONS

Farmers in moisture limited areas hesitate to make any substantial investment in grain crops because of the high risk of crop failure and thus keep their lands fallow. Drought and salt tolerant varieties are a possible solution to their problem In this regard out yielded wheat cultivar/line NARC-9, NARC-26 and MEHRAN-89 yield potential of 1790 Kg/ha and 1604.67 Kg/ha having 125, 130 and

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132 maturity days respectively for Thatta; whereas 90-C-043 and AZARI-W2, maturity days 118 and 120; having yield record of 1259 Kg/ha and 1197.67 Kg/ha respectively are recommended for Dokri. Barley followed by rice crop at residual moisture can be grown successfully taking ROHO/MAZURKA ICB77-0169-4AP-5AP-1AP-W12198/ER/ APM (1305.67 Kg/ha) and HARMA-02/11012-2/MZQ/3/MA6102/VOLLA/WW63191CB84-0422-9AP-OAP (1305.67 Kg/ha) entries for Thatta and B-88059 and AZARI-B2 having grain yield of 1166.67 Kg/ha are recommended for Dokri.

LITERATURE CITED

BLUM, A. 1991. Breeding Crop Varieties for Stress Environments. IN CRS Critical Review's in Plant Sciences. Vol-2, Issue 3. PP. 208.

BLUM, A. 1979. Genetic Improvement of drought resistant in crop plants. A case of sorghum. IN stress physiology in crop, H. Mussel and R.C. Staples, eds. Willey Interscience, New York, USA, PP. 429 - 445.

BLUM, A., GOZLAN, G., AND MAYER, J. 1981. The manifestation of dehydration avoidance in wheat breeding germplasm. Crop Sci. 21, 459.

BYERLEE, D., SHEIKH, A.D., ASLAM, M., AND HOBBS, P.R. 1984. Wheat in rice based farming systems of the Punjab. Implications for research and extension. Wheat Programme NARC, Islamabad. PARC/Cimmyt report, October, 1984, Pakistan Agric. Res. Council, Islamabad.

FISCHER, K.S., E.C. JOHNSON, AND T.O. EDMEADES. 1983. Breeding and selection for drought resistance in tropical maize. CIMMYT, EL BATAN, Mexico.

BEST AVAILABLE DOCUMENT

165

KEIM, D.L., AND KRONSTAD, W.E. 1981. Drought response of winter wheat cultivars grown under field stress condition.Crop Sci., 21, 11.

MacDOWALL, F.D.H. 1973. Growth kinetics of Marquis wheat versus morphogenic dependence. Canadian Journal of Botany 51 : 1259-65.

MAY, L.H., AND R.L.MILTHORPE.1962. Drought resistance of crop plants. Field Crop Abstracts, 15:171-179

ORAKWUE, F.C. 1984. Wheat germplasm development for heat and drought tolerance for Nigeria. Pages 100-106. Proceedings of the International Symposium. Wheats for more tropical environments. United Nations Development Programme and CIMMYT.

QUISENBERRY, J.E. 1982. Breeding for drought resistance and plant water efficiency. In breeding plants for less favorable environments. M.N. Christensen and CF. Lewis, eds. John Willey and Sons, Inc., New York, USA, PP. 193-212.

RAJARAM, S., AND W.L. NELSON. 1982. Wheat production system in arid and semi-arid regions. IN Proceedings Int. Summer School on Agri., Royal Society of Dublin. pp. 305-324.

SHEORON, I.S. AND GRAG, O.P. 1981. Effects of Cl and SO4 salinity on germination and early seeding growth of mong (Phaseolus aureus), Acta Botnica, Indica, 6 : 84 - 89.

SOJAKA, R.E., STOLZY, L.H., AND FISCHER, R.A. 1981. Seasonal drought response of selected wheat cultivars, Agron. J. 73, 838. 1981.

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ROLE OF N_2-FIXING AND GROWTH HORMONE-PRODUCING BACTERIA IN IMPROVING GROWTH OF WHEAT AND RICE

K.A MALIK[1], G. RASUL[1], UZMA HASSAN[1], SAMINA MEHNAZ[1] & M. ASHRAF[2]

1. National Institute for Biotechnology & Genetic Engineering P.O. Box No. 577, Jhang Road, Faisalabad, Pakistan.

2. Department of Biological Sciences, Quaid-e-Azam University, Islamabad, Pakistan.

Key Words: *In situ* ARA, Pseudo-nodule, 2,4-D, *Azospirillum, Azotobacter,* ^{15}N, IAA, Attachment, Colonization.

Abstract

The *in situ* acetylene reduction assay values for rice grown in nitrogen depleted soils were higher than the values obtained for varieties grown in fertile soils. Isolations carried out on soil extract media showed a preponderance of *Azospirillum, Flavobacterium* and *Azotobacter*. Of 19 strains tested, 9 produced indoleacetic acid. *Pseudomonas* sp. produced the maximum quantity of indoleacetic acid (35 μg/ml) in liquid medium containing NH_4Cl. The production was reduced in semisolid medium without NH_4Cl. *Azotobacter* strains tested did not produce indoleacetic acid. The 2,4 dichlorophenoxy acetic acid (0.5 and 1 mg/l) with diazotrophic bacteria induced nodule like structures on the wheat roots. The bacteria were found in the nodules in the form of micro colonies or bacterial aggregates. ^{15}N dilution data indicated that 12.5-46.5% atmospheric N was incorporated in the nitrogen pool of inoculated plants. The proliferation of *Azotobacter, Azospirillum,* and *Rhizobium* spp. was maximum at 4-6 days, and the proliferation of *Azotobacter* and *Azospirillum* was better as compared with *Rhizobium* sp.

Introduction

Nitrogen fixation associated with roots of grasses has been recognized as a possible significant component of the nitrogen cycle in a range of ecosystems including several extreme environments (Dart, 1986). Salinity represents an extreme environment and is characterized by low organic matter and very low nitrogen content in the soil. However, certain plants have been found to grow well under these conditions (Malik et al; 1986). Since the development of acetylene reduction assay (ARA), many plants have been shown to harbor diazotrophs in and around their roots (Jagnow, 1983; Malik et al. 1991; Patriquin and Döbereiner 1978). Among the plants growing in saline environments, rice and wheat have also exhibited high nitrogenase activities as estimated by ARA (Malik et al. 1991).

In addition to the beneficial effects of N_2-fixing bacteria associated with the roots of cereal crops, these bacteria are also reported to produce growth-promoting substances that help in increasing crop yield (De-Freitas et al. 1990; Lifshitz et al. 1987).

More recently, nodule like structures have been produced on the roots of wheat by the use of the synthetic hormone 2,4 dichlorophenoxy acetic acid (2,4-D). These pseudonodules have been reported to reduce acetylene (Ridge et al. 1992; Zeman et al. 1992).

Wheat and rice are crops of great economic importance. They have a vital role as food grain and are particularly important for Asia and Africa. They are cultivated on large areas and require chemical nitrogenous fertilizer input. The objective of adequate the present study was to isolate and characterize plant growth-promoting rhizobacteria (PGPR) that could be used as inoculants (biofertilizer) for rice and wheat crop to reduce chemical fertilizer input and improve yields. The N_2-fixing potential associated with these crops grown in different soil habitats was determined using *in situ* ARA of rice grown in saline soils and wheat grown at different fertility levels. Bacterial isolates from the rhizospheres were screened to determine their ability to produce indoleacetic acid (IAA) and their effects on root growth. The survival and proliferation of these bacteria on and around root surfaces under the influence of 2,4-D and cellulase enzymes was also studied.

Materials & Methods

In SITU ARA OF RICE AND WHEAT

The *in situ* ARA was carried out in the rice field by enclosing rice plants in polyethylene canopies that were tightly fixed on PVC tubes 12 cm in diameter and 15 cm long. Nearly 10 cm of this tube was driven into the soil enclosing the rice plant. A hole was made in the PVC tube near the soil surface to fix a suba seal for injecting C_2H_2 and for taking gas samples. After the volume of the polyethylene canopy was estimated, 10% C_2H_2 atmosphere was provided. The gas samples were collected in 10 ml vacutainers after 16-20 h and later analyzed for ethylene production on a Carlo-Erba Gas chromatograph fitted with flame ionization detector. The plants without acetylene were used as a control. Activities were described on per plant basis (Bilal et al. 1990ab).

Similarly, *in situ* estimation of ARA was carried out with wheat grown on plots that got regular farm yard manure (FYM) or nitrogenous fertilizer application. In the control treatment, no fertilizer or organic matter had been applied for the last 10 years, while in the other treatments, different amounts of FYM or urea had been applied annually. After ARA, the plants were harvested, and their organic carbon content was determined as described by Malik and Azam (1985). Activities were described on the basis of per gram photosynthetic carbon.

ISOLATION AND IDENTIFICATION

First, 200 g dried and sieved soil from a rice field at the Biosaline Research Station (BSRS), Lahore was shaken for 24 hours with 1 l of distilled water. Soil particles were removed by centrifugation. The supernatant was autoclaved separately either with 5 g/l malate or with 5 g sucrose, 5 g mannitol, and 0.5 ml lactic acid/l. For semisolid medium, 2 g/l agar was added, and 5 ml of this medium was poured into 17 ml McCartney vials. Two to three excised, 2-3 cm long root pieces (for rhizoplane fraction) and a few milligrams of root-adhering soil (for rhizosphere fraction) were inoculated into vials, which were incubated at 30°C. These vials were observed for diffused growth or pellicle formation after 24 and 48 hours of incubation. Vials exhibiting diffused growth or pellicle formation were subjected to ARA. A loopful from the ARA$^+$ vials was used for second and third enrichments. The third-enrichment vials were streaked on soil extract agar medium plates. The isolated colonies were separately subjected to determine acetylene-reducing potential, and purity was checked by streaking on potato dextrose agar and nutrient agar plates. The cultures were stored at 4°C on nutrient agar slants. Isolated and purified colonies were identified up to genus level (Bilal et al. 1990 b).

SCREENING FOR IAA-PRODUCING DIAZOTROPHS

For detection and quantification of IAA production by diazotrophic bacteria, cultures were grown in Okon's malate medium (Okon et al. 1977) enriched with 5 g sucrose, 5 g mannitol, and 1 g ammonium chloride per litre of the medium. Tryptophan 100 mg/l was added as IAA precursor. After 4 weeks of growth, qualitative estimation of IAA was done using the Fe-$HClO_4$ and Fe-H_2SO_4 methods (Gordon and Weber 1951). Isolates positive for IAA were subjected to quantitative estimation by ethyl acetate extraction (Tien et al. 1979).

EFFECT OF IAA-PRODUCING BACTERIA AND SYNTHETIC HORMONE IAA ON ROOT MORPHOLOGY.

Wheat and rice seedling roots 2-5 days old were dipped into bacterial suspension (10^7 cells/ml) for 10 minutes. Nine PGPR were selected for these studies on the basis of their ability to produce appreciable amounts of IAA in the culture medium (Table 3). A positive control of 50 μg/ml of IAA in the plant growth medium was used to determine IAA effects on root growth in wheat and rice. The seedlings were transferred to sterile plastic growth pouches containing autoclaved 1:5 soil extract provided with 5 g/l sucrose. The plants were harvested after 15 days, and the root length, including seminals and nodals, was measured.

A control experiment was carried out to determine the most effective concentration of IAA in the growth medium. The rice plants were grown in long glass tubes using Hoagland liquid medium containing 0.5-8 μg/ml of IAA. The medium without IAA was used as a control. The plants were harvested after 6 weeks and observed for root, shoot, and total plant dry weight. The effect on the number

of nodal axis (roots) was also noted.

QUANTIFICATION OF N_2 FIXED BY ^{15}N ISOTOPE DILUTION

Wheat plants were grown in N-free half-strength Hoagland liquid medium containing 0, 1, or 2 mg/l 2,4-D in long tube assemblies (Bilal et al. 1993). The seedlings were inoculated with different diazotrophic bacterial strains, and 16 μg/ml ^{15}N of 10% atom excess in the form of $(^{15}NH_4)_2SO_4$ was provided in every tube for quantification of N_2 fixation. Treatment without inoculum was taken as the nonfixing control.

After 5 weeks of growth in the long tube assemblies, the cotton plugs were replaced with suba seals. The atmosphere above the plants was replaced with 10% C_2H_2 v/v. The gas samples were taken after 2 h incubation in light and analyzed for C_2H_2 on a gas chromatograph as described earlier (Bilal et al. 1990b). After 6 weeks, nitrogen content was determined by the Kjeldhal method. The percentage of excess ^{15}N was determined on a mass spectrometer (Malik et al. 1991).

ANATOMY OF PSEUDONODULES

The plants with roots attached were harvested after 6 weeks. Root morphology was studied both by the naked eye and under the low-power magnification of a stereomicroscope. The number of nodule-like structures present was counted.

Pseudonodules formed by the action of 2,4-D were fixed in a solution containing 2.5% glutaraldehyde and 2.5% formaldehyde in 0.1 M phosphate buffer at pH 7.4 for 2h (Karnobsky, 1965). After three rinses in the same buffer, the specimens were post-fixed in 2% OsO_4 for 1 h. They were then stained in uranyl acetate for 1-2 h, after which they were dehydrated in a gradual ethanol series. After dehydration, the specimens were infiltrated with epoxy resin gradually for 3 days. They were then embedded in fresh resin and polymerized at 35°C for one night, after which they were kept at 60°C for two nights. The sections were cut on ultramicrotome and stained with methylene blue and fluorescent antibody (FA) stains prepared against relevant diazotrophic strains.

SURVIVAL AND PROLIFERATION STUDIES

To study the proliferation of inoculated bacteria on the plant root surfaces, the Fahraeus slide system as described by Somesagaran and Hoben (1985) was used. Wheat and rice seedlings 2-5 days old were soaked in bacterial suspensions (10^9 cells/ml) of *Azospirillum* sp., *Azotobacter* sp., and *Rhizobium* sp. for 30 minutes; gently washed with phosphate buffer saline (PBS); and then transferred to Fahraeus slides. Equal volumes of all three suspensions were mixed for the mixed treatment. The tubes containing Fahraeus slides were incubated at 30°C (day) and 26°C (night) with a day length of 16 h and light intensity of 400 u $E.m^2.S$. Fahraeus slides were observed after various intervals over the next week. Roots were removed from the agar and put in conical flasks (250 ml) containing 45 ml saline with sharp pebbles. The bacteria were dislodged from the roots with pebbles by shaking for

6 hours. Next, 1 ml of this suspension was appropriately diluted (serial dilution), and cells were counted by haemocytometer (Bilal, 1988; Bilal et al. 1990b). Proliferation studies were also carried out on the wheat and rice roots using the Fahraeus slide system containing 1 mg/l of 2, 4-D in the growth medium.

In another experiment, wheat seedlings were first dipped in 1% cellulase and 0.1% pectolyase suspension for 10 minutes, and then transferred to the Fahraeus slides. The slides were transferred to the tubes containing Fahraeus medium with 1 mg/l of 2,4-D.

Results

IN SITU ARA OF RICE

Three salt-tolerant rice varieties grown at BSRS Lahore were tested for their acetylene-reducing potential by whole plant canopy assay. The rice was growing in saline soils having a pH of 9.0 and EC of 20 $mScm^{-1}$. The *in situ* ARA was carried out at the panicle initiation stage. The results are presented in Table 1. The values given in the table are averages of three replicates. NIAB-1 variety showed the highest acetylene reduction among the three varieties tested.

Table 1: Whole plant canopy assay (*in situ*) ranges of different varieties of rice grown in saline sodic soils at BSRS Lahore, Activities are given as umol $C_2H_4.pl^{-1}.\ 24h^{-1}$.

Variety	Activity
NIAB-1	67-74 (71)
95	24-35 (30)
96	66-70 (69)

The values given in the parentheses are averages of three replicates.

Similarly, *in situ* estimation of ARA was carried out with one wheat variety grown at the National Institute for Agriculture and Biology (NIAB), Faisalabad. The results are presented in Table 2. The values given in the table are averages of three replicates. Maximum ARA values were obtained in the case of FYM at 16 kg/ha, whereas in th case of urea treatment, lower values than even control were found. This is indicative of the fact that increased application of nitrogenous fertilizers inhibits diazotrophic activity.

ISOLATION AND IDENTIFICATION OF BACTERIA

Organisms associated with the roots of rice plants grown at BSRS, Lahore, isolated on semisolid soil extract medium, belonged to the genera *Xanthobacter, Pseudomonas, Zoogloea, Flavobacterium,*

Azospirillum, and *Azotobacter*. The populations of *Pseudomonas* and *Flavobacterium* were abundant as compared with the others. Table 3 gives 21 randomly selected and identified isolates.

Table 2: *In Situ* ARA ranges of wheat plants supplied with different rates of urea or farm yard manure or grown in normal fertile soil at NIAB, Faisalabad. Activities are given as nmol C_2H_4 h^{-1} gmC^{-1}.

	Plant dry weight(g)	Photosynthetic C.	nmol C_2H_4 h^{-1} gmC^{-1}
Control	6.46	2.58	18-48 (29)
F16	5.78	2.30	58-196 (132)
F32	9.40	3.76	75-79 (76)
N100	12.08	4.80	2-3 (3)
N200	17.90	7.16	3-18 (10)

F16 = 16 kg/h FYM; F32 = 32 kg/h FYM; N100 = 100 kg/h urea; N200 = 200 kg/h urea. The values given in the table are averages of three replicates.

SCREENING OF IAA^+ STRAINS

Of 19 strains tested for IAA production, 10 were able to produce IAA in the presence of combined nitrogen. The majority of the strains of *Psuedomonas*, *Azospirillum,* and *Flavobacterium* produced appreciable quantities of IAA when grown in liquid medium with combined nitrogen. Under nitrogen-fixing conditions, the amount of IAA produced was reduced. Only three strains were able to produce any IAA under semisolid conditions (Table 3).

EFFECT OF PGPR ON PLANT GROWTH

The re-inoculation studies of PGPR were carried out in N-free, half-concentration Hoagland liquid medium. The results are presented in Table 4. For wheat *Zoologea* sp. KY-1 was the most effective strain, with an increased root length (33 cm) observed as compared with the IAA^- control (14 cm). Similar observations were made for rice with an observed root length of 26 cm as compared with 8.5 cm for the IAA^-.

Table. 3: IAA production by diazotrophic bacteria. Quantities are described as μg IAA ml^{-1} in culture medium.

N_2-Fixing Bacteria		CCM+Tryp (Liquid)		CCM+Tryp (Semisolid)	
		$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$
Azospirillum	sp.(N-21)	9.67	0.33	0.27	2.00
Azospirillum	sp.(Iso-2)	-	-	-	-
Azospirillum	sp.(N-4)	6.30	0.34	2.23	-
Azotobacter	sp.(Iso-1)	-	-	-	-
Azotobacter	sp.(95-43)	-	-	-	-
Azotobacter	sp.(Br2ii)	-	-	-	-
Flavobacterium	sp.(RRN-29)	-	-	-	-
Flavobacterium	sp.(RRN-30)	-	-	-	-
Flavobacterium	sp.(96-57(2)	-	-	-	-
Flavobacterium	sp.(96-57)	11.87	1.01	3.00	-
Flavobacterium	sp.(96-57ii)	8.75	0.67	2.60	-
Pseudomonas	sp.(96-51)	35.70	0.31	0.25	-
Pseudomonas	sp.(39NA)	31.50	0.44	0.37	4.00
Pseudomonas	sp.(RRN-54)	22.50	0.25	0.90	-
Pseudomonas	sp.(N-15)	7.10	0.33	0.75	-
Pseudomonas	sp.(39NAI)	-	-	-	-
Pseudomonas	sp.(RCC46)	-	-	-	-
Xanthobacter	sp.(N-6)	-	-	-	-
Zoogloea	sp.(KY-1)	5.11	0.34	2.90	0.60

CCM = Combined Carbon Medium; Tryp = Tryptophan; - = No IAA
SD ± (0.04-0.67) $\mu g/ml$.

control. The four *Pseudomonas* strains (N-15, 96-51, RRN-54, and 39-NA) were equally good for wheat, but for rice only 96-51 and N-15 were effective. *Azospirillum* sp. N-4 was relatively more effective for wheat and had a moderate effect on rice (Table 4). *Pseudomonas* sp. 39-NA and RRN-54 and *Flavobacterium* strains 96-57 and 96-57i showed a positive response in wheat, but reduced growth in rice as compared with the IAA$^-$ control.The positive control (50 $\mu g/ml$ IAA) also showed a positive response in wheat, but inhibited rice growth as compared with the IAA$^-$ control.Of the the nine strains tested, four *Azospirillum* sp.(N-21), *Zoogloea* sp. (KY-1), *Pseudomonas* sp. (N-15), and *Pseudomonas* sp. (96-51) gave a positive response with rice. All the other strains showed a negligible or negative response for rice growth (Table4). Therefore, a control experiment was carried out to determine the most effective concentration of IAA in the growth medium. It was observed that 2 $\mu g/ml$ IAA in growth medium increased the root shoot length and dry weight.An increase in the number of nodal axes (roots) was also noted (Table 5).

Table 4: Effect of PGPS-producing bacteria on root morphology of wheat and rice.

Treatments	Root length (cm), including nodal axes (roots)	
	Wheat	Rice
IAA (50 $\mu g\ ml^{-1}$)	30.5	11.5
Control	14.0	8.5
Azospirillum sp.(N-21)	11.0	18.2
Azospirillum sp.(N-4)	24.2	11.0
Flavobacterium sp.(96-57)	9.1	9.1
Flavobacterium sp.(96-57ii)	21.4	6.0
Pseudomonas sp.(RRN54)	27.9	4.5
Pseudomonas sp.(39NA)	21.3	7.5
Pseudomonas sp.(96-51)	26.3	16.4
Pseudomonas sp.(N-15)	18.2	19.8
Zoogloea sp.(KY-1)	33.0	26.1

The SD range for wheat was ± (1.0-3.8)cm and for rice was ± (0.5-3.9) cm. The values given in the table are the averages of six replicates.

Table 5: Effect of indoleacetic acid on rice plant growth:

Concentration of indoleacetic acid (μg/ml)	Shoot weight (mg/plant)	Root weight (mg/plant)	Whole plant weight (mg/plant)
0.0	23.1 ± 2.7	5.2 ± 1.2	28.5 ± 2.5
0.5	18.6 ± 3.3	6.0 ± 1.0	24.6 ± 1.6
1.0	21.4 ± 3.7	7.1 ± 1.1	28.8 ± 2.2
2.0	25.1 ± 1.5	8.7 ± 0.9	34.0 ± 3.5
4.0	21.5 ± 2.1	7.3 ± 0.9	29.0 ± 2.9
8.0	21.1 ± 2.3	6.9 ± 1.0	27.8 ± 3.6

The values given in the table are averages of six replicates; and ± values indicate standared deviations from the mean.

EFFECT OF SYNTHETIC AUXIN 2,4-D ON NITROGENASE ACTIVITY AND PLANT GROWTH

The 2,4-D is a synthetic auxin. It produced nodule-like structures on wheat roots irrespective of bacterial inoculation. The numbers of nodule-like structures present on the roots under different treatments are given in Table 6. As the growth was under axenic conditions, no acetelylene reduction was observed in the control. However relatively high values of ARA were detected in the 1 and 2 μg/ml treatments of *Azotobacter* Iso-1 and *Azospirillum* Iso-

2. The 2,4-D addition had no significant effect on ARA values. Similarly, nitrogen uptake results showed no significant difference.

Table 6: Effect of different concentrations of 2,4-D on pseudonodulation and nitrogen fixation associated with wheat plants inoculated with different diazotrophic strains.

Isolate Codes	Conc. of 2,4-D*	Dry wt. (mg tube^{-1})	Total N*	%NDFA	No.of Nodules	ARA
Control	0.0	64.50	3.90	Nil	0	0
	0.5	50.00	4.80	Nil	12	0
	1.0	81.60	4.62	Nil	18	0
	2.0	67.00	2.94	Nil	0	0
Iso-1	0.0	64.00	5.18	Nil	0	62-417 (199)
	0.5	54.40	4.90	Nil	16	ND
	1.0	141.40	4.14	23.8	0	34-350 (150)
	2.0	40.60	3.60	39.7	4	333-384 (351)
Iso-2	0.0	64.90	5.38	Nil	0	50-283 (153)
	0.5	60.90	5.24	46.9	8	116-356 (236)
	1.0	54.50	4.10	24.7	26	84-231 (153)
	2.0	39.60	2.46	42.6	2	56-252 (1540
K - 1	0.0	ND	ND	ND	0	15-20 (18)
	0.5	54.80	4.37	19.8	32	34-57 (44)
	1.0	59.30	4.76	18.3	26	ND
	2.0	58.30	4.51	12.5	32	33 (17-64)
Iso-1+ Iso-2+ K-1	0.0	88.30	5.10	Nil	0	70-308 (189)
	0.5	61.50	3.40	Nil	14	266-406 (330)
	1.0	50.10	6.02	Nil	16	118-529 (326)
	2.0	54.60	5.04	Nil	2	ND

2,4-D = mg l^{-1} ;%NDFA = % Nitrogen derived from atmosphere; ARA = nmol C_2H_4 tube^{-1} h^{-1}. values outside parentheses indicate the ranges of six replicates, and values inside parenthes are the averages of six replicates. Total N = mg tube^{-1}; ND = not determined;Nil = % NDFA zero.

However, the estimation of the prcentage of nitrogen derived from the atmosphere (% NDFA), based on ^{15}N isotopic dilution, indicated significant plant uptake of the bacterially fixed nitrogen, with a range of 12-46 %NDFA. All these estimates were made by keeping the uninoculated treatment as a control. Relatively higher NDFA values were obtained in the case of *Azospirillum* Iso-2 inoculation.

NODULE MORPHOLOGY, STRUCTURE AND BACTERIAL COLONIZATION

The plants with nodules were harvested after 6 weeks. A schematic presentation of the nodules is given in Figure 1. Many of these nodules, on examination, looked like aborted lateral root primordia with a preponderance of root hair all around them.

Ultrathin sections (Figure 2 a and b) showed fairly disorganized cellular structure. The outer layer of cells seemed to be very loose and disjointed. The bacterial presence inside these nodular cells was observed and confirmed by flourescent antibody staining. *Azotobacter* cells were found in abundance in the outer surface cells of the nodular structures in round-shaped aggregates consisting of eight cells. (Figures 2 a and b).

SURVIVAL AND PROLIFERATION STUDIES

The proliferation of different bacteria on the root surfaces was studied using the Fahraeus slide system. Fahraeus medium with and without 2,4-D was used for plant growth. In the Fahraeus medium with 2,4-D, survival and proliferation studies were carried out on the wheat roots treated with and without cellulose-degrading enzymes (Table 8). It was observed that in rice, the maximum bacterial proliferation of inoculated bacteria was 4-6 days. However, the proliferation of *Rhizobium* sp. was much less than that of *Azospirillum* sp. and *Azotobacter* sp. On the other hand, the presence of 2,4-D in the medium increased the *Rhizobium* population and decreased the *Azotobacters* population. *Azospirillum* and the mixed treatment remained unaffected by 2,4-D (Table 7).

In wheat, the maximum proliferation of inoculated bacteria was observed on the fourth day after inoculation. The presence of 2,4-D in the medium increased the proliferation of *Azospirillum* and *Azotobacter* sp., but decreased proliferation in *Rhizobium* and mixed treatment. Enzyme treatment to the seedlings raised the numbers of all three strains. However, in this treatment, maximum proliferation was achieved 24 hours earlier in all the species (Table 8).

Discussion

With the development of the acetylene reduction methodology, several plant-bacteria systems have been reported to support associative nitrogen fixation. However, most of the studies have been based on excised root acetylene reduction assay. There are relatively few reports on the *in situ* ARA of various crop plants (Roger and Ladha 1992). Use of ARA for quantification gives erroneous results (Van Berkum, 1980; Lethbridge et al. 1982), but this methodology is useful for screening purposes. The ARA values obtained for different salt-tolerant rice varieties were much higher than values obtained with other rice varieties grown in fertile nonsaline soils (data not presented). The saline habitat, being a low fertility environment, provides an ideal ecological niche for nitrogen fixation. Similar results have been obtained with kallar grass grown in saline soils (Bilal, 1988; MaliK and Bilal, 1988).

Fig. 1 Nodule-like structures (pseudonodules) induced on wheat roots treated with 1.5 μg/ml 2, 4-D.

Fig.2 Transverse section of ultrathin section of wheat nodules (a) (b) showing the micro colonies of *Azotobacter* Iso-1 (a = 1250 X, b = 10,000 X)

Table 7: Attachment, colonization, and proliferation of diazotrophic bacteria on root surfaces of rice. The numbers given in the table x 10^8 indicate the total bacteria attached per gram fresh weight root.

Diazotrophic Bacteria	Observ. (Days)	Inoculum	Inoculum + 2,4-D 1 mg/l)
Azospirillum sp.	1	27 ± 2	31 ± 4
	2	208 ± 13	187 ± 16
	3	556 ± 30	267 ± 11
	4	1169 ± 86	1172 ± 52
	5	2409 ± 107	1590 ± 79
	6	3319 ± 50	8261 ± 94
	7	1961 ± 53	3580 ± 85
Azotobacter sp.	1	110 ± 10	140 ± 15
	2	227 ± 6	473 ± 12
	3	541 ± 20	2050 ± 23
	4	2148 ± 141	2176 ± 63
	5	3605 ± 125	2126 ± 38
	6	5718 ± 67	1698 ± 35
	7	2096 ± 54	941 ± 33
Rhizobium sp.	1	20 ± 1	50 ± 1
	2	24 ± 3	88 ± 5
	3	23 ± 2	172 ± 4
	4	37 ± 4	250 ± 11
	5	41 ± 6	190 ± 9
	6	30 ± 4	142 ± 20
	7	18 ± 3	102 ± 17
Azospirillum + *Azotobacter* + *Rhizobium* sp. (Mix)	1	70 ± 12	118 ± 8
	2	161 ± 16	150 ± 8
	3	266 ± 14	447 ± 11
	4	2711 ± 103	2742 ± 96
	5	10350 ± 289	10256 ± 199
	6	4503 ± 49	8531 ± 62
	7	2872 ± 295	2436 ± 120

The values given in the table are averages of three replicates; ± values indicate the standard deviations from the mean.

Table 8: Attachment, colonization, and proliferation of diazotrophic bacteria on root surface of wheat. The numbers given in the table x 10^8 indicate the total bacteria attached per gram fresh weight root.

Diazotrophic Bacteria	Observ. (Days)	Inoculum	Inoculum + 2,4-D	Inoculum + 2,4-d + Enzyme
Azospirillum sp.	1	8	10	96
	2	29	21	293
	3	36	42	451
	4	74	177	258
	5	71	66	61
	6	11	24	47
	7	6	51	66
Azotobacter sp.	1	9	10	69
	2	20	19	188
	3	32	71	411
	4	111	148	125
	5	81	89	43
	6	28	28	14
	7	1	70	19
Rhizobium sp.	1	10	9	72
	2	17	9	219
	3	33	67	257
	4	124	116	116
	5	59	117	63
	6	69	54	50
	7	22	16	18
Azospirillum + *Azotobacter* + *Rhizobium* sp. (Mix)	1	9	10	36
	2	16	16	281
	3	69	29	457
	4	129	79	612
	5	72	56	61
	6	13	15	28
	7	38	68	11

2,4-D = (1 mg/l); Enzyme = (1% cellulase and 0.1% pectolyase).

A number of diazotrophic bacteria have been isolated from roots using various nitrogen free, media (Rennie, 1981). For the present study, soil extract medium was used, supplemented with carbon source/s only. This allowed the isolation of several strains of *Pseudomonas* and *Flavobacterium* that had not previously been isolated from plants growing in saline soils. For testing the IAA producing ability, Fe-H_2SO_4 proved better than Fe-$HClO_4$. There are reports about the IAA producing ability of *Azotobacter*, *Azospirillum* and *Pseudomonas* (Martinez-Toledo et al., 1988; Gonzalez-Lopez et al., 1986; Zimmer et al., 1988), but the *Azotobacter* strains reported in this study did not produce any IAA (Table 3).

Auxins are growth regulators that are essential for plant growth. Some bacteria are also reported to produce these growth hormones, affecting plant growth (Zimmer et al., 1988; Lifshitz et al., 1987; Bric et al., 1991; Tien et al., 1979). The bacteria are reported to change root morphology (i.e., increase in root length, and number of nodal and lateral roots), hence enhancing the nutrient uptake to improve plant growth (Kapulnik et al., 1985; Rodriguez-Barrueco, 1991; Harari et al., 1989; Malik and Bilal, 1988). We observed the increase in root length of wheat inoculated with different diazotrophic strains (Table 5). We did not observe any significant increase in the root length of rice. IAA, 50 µg/ml in growth medium also did not give a positive response in rice, though this concentration produced a good response in wheat. In testing the effective concentrations for rice, it was noted that 2 µg/ml IAA in the medium improved root shoot dry weight and length. The number of nodal axes, lateral roots, and root hairs also increased. These observations confirm that IAA requirements vary among plant species.

Using 2,4-D, nodule-like structures were only induced on wheat roots and not on rice though thickening of the roots occurred. Recently, Ridge et al, (1992) studied the induction of nodule-like structures on wheat roots. They considered these nodules to be lateral root primordia because they observed vascular tissues in anatomical sections. In our studies, a concentration of root hair was found around the nodular structures. A preponderance of bacterial colonization was observed on these root hairs. Similarly, bacterial aggregates were observed on the root hairs and inside the nodular structures when inoculated with *Azotobacters*. Similar observations for kallar grass have already been reported (Bilal et al, 1993). The detection of nitrogenase activity as estimated by the ARA and ^{15}N isotope dilution methods suggests that the inner cells of pure bacterial aggregates or microcolonies are responsible for nitrogen fixation.

The presence of associative nitrogen fixation in non-legumes is a random process. Although much work related to the re-inoculation of diazotrophs has been done on grass rhizosphere; our knowledge about root colonizations is still not complete. Information is available about the role of motility, chemotaxis response, carbohydrate utilization, and presence of binding and receptor structures, but little is known about the mechanism of

initial attachment of bacteria to the root surfaces. There are probably specific sites on the roots where colonization is initiated. The use of 2,4-D or such growth-promoting substance of microbial origin may be able to create preferential sites for colonization, in turn increasing the beneficial effects on plant growth. More work is needed to prove or disprove these hypotheses.

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References

Bilal, R. 1988 Ecology of root associated diazotrophs. In: Associative nitrogen fixation in plants growing in saline soils. Ph.D thesis Punjab University, Lahore, Pakistan.

Bilal R., Rasul, G., Arshad, M. and Malik, K.A. (1993). Attachment, colonization and proliferation of *Azospirillum brasilense* and *Enterobacter* spp. on root surface of grasses. World J. Microbiol. Biotechnol. 9, 63-69.

Bilal, R., Rasul, G., Qureshi, J. A. and Malik, K. A. (1990 a) Characterization of *Azospirillum* and related diazotrophs associated with roots of plants growing in saline soils. World J. Microbiol. Biotechnol. 6, 46-52

Bilal, R., Rasul, G., Mahmood, K. and Malik, K. A. (1990 b) Nitrogenase activity and nitrogen fixing bacteria associated with the roots of *Atriplex* spp. growing in saline sodic soils of Pakistan. Biol Fertil Soils. 9, 315-320

Bric, J. M., Bostock, R. M. and Silverstone, S. E. (1991) Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol. 57, 535-538

Dart PJ 1986. Nitrogen fixation associated with non-legumes in agriculture. Plant and Soil. 90: 303-334.

DeFreitas, J.R. And Germida, J.J. (1990). Plant growth promoting rhizobacteria for winter wheat. Can. J. Microbiol. 36, 265-272.

Gonzalez-Lopez, J.Salmeron, V. Martinez-Toledo, M. V. Ballesteros, F. and Ramos-Cormenzana, A. (1986) Production of auxin, gibberellins and cytokinins by *Azotobacter vinelandii*. ATTC 12837 in chemically defind media and dialysed soil media. Soil Biol. BIOchem. 18, 119-120

Gordon, S. A. and Weber, R. P. (1951) Colorimetric estimation of

indoleacetic acid. Plant Physiol. 26, 192-195

Harari, A., Kigel, J. and Okon, Y. (1989) Involvement of IAA in the interaction between *Azospirillum brasilense* and *Panicum miliaceum* root. In: Nitrogen Fixation with Non-Legumes. F. A. Skinner, R. M. Body and I. Fendrik. pp. 310-310 Kluwer Academic Publishers.

Jagnow, G. (1983) Nitrogenase (C_2H_2) activity in non-cultivated and cereal plants: Influence of nitrogen fertilizer on population and activity of nitrogen fixing bacteria. Z. Pflanzenernaehr. Bodenkd. 146, 217-227

Karnovsky, M. J., (1965). A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy, J. Cell. Bill. 27, 137a-138a.

Kapulnik, Y., Feldman, M., Okon, Y. and Henis, Y. (1985) Contribution of nicrogen fixed by *Azospirillum* to the N nutrition of spring wheat in Israel. Soil Biol. Biochem. 17: 509-516.

Lethbridge, G., Davison , M. S. and Sparling, G. P. (1982) Critical evaluation of the acetylene reduction test for estimating the activity of nitrogen fixing bacteria associated with the roots of wheat and barley. Soil Biol. Biochem. 14, 27-35

Lifshitz, R., Kloepper, J.W., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E.M. and Zaleska, I. (1987). Growth promotion of canola (repeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Can. J. Microbiol. 33, 390-395.

Malik, K. A. and Azam, F. (1985) Effect of humic acid on wheat (*Triticum aestivum*) seedling growth. J. Appl. Expt.Botany. 25, 245-252

Malik, K.A. and Bilal, R. (1988) Survival and colonization of inoculated bacteria in kallar grass rhizosphere and quantification of N_2-fixation. Plant and Soil. 110: 329-338.

Malik, K. A., Bilal, R., Rasul, G., Mahmood, K. and Sajjad, M. I. (1991) Associative N_2-fixation in saline sodic soils and its relative quantification based on ^{15}N natural abundance. Plant and Soil. 137, 67-74

Malik, K. A., Zahoor, A. and Naqvi, S. H. M. (1986) Kallar grass, a plant for saline land. pp. 93. Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad.

Martinez-Toledo T., De La Rubia., J Moreno and J Gonzalez-lopez (1988). Root exudates of *Zea mays* and production of auxins, gibberellins and cytokinins by *Azotobacter chroococcum*. Plant and Soil. 110: 149-152.

Okon, Y., Albrecht, S. L. and Burris, R. H. (1977) Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. Appl. Environ. Microbiol. 33, 85-88

Patriquin, D.G. and Dobereiner, J. (1978) Light microscope observation of tetrazolium reducing bacteria in the endorhizosphere of maize and other grasses in Brazil. Can. J. Microbiol. 24: 734-742.

Rennie R. J. 1981 A single medium for isolation of acetylene reducing (dinitrogen fixing) bacteria from soil. Can. J. Microbiol. 27, 8-14

Ridge, R.W., Bender, G.L. and Rolfe, B.G. (1992). Nodule like structures induced on the root of wheat seedlings by the addition of the synthetic auxin 2,4-dichlorophenoxyacetic acid and the effect of microorganisms. Aust. J. Plant Physiol. 19, 481-492.

Rodriguez-Barrueco, C., Cervantes, E., Subbarao, N.S. and Rodriguez-Caceres, E. (1991). Growth promoting effect of *Azospirillum brasilense* on *Casuarina cunninghamiana* Miq. Seedlings. Plant and Soil. 135, 121-124.

Roger, P. A. and Ladha, J. K. (1992) Biological N_2 fixation in wetland rice field: Estimation and contribution of nitrogen balance. Plant and Soil. 141, 41-55

Somasegarn, P. and Hoben, H.J. (Eds)(1985) To perform agglutination reaction with pure cultures of *Rhizobium* (exercise 7). In: Methods in Legume-*Rhizobium* Technology, NifTAL., USA. P. 82-88.

Tien, T.M., Gakins, M. H. and Hubbell, D. H. (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl milet (*Pennisetum americanum* L.). Appl. Environ. Micrbiol. 37: 1016-1024.

Van Berkum, P. (1980) Evaluation of acetylene reduction by excised roots for the determination of nitrogen fixation in grasses. Soil. Biol. Biochem. 12: 141-145.

Van Berkum, P. and Bohlool, B.B. (1980). Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses. Microbiol. Rev. 44: 491-517

Zeman, A.M.M., Tchan, Y.T., Elmerich, C. and Kennedy, I.R. (1992). Nitrogenase activity in wheat seedlings bearing para-nodules induced by 2,4-dichlorophenoxyacetic acid (2,4-D) and inoculated with *Azospirillum*. Res. Microbiol. 143, 847-855.

Zimmer, W., Roeben, K. and Boothe, H. (1988) An alternative explanation for plant growth promotion by bacteria of the genus

SELECTION OF COTTON VARIETIES TOLERANT TO SALINITY

Akhtar Nawaz Khan and Muhammad Aslam

Faculty of Agriculture, Gomal University,

Dera Ismail Khan, Pakistan.

ABSTRACT

The salt tolerance of 20 cotton (G. hirsutum L. and G. arboreum L.) cultivars was studied in several experiments that included both hydroponic and soil culture in different saline growth conditions. Screening at the seedling stage in a hydroponic system provided a reproducible technique for mass screening of cotton genotypes for salt tolerance. Fresh shoot weight was the main selection criterion used to determine the salinity tolerance of cotton cultivars. Cultivars exhibited a wide range in salinity tolerance, with Rehmani and S-12 being the most tolerant.

Introduction

On a world wide basis, soil salinity/sodicity is among the major factors responsible for low crop production, with 400 to 950 x 10^6 ha of soils being salt-affected (Epstein et al., 1980). These areas are located primarily in arid or semi-arid regions where the problem may become further exacerbated by use of low-quality (i.e., saline) irrigation water. Excessive accumulation of salts in the root zone is a problem of great agricultural, economic, and social significance in these regions.

About 5.6 x 10^6 ha of agricultural land is salt-affected in Pakistan (Chaudhary et al., 1978). The magnitude of the resultant reported reduction in seed cotton yield was 41% for slightly affected land and 73% for moderately affected land. An annual loss of over 14 billion rupees was estimated for the entire irrigated area of the country as a result of salinity-induced production loss (Qayyum and Malik, 1985).

Two approaches are available for overcoming the problems of salt-affected lands: first is to use the available technologies for reclaiming these soils; the second employs biological exploitation of such soils through the use of salt-tolerant plant species. The former approach aims to make the soil environment suitable for common crop growth through hydrological (leaching) management. This approach has been the primary method used in Pakistan; however, the associated high costs can limit reclamation efforts in many parts of Pakistan. The approach of employing biological techniques involves the selection and breeding of salt-tolerant crop cultivars for use in saline areas. This paper presents a procedure useful in identifying cotton cultivar tolerance to salinity through the screening of cotton germplasm in hydroponic culture and natural saline field conditions.

MATERIALS AND METHODS

Experiment #1

Seedlings were grown in plastic-coated iron trays (60 x 30 x 8 cm), filled with quartz sand (4 cm depth). Distilled water was

applied for germination; half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950) was applied until the seedlings were transplanted. Two leaf seedlings were transferred into galvanized painted plastic-lined iron growth tanks (99 x 99 x 24 cm) supported on iron stands 90 cm above ground containing aerated half-strength Hoagland solution. The iron tanks were covered with thermopal sheets having holes, which kept the plant roots suspended in the Hoagland solution. The solution was aerated using an air compressor. Two days were allowed after transplanting for the seedlings to become established, and the medium was then salinized with NaCl salt in increments of 50 mol m^{-3} per day until the final salinity levels were reached (0, 100, 160, 220 mol m^{-3} NaCl). The experimental design was a completely randomized design with three replications. Seedlings were harvested at 15 days of growth after salination for measurement of fresh and dry weights of shoot.

Experiment #2

In this trial, the experiment #1 procedure was modified so that desired salt concentrations of 100, 160, and 220 mol m^{-3} NaCl were prepared before the seedlings were transplanted, instead of the salinity being gradually increased up to desired levels. The remaining facilities and procedures were the same as for experiment #1.

Experiment #3

A field experiment was conducted with natural soil salinity levels, electrical conductivity of saturation extract (EC_e) between 7 and 16 dS m^{-1}, and pH of 7.8. The experimental design was a

randomized complete block design with three replications using a plot size of 6 x 30 m. Twenty cultivars were hand-drilled after seed beds were prepared, each in 2 rows, 6 m long, with a row spacing of 75 cm and intrarow plant spacing of 30 cm. Fertilizers (urea and DAP) were applied at the rate of 100 kg N ha^{-1} and 50 kg P ha^{-1}, as recommended. All of the P fertilizer and half of the N fertilizer were applied at planting, with the remaining N being applied by the broadcast method with the second irrigation.

Irrigation was performed according to the plant requirements, assessed by visual observation. Normal agronomic practices were followed throughout the growing season. Picking was done by hand at maturity for determining yield data on the cultivars.

RESULTS AND DISCUSSION

In this study, absolute shoot fresh yield was used as a criterion to determine the salinity tolerance of cotton crops. Various workers have used this parameter as a tool in determining the salt tolerance of plants (Shannon, 1984; Khan, 1987). From the farmers' point of view, the maximum growth or economic yield potential of a plant species under given salinity is important, regardless of any growth/yield depression as compared with nonsaline conditions. Also, actual crop yields are useful when comparing different varieties of the same crop under a given set of conditions. However, the relative performance of different varieties will depend upon the level of salinity, and a variety giving higher yield in a saline environment may not perform better

than others under nonsaline conditions.

Data on growth parameters for experiment #1, shoot fresh yield and shoot dry weight, are given in Tables, 1 and 2, respectively. Both parameters decreased significantly with an increase in salt concentrations in the growth media although the differences between the 100 and 160 mol m^{-3} concentrations were nonsignificant. Average reductions of 30, 40 and 53% were found in shoot fresh yield at salt concentrations of 100, 160, and 220 mol m^{-3} NaCl, respectively. The highest absolute shoot yield of Rehmani was significantly higher than that of the other cultivars, except for K-68-9, DNH-1, and CIM-70. The interaction effects of salinity and variety were significant (P = 0. 05) in that the performance of the cultivars varied at different salt concentrations.

Using plant mortality percentage (experiment #2) as a criterion for salt tolerance evaluation, S-12 and Rehmani proved to be somewhat more tolerant at salt concentration of 220 mol m^{-3} NaCl, expressing only 40 and 50 percent plant mortality, respectively (Table 3). At a salt concentration of 160 mol m^{-3} NaCl, the outstanding cultivars were Rehmani, S-12, BH-41 and CIM-109, having 10, 20, 20, and 30% mortality, respectively. CIM-70, RH-1, NIAB-86, Ravi, D-9, Rohi, and DNH-1 were the most sensitive at 160 mol m^{-3} NaCl.

When compared on a relative yield basis (experiment #2), i.e., shoot fresh weight expressed as a percentage of the respective control yields, S-12 and Rehmani were again the most tolerant while the cultivars mentioned above were the most sensitive (Table-4). At

a salinity level of 160 mol m^{-3} NaCl, the most sensitive cultivars were CIM-70, RH-1, NIAB-86, Ravi, Rohi, DNH-,1 and FH-682, while S-12, Rehmani, BH-41, CIM-109, and FH-78 were salt tolerant at this salinity level. These trends closely follow those of the plant mortality percentage.

Analysis of variance indicates that in experiment #3, varietal differences in terms of seed cotton yield were statistically significant (Table 5). The maximum seed cotton yield was obtained from Rehmani which had the highest yield of all cotton cultivars in the trial, but this yield was not statistically greater than that of Gohar-87, NIAB-78, S-12 and NIAB-86.

CONCLUSIONS

Various researchers (Maas and Poss, 1989; Shannon, 1984; Epstein et al., 1980; Rush and Epstein, 1981; Shannon, 1978; Nieman and Shannon, 1977) have described or developed quick methods for screening different crops for salt tolerance at early growth stages. A screening program for appraising the salinity tolerance of existing crop plants is necessary to meet the present needs of the salt-affected areas of the world. The results can be useful to farmers as a basis for choosing crops for commercial production on salt-affected soils.

Various screening techniques (e.g., nutrient culture and soil culture) have been compared to determine their relative value as general procedures for mass screening of cotton cultivars for tolerance to salinity. Screening at the seedling stage in solution

culture was easy and provided an effective method for measuring the salt tolerance of the cultivars. The solution culture method was highly correlated with field study results (Tables 1 to 5). Shoot fresh weight at the seedling stage was found to be most suitable for appraising salt tolerance. Absolute shoot fresh yield (solution culture) was highly correlated with seed cotton yield at low and high salinities under field conditions (Table 6). These results indicate that screening at the seedling stage in solution culture is a reasonably reliable technique for mass screening of cotton germplasms.

The validity of the solution culture technique is authenticatd by the high degree of similarity between the results of this technique and those from field studies. In both cases Rehmani and S-12 proved to be the most tolerant, while D-9, Ravi, Rohi, CIM-70 and RH-1 proved to be the most sensitive.

TABLE 1 Shoot fresh yield (g per plant) of various cotton cultivars exposed to different salt concentrations for 15 days in nutrient solution.

CULTIVARS	NaCl mol m^{-3}				
	Control	100	160	220	Mean
Rehmani	4.96	3.41	3.08	2.66	3.53 a
K-68-9	5.04	3.61	2.77	2.25	3.42 ab
DNH-1	4.68	3.27	2.79	2.20	3.23 ac
CIM-70	4.58	3.26	2.87	2.05	3.19 ad
S-12	4.57	3.12	2.70	1.93	3.08 be
Shaheen	4.42	2.91	2.56	2.07	2.99 bf
FH-87	4.47	3.03	2.52	1.83	2.96 cf
RH-1	4.60	2.67	2.35	2.08	2.92 cf
BH-41	4.10	2.78	2.50	1.95	2.83 cf
Gohar-87	3.63	2.84	2.53	2.03	2.77 df
DNH-25	3.73	2.84	2.59	1.88	2.76 df
NIAB-78	3.72	2.71	2.44	2.04	2.73 ef
CIM-262	4.10	2.73	2.19	1.80	2.71 ef
BH-36	3.66	2.80	2.43	1.81	2.67 ef
NIAB-86	3.63	2.79	2.40	1.83	2.66 ef
FH-682	3.87	2.65	2.14	1.73	2.59 f
CIM-109	3.68	2.61	2.15	1.70	2.54 f
Ravi	1.88	1.58	1.42	1.11	1.50 g
D-9	2.12	1.40	1.10	0.89	1.38 g
Rohi	1.95	1.36	1.10	0.63	1.26 g
Mean	3.87 a	2.72(70) b	2.33(60) b	1.82(47)c	

Means followed by the same letter are not significantly different according to the Duncan's Multiple Range Test (P = 0.05). Letters other than the first and the last have been omitted to simplify the results.

Values in parentheses represent percentages of the respective controls.

TABLE 2 Shoot dry yield (g per plant) of various cotton cultivars exposed to different salt concentrations for 15 days in nutrient solution.

CULTIVARS	NaCl mol m^{-3}				
	Control	100	160	220	Mean
S-12	0.84	0.50	0.37	0.27	0.50 a
CIM-70	0.68	0.53	0.40	0.33	0.49 a
Rehmani	0.60	0.50	0.44	0.33	0.47 ab
DNH-1	0.66	0.48	0.41	0.29	0.46 ab
K-68-9	0.56	0.49	0.42	0.31	0.45 ab
BH-36	0.58	0.48	0.40	0.33	0.45 ab
Shaheen	0.56	0.44	0.41	0.34	0.44 ab
NIAB-86	0.56	0.42	0.42	0.35	0.44 ab
RH-1	0.59	0.44	0.37	0.31	0.43 ab
CIM-109	0.54	0.47	0.40	0.30	0.43 ab
CIM-262	0.56	0.45	0.36	0.27	0.41 ac
NIAB-78	0.60	0.45	0.35	0.29	0.41 ac
DNH-25	0.52	0.45	0.38	0.28	0.41 ac
FH-87	0.60	0.50	0.33	0.19	0.40 ad
Gohar-87	0.56	0.42	0.35	0.20	0.40 ad
FH-682	0.53	0.41	0.32	0.25	0.38 bd
BH-41	0.47	0.43	0.39	0.22	0.38 bd
Ravi	0.45	0.34	0.29	0.18	0.32 cd
D-9	0.50	0.35	0.23	0.18	0.31 cd
Rohi	0.40	0.29	0.27	0.21	0.31 cd
Mean	0.57 a	0.44(78)b	0.36(64)b	0.28(48)c	

Means followed by the same letters are not significantly different according the Duncan's Multiple Range Test (P = 0.05). Letters other than the first and the last have been omitted to simplify the results.

Values in parentheses represent percentages of the respective controls.

TABLE 3 Plant mortality (%) of various cotton cultivars exposed to different salt concentrations for 15 days in nutrient solution.

CULTIVARS	NaCl mol m^{-3}			
	Control	100	160	220
Rehmani	0	10	10	50
S-12	0	10	20	40
CIM-109	0	10	30	70
DNH-1	0	20	60	80
BH-41	0	10	20	80
FH-682	0	20	60	80
Shaheen	0	40	50	90
NIAB-86	0	20	90	90
CIM-70	0	60	100	100
RH-1	0	20	90	100
Gohar-87	0	40	60	100
FH-87	0	10	40	100
Rohi	0	20	60	100
NIAB-78	0	20	40	100
D-9	0	50	70	100
DNH-25	0	30	60	100
Ravi	0	40	70	100
K-68-9	0	40	60	100
BH-36	0	10	50	100
CIM-262	0	30	60	100

TABLE 4 Shoot fresh yield (% of control) of various cotton cultivars exposed to different salt concentrations for 15 days in nutrient solution.

CULTIVARS	Control	NaCl mol m^{-3} 100	160	220
	g per plant	Percent of control		
S-12	2.55	81	60	32
Rehmani	2.84	81	58	29
CIM-109	2.80	81	54	14
NIAB-86	2.37	70	8	11
BH-41	2.63	81	56	11
DNH-1	2.70	65	19	7
Shaheen	2.51	59	37	6
FH-682	2.63	54	21	3
Rohi	1.63	68	18	1
CIM-70	3.30	31	0	0
RH-1	2.87	59	2	0
Gohar-87	2.66	50	29	0
FH-87	2.63	84	46	0
NIAB-78	2.09	75	34	0
D-9	1.56	49	20	0
DNH-25	2.00	41	24	0
Ravi	1.55	56	10	0
K-68-9	3.10	44	28	0
BH-36	2.16	81	31	0
CIM-262	2.29	44	30	0

TABLE 5 Seed cotton yield (kg ha^{-1}) of various cotton cultivars under natural saline field conditions.

CULTIVARS	Salinity levels, EC_e, dS m^{-1}			
	7-8	10-12	13-16	Mean
Rehmani	990	650	470	703 a
Gohar-87	987	600	404	664 ab
NIAB-78	925	595	390	637 ac
S-12	920	525	412	619 ad
NIAB-86	963	489	375	609 ad
K-68-9	940	480	360	593 bd
RH-1	903	532	334	590 be
Shaheen	897	464	397	586 be
CIM-109	880	470	390	580 be
FH-87	878	528	329	578 be
FH-682	890	510	310	570 be
CIM-70	840	516	355	570 be
BH-41	880	490	330	567 be
CIM-262	875	490	335	567 be
BH-36	860	475	340	558 ce
DNH-25	865	495	290	550 ce
DNH-1	869	451	301	540 ce
Rohi	827	445	271	514 de
D-9	851	465	200	505 de
Ravi	880	353	205	479 e

Means followed by the same letter are not significantly different according to the Duncan's Multiple-Range Test (P = 0.05). Letters other than the first and the last have been omitted to simplify the results.

TABLE 6 Relationship between absolute shoot yield and seed cotton yield of cotton cultivars grown in different saline media.

VARIABLE			CORRELATION COEFFICIENT
SOLUTION CULTURE (Experiment #1) 100 mol m^{-3}		SALINE FIELD (Experiment #3) 7-8 dS m^{-1}	
Absolute shoot fresh yield	vs	Yield of seed cotton	0.4603*
SOLUTION CULTURE (Experiment #1) 160 mol m^{-3}		SALINE FIELD (Experiment #3) 10-12 dS m^{-1}	
Absolute shoot fresh yield	vs	Yield of seed cotton	0.3920NS
SOLUTION CULTURE (Experiment #1) 220 mol m^{-3}		SALINE FIELD (Experiment #3) 13-16 dS m^{-1}	
Absolute shoot fresh yield	vs	Yield of seed cotton	0.7676**

NS = Nonsignificant
* = Significant (P = 0.05)
** = Highly Significant (P = 0.01)

REFERENCES

Chaudhry, M.B., M.A. Mian, and M. Rafiq. 1978. Nature and magnitude of salinity and drainage problems in relation to agricultural development in Pakistan. Pak. J. Forestry 28(2):70-72.

Epstein, E., J.D. Norlyn, D.B. Kelley, G.A. Cunningham, and A.F. Wronn. 1980. Saline culture of crops. A genetic approach. Science. 210:399-404.

Hoagland, D.R., and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Cir. 347. P. 32.

Khan, A.N. 1987. Mechanism of salt tolerance in cotton (G. hirsutum L. and G. arboreum L.) Ph.D. Thesis, Univ. Agri., Faisalabad.

Maas, E.V., and J.A. Poss. 1989. Soil sensitivity of wheat at various growth stages. Irrig.Sci.10: 29-40.

Nieman, R.H., and M.C. Shannon. 1977. Screening plants for salinity tolerance. IO:M. J. Wright (ed.), Plant Adaptation to Mineral Stress in Problem soils. Proc., Work shop at Nail.Agric.Library, Beltsville, MD, 1976: 359-367.

Qayyum, M.A., and M.D. Malik. 1985. Farm production losses in salt affected soil. First National Congress of Soil Science, Soil Sci. Soc. of Pakistan.

Rush, D.W., and E. Epstein. 1981. Comparative studies on the sodium, potassium and chloride relations of a wild halophytic and a domestic salt sensitive tomato species. Plant Physiol. 68:1308-1313

Shannon, M.C. 1978. Testing salt tolerance variability among tall wheat grass lines. Agron.J. 70:719-722.

Shannon, M.C. 1984. Breeding, selection and the genetics of salt tolerance. In: R.C. Staples and G.H.Toenniessen (Eds.) Salinity tolerance in plant strategies for crop improvement. John Wiley and Sons, New York. pp.231-235

SOIL FERTILITY

GROUP ADVISOR:

Dr. Jamal Oster
Department of Soil & Environmental Sciences
University of California
Riverside, California

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Comparative Use of Water from Good- and Poor-Quality Sources to Extent Irrigated Area and Studies Physical Problem in Salt-Affected Soils. I. Hussain, M. Akhtar Abbas, F. Ahmad, and M. Jamil.

Potassium Variance Characteristics of Soil and Silt in Relation to Mineralogy. I. Selected Khaddar Varieties of Lower Indus. M.S. Akhtar, Z.I. Awan, and M. Arshad.

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Pre-Flowering Tillage Techniques on Water and Crop Yield Production in a Semi-Arid Climate of Pakistan. R.U. Awan, Ia. Awan, Z.U. Awan, and M.A. Khan.

CONJUNCTIVE USE OF WATER FROM GOOD- AND POOR-QUALITY SOURCES TO EXTEND IRRIGATED AREAS AND REDUCE DISPOSAL PROBLEMS IN SALT-AFFECTED SOILS

T. Hussain, M. Akhtar Abbas, R. Ahmad and G. Jilani
Dept. of Soil Science, Univ. of Agriculture, Faisalabad, Pakistan

ABSTRACT

Field experiments were carried out to evaluate rice-wheat yields using brackish water for irrigation. The studies consisted of evaluating cyclic vs. blended canal and brackish tubewell water and the use of various chemical (gypsum, H_2SO_4, and HCl), and organic (poultry manure, green manure, pressmud) amendments for amelioration of brackish water. The results indicate that the cyclic use strategy resulted in better yields than the use of blended canal and brackish waters. The addition of H_2SO_4 resulted in higher yields, but differences among various chemicals were statistically nonsignificant. As regards the organic amendments, poultry manure gave better yields during the first season, while in the following seasons, the pressmud treatment showed significant superiority. From these studies, it can be concluded that brackish waters of various qualities can be successfully used for crop production under proper management strategies.

INTRODUCTION

One of the major constraints on agricultural production is the limited supply of quality water for surface irrigation. In Pakistan, it results in 11.9 m ha lying as culturable waste (Federal Bureau of Statistics, 1987). An increase in canal water supplies is not possible in Pakistan beacuse of limited resources. Canal water supplies are being supplemented by groundwater, but the quality of this groundwater is questionable beacuse of its high concentration of salts (Khan et al., 1990).

It is a common practice among the farmers in Pakistan to use brackish groundwater after mixing it with the available canal water. This practice often deteriorates the water quality without contributing additional water for crop production. Cyclic use of waters of low and high salinity, as conceived by Rhoades (1984), prevents the soil from becoming saline and allows for the substitution of brackish water for 50% of irrigation needs. The quality of brackish waters can be improved by the use of certain amendments, such as gypsum, sulphuric acid, hydrochloric acid, and other acid formers (Qureshi et al., 1975; Ahmad et al., 1979; Ayers and Westcot, 1985; Ghafoor et al., 1987). Moreover, regular additions of organic material to the soil are extremely important

in maintaining good physical condition and fertility of the soil when using brackish or blended water (Bhatti et al., 1985; Chaudhry et al., 1985; Hornick and Parr, 1987). The present experiments were undertaken to study the possibilities for using brackish waters for crop production under the agro-ecological conditions of Pakistan.

MATERIALS AND METHODS

The following three experiments were a part of a project developed between the Board on Science and Technology for International Development (BOSTID) of the U.S.National Academy of Sciences and the Pakistan Agricultural Research Council. The studies were conducted on a farmer's field 2 km upstream from Khurrianwala on Madhuana Drain on Faisalabad-Lahore Road, Faisalabad.

Experiment I: <u>Cyclic vs. Blended Use of Canal and Brackish Water for Crop Production</u>

The experiment was initiated during the summer of 1992 in a rice-wheat cropping system. In the first crop of rice, there were four treatments: canal water (CW) alone; tubewell water (TW) alone; and cyclic or blended use of CW and TW. The ratio of the blend was adjusted to achieve an EC of 2.0 dS m^{-1} in the water. For the wheat crop (1992-93), the ratios of the blend were used to adjust the SAR and RSC of the brackish water. Thus, there were two blends: Blend I (40% CW, 60% TW) with SAR 10.0 (mmol $L^{-1})^{1/2}$ and Blend II (80% CW + 20% TW) with RSC 2.5 me L^{-1}. In the case of cyclic use, irrigation with TW was applied as conceived by Rhoades (1984), and the resulting change in EC of the soil was determined. The CW water required to bring the EC to the original level was determined by the formula of Dielman et al., (1979). The experiment was replicated thrice following an RCB design. Individual plot size was 5 m x 8 m. In both the rice and wheat crops, preplanting irrigations with CW were applied in all the treatments. The CW had EC 0.28 dS m^{-1}, SAR 2.10 (mmol $L^{-1})^{1/2}$, and the RSC 0.8 me L^{-1}, whereas the corresponding values for TW were 2.72 dS m^{-1}, SAR 12.05 (mmol $L^{-1})^{1/2}$, and RSC 10.4 me L^{-1}, respectively. Sixteen irrigations were applied to the rice crop with a week interval (7.5cm each time), whereas five irrigations were applied in the wheat crop. Recommended plant protection and agronomic practices,such as fertilization, were adopted equally in all the treatments. A

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basal dose of N, P_2O_5 and K_2O were applied @ 120,90 and 60,kg ha^{-1} respectively at the time of transplanting/sowing of the crops.Data on the yield parameters was collected at maturity. Soil samples up to a depth of 30 cm after the harvest of each crop were analyzed for pH, EC, and SAR determinations following methods given in Handbook No. 60 (U.S. Salinity Laboratory Staff, 1954). Physical measurements such as bulk density and soil strength were determined from undisturbed soil samples.

Expriment II: Use of Brackish Water Through Organic Amendments

This experiment was conducted on a rice-wheat cropping rotation. There were again four treatments withthreereplications using an RCB design. The treatments werc 1.TW (same quality as in experiment I),2. TW plus sesbania green manure @ 10 t ha^{-1}, 3.TW plus pressmud (sugar industry waste with EC 2.2 dS m^{-1}, pH 6.6, (soluble Ca + Mg 4%) added on the basis of its (Ca + Mg amount),and 4.TW plus poultry manure (Ca + Mg = 5.2%) @ 10t ha^{-1}. Green manure and poultry manure were incorporated before transplanting, while pressmud was applied with each irrigation. A basal dose of N, P_2O_5 and K_2O were applied @ 60,90 and 60 kg ha^{-1}, respectively at the time of transplanting/sowing of the crops. Other production practices were as described for experiment I.

Experiment III:Use of Brackish Water Through Chemical Amendments

There were again four treatments: TW, TW amended with gypsum calculated by Eaton's formula (Eaton, 1965), TW treated with H_2SO_4 on the basis of titratable basicity, and TW treated with HCl as in the H_2SO_4 treatment. The plot size was 5 m x 8 m. The treatments were repeated thrice with an RCB design. The crop rotation was rice (Bas-385) followed by wheat (LU-26S) and again rice (Bas-370). Other production practices were as in experimrnt I.

RESULTS AND DISCUSSION

Study I

Crops Yield

Data in Table 1 show that the maximum response of significantly higher crop yields was achieved with the CW treatment. The data further reveal that blended use of canal and

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brackish water resulted in lower yields as compared with their use in the cyclic mode. Better yields from the cyclic treatment can be attributed to the proper maintenance of salt balance under this treatment. In the case of blended use, the plant can extract only good quality water from the mix. This extraction is done by the expenditure of energy that otherwise could be used by the plants for biomass production. Bradford and Letey (1992) reported that cyclic use strategy produced higher simulated yields than the blended strategy.

Irrigation with TW alone resulted in the lowest yields, due to the higher sodic hazard of irrigation water (high RSC). Reduction in crop yields under stress has also been reported by Maas and Hoffman (1977) and Aslam et al., (1988).

Plant Tissue Analysis

Rice/wheat grain samples were analyzed for concentrations of Ca, Na, K, and Cl. The data are given in Table 2. The table shows that higher uptakes of Na and Cl by the plants were observed in the plots irrigated continuously with brackish water. This was due to the relatively high sodic hazard of the water. The uptake of Ca and K was reduced because of the increased competition between Na and Ca (Suarez and Grieve, 1988). Maximum uptake of K and Ca was observed with CW treatment. The cyclic use strategy generally resulted in lower uptake of Na and Cl as compared with the blended use strategy. Although the data suggest that the blended use extends water supplies, comparatively better results can be obtained from the cyclic use strategy.

Soil Characteristics

The ionic contents of the soil extracts increased appreciably with the application of TW, which resulted in increased solute concentration EC and SAR (Table 3).in the soil solution. There was, however, less development of soil salinity/sodicity with the cyclic or blended use treatments, thus indicating a possibility for the limited substitution of good-quality CW for the brackish TW. The soil strength of the undisturbed soil samples was also noted at the time of crop harvest. Brackish water resulted in more hardening of the soil, which was further elucidated by the data of on bulk density (Table 4).

Study II

Crop Yields

Data on the effects of various brackish water management strategies on the yield of rice and wheat crops are given in Table 5. The maximum yield of the first crop of rice (1992) was obtained from the treatment where brackish water was amended with poultry manure, followed by the green manure treatment. The differential response to various organic manures may be attributed to their different decomposition rates and the percentage concentration of various nutrients in the manures. The increased growth and yield of the rice crop were attributed to the increased pCO_2 due to the plant root respiration and decomposition of organic matter (Gupta et al., 1989; Robbins, 1986; Chhabra and Abrol, 1977), which thereby increases the soil salinity and decreases the soil pH in calcarious soils. The manures added to the soil increase the soil CEC and thus reduce the amount of sodium per unit area. The faster the decomposition of manure in the soil, the more rapidly various nutrients are taken up by the plants. Maintenance of adequate fertility under salt stress conditions to increase crop yields has been reported by many workers (Ravikoritch and Porat, 1967; Aslam and Muhammad, 1972). During the first crop of rice (1992), maximum yield was observed from the poultry manure treatment, which contained 3.4% Ca. However, in the following crops, the pressmud treatment became progressively worse as compared with the other treatments, indicating more residual effects from the pressmud treatment. The pressmud contained enough phosphorus, in addition to 3.8% Ca. Since the pH of the saturated paste of pressmud was 6.6, the pressmud produced more residual acidity and increased the availability of more micronutrients,as well as of P. From the data, however, it can be suggested that brackish waters of varying qualities can be used successfully for crop production using various organic management strategies.

Plant Tissue Analysis

Rice/wheat grain samples were analyzed for their ionic uptake. The data are given in Table 6. Both sodium and chloride uptake was reduced by the application of manure. A decrease in the ionic concentration of Na can be attributed to the release of Ca from the decomposition of green manures, as well as the solubilization of native $CaCO_3$ (Gupta et al., 1989; Robbins, 1986). Calcium, competes with Na for ion exchange reactions at the root-soil interface. Calcium being divalent in nature, possesses a higher affinity than

Na for absortion on the plant root surface. Brackish water with high SAR and RSC reduced the Ca uptake. Suarez and Grieve (1988) have reported that as the activity of Na^+ in the substrate increases, the system becomes less discriminating, and the selectivity of Ca^{2+} is impaired. Increased root permeability, caused by reduction in the availability of external Ca^{2+}, may lead to the increased Cl^- uptake; Cl^- uptake increased under the brackish water treatment.

Soil Characteristics

The soil samples were analyzed for EC, SAR, and pH determination after the harvest of each crop. The data are presented in Table 7. They show a considerable development in soil salinity/sodicity as a result of brackish water irrigation without the application of any amendment. There was less development in soil sodicity with the application of various manures because of the increased Ca^{2+} concentration in the soil solution resulting from the decomposition of the manures. As a result, the permeability of the soil was improved as Na accumulated in the soil profile when the brackish water was leached downward. Data on the soil crusting/soil strength (Table 8) also reveal more compactness in the soil where TW was applied continuously. Bulk density was also low in the plots where organic amendments (manures) were added. The effectiveness of various organic amendments in improving the quality of brackish water has also been reported by Wahab (1968) and Chillar and Swarup (1984).

Study III

Crop Yield

The ameliorative effects of various amendments on the quality of brackish water irrigation are shown in Table 9 which gives yield data on the rice and wheat crops. The application of acids or gypsum significantly enhanced yields for both crops. This was due to the amelioration of the brackish water with the application of these amendments. The addition of gypsum to the soil increased permeability by increasing the electrolyte concentration and by reducing the exchangeable sodium (Keren and Shainberg, 1981; Loreday, 1976). Because of the competitive behavior of Ca and Na in the soil in various exchange reactions, the addition of gypsum to the soil resulted in the leaching of Na out of the soil profile. Acids added to the irrigation water reduced the concentration of HCO_3^- and contributed to an acidity on

the soil surface that released calcium (Ayers and Westcot, 1985). Because of the double action of acids when added to soil/water (i.e. neutralization of the CO_3 and HCO_3 of the irrigation water, and release of native Ca in the calcarious soils), the acid treatments resulted in better yields in the present study.

Plant Tissue Analysis

Rice/wheat grain samples were analyzed for the uptake of various ions (Table 10). In the first crop of rice (1992) calcium uptake by the plants increased significantly over the control with the application of gypsum or acids. This was due to the increased concentration of soluble Ca in the soil solution as a result of the application of amendments. Increased Na concentration in the soil solution as a result of TW application alone resulted in lower uptakes of Ca in the control. Sodium-induced Ca^{2+} deficiencies have also been observed in earlier studies by Grieve and Mass (1988) and Maas and Grieve (1987).

Potash concentrations in both the paddy and straw were increased with the application of gypsum/acids. Brackish water irrigation resulted in lowest K concentrations in the plant tissues. Janzen and Chang (1987) found that barley plants exposed for Na_2SO_4 salinity contained only one-third the concentration of K in their roots of those grown in nonsalinized solutions.

Soil Characteristics

The effects of brackish water irrigation and the various management strategies on the soil chemical characteristics after the harvest of each crop are reported in Table 11. An increase in EC values, was observed in the plots amended with gypsum or acids, though this increase was not comparable to the increase in EC in the brackish water irrigated plots. The possible reason could be the release of Ca in soil. The SAR of the soil increased from 10.58 to 18.59 $(mmol\ L^{-1})^{1/2}$ in the plots irrigated with brackish water. Soil bulk density (Table 12) was affected significantly, though not more than in plots irrigated continuously with TW without the application of amendments. Soil crusting was significantly increased with brackish water irrigation. The sodic hazard was nullified, however, when gypsum or acids were added to the irrigation water.

CONCLUSIONS:

These experiments suggest a possibile use for brackish water under proper management strategies for sustaining crop yields. These strategies include the cyclic use of canal and brackish waters, or the use of certain chemical amendments (e.g., gypsum, H_2SO_4 or HCl) or organic amendments (e.g., organic manures such as sesbania, poultry manures, or pressmud). Although the acid treatments resulted in relatively higher yields than the gypsum treatments, the use of gypsum as an alternative amendment is recommended for the amelioration of brackish waters.

REFERENCES

Ahmad, B.,W.D.Kemper, G.Haider and M.A.Niazi. 1979. Use of gypsum to lower the SAR of irrigation water. Soil Sci. Soc. Am. J. 43:698-702

Aslam, M., and S. Muhammed. 1972. Effect of various nitrogen carriers at various salinity levels. Pak. J. Sci. Res. 24: 244-251.

Aslam, M., R.H. Qureshi, N. Ahmad and S. Muhammed. 1988. Response of rice to salinity shock at various growth stages and type of salinity in rooting medium. Pak. J. Agri. Sci. 25(3): 199-205.

Ayers, R.S. and D.W. Westcot. 1985. Water Quality for Agriculture. FAO irrigation and drainage paper 29. Rev. 1. FAO,eRom

Bhatti, H.M., M. Yaseen and M. Rashid. 1985. Evaluation of Sesbania green manuring in rice-wheat rotation. Proc. Intl. Symp. on Nitrogen and Environment. Lahore, Pakistan. July 7-12, 1984. 99. 275-284.

Bradford, S., and J. Letey. 1992. Cyclic and blending strategies for using non saline and saline waters for irrigation. Irrigation Science 13: 123-128.

Chillar, P.K., and A. Swarup. 1984. Effect of continuous cropping and fertilizer use on soil properties and yield of rice and wheat in sodic soil. Ind. J. Agri. Sci. 54(6): 461-466.

Chhabra, R., and I.P. Abrol. 1977. Reclaiming effect of rice grown in sodic soil. Soil Sci. 124: 49-55.

Chaudhry, M.R., M.S. Rafiq, A. Haider and L.A. Shahid. 1985. Ameliorative effect of gypsum on soil properties and crop yield irrigated with high SAR water. Mona Rec. Expt. Project. WAPDA, Pub. No. 144: 8-14.

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Dieleman, P.J. 1979. East Khairpur Tile Drainage Project. Revised Plan Report, WAPDA, South, ILACO. p. 96.

Eaton, F.M. 1965. Waterlogging and salinity in the Indus Plains. Pakistan Dev. Rev. 5: 391-392.

Federal Bureau of Statistics. 1987. Agricultural Statistics of Pakistan. MINFAC, Govt. of the Pakistan, Islamabad.

Ghafor, A., S. Muhammad and M. Yaqub. 1987. Use of saline-sodic water for reclamation of a salt affected soil and for crop production. Pak. J. Soil Sci. 2(1-4): 17-21.

Grieve, C.M., and E.Y. Maas. 1988. Differential effects of sodium/calcium ratio on sorghum genotypes. Crop Sci. 28: 659-665.

Gupta, R.K., R.R. Singh and I.P. Abrol. 1969. Influence of simultaneous changes in sodicity and pH on the hydraulic conductivity of an alkali soil under rice culture. Soil Sci. 147: 28-33.

Hornick, S.B. and J.F. Parr. 1987. Restoring the productivity of marginal soils with organic amendments. Amer. J. Alt. Agri. 11(2): 64-68.

Janzen, H.H., and C. Chang. 1987. Cation nutrition of barley as influenced by soil solution composition in a saline soil. Cans. J. Soil Sci. 67: 619-629.

Keren, R., and I. Shainberg. 1981. The efficiency of industrial and mined gypsum in reclamation of sodic soil-rate of dissolution Soil Sci. Soc.Am.J.45:103-107

Khan, G.S., M.T. Afzal and M. Ikram. 1990. Quality of groundwater, land form and drainage relationship in semi-arid tract around Lahore (Pakistan)-1. Pak. J. Soil Sci. 5(1-2): 25-28.

Loreday, J.1976. Relative significance of electrolyte and cation exchange effects when gypsum is applied to sodic clay soil. Aust. J. Soil Res. 14:361-371

Maas, E. V., and C.M. Grieve. 1987. Sodium induced calcium deficiency in salt stressed corn. Plant Cell Envirobn. 10:559-564.

Maas, E. V., and G. J. Hoffman 1977. Crop salt tolerance-current assessment. J. Irrigation and Drainage Div. ASCE 103:115-13

Qureshi, R.H., M. Hanif, M.I. Rajoka and G.R. Sandhu. 1975. Use of saline sodic waters for crop production. In: The "Optimum Use of Water in Agriculture, CENTO Sci. Program, Turkey. pp. 123-197.

Ravikoritch,S., and A. Porat. 1967. The effect of nutrients on salt tolerance of crop. Plant and Soil. 26:49-71

Rhoades, J.D.1984. Use of saline water for irrigation. California Agriculture 38(10):42-43

Robbins, C.W. 1986. Sodic calcareous soil reclamation as affected by different amendments and crops. Agron.J. 78: 916-920

Sharma, D. P., K.N.Singh, K.V.G.K. Rao and P.S. Kumbhare. 1991. Recycling drainage water for irrigation In: Annual Report(1990-91). Central Soil Salinity Res. Inst., Karnal, India.

U.S.Salinity Laboratory Staff.1954. Diagnosis and improvement of saline and alkali soils. USDA Agric. handbook No. 60

Suarez, D.L., and C.M. Grieve. 1988. Predicting cation ratios in corn from saline solution composition. J.Exp. Bot.39: 605-612

Wahab,A. 1968. Amelioration of 'Bara' soil with chemical amendments. Fifty years of Agri. Education and Research. Vol.II. Dept. Agri. West Pakistan.

Table 1: **Effects of cyclic vs. blended use of canal and brackish water on the yield of a rice-wheat cropping system.**

Treatment	Rice(1992)	Wheat(1992-93)	Rice(1993)
	t ha^{-1}		
T_1.Canal water alone	5.59 a	2.61 a	5.67 a
T_2.Cyclic use	5.13 ab	2.56 b	5.17 b
T_3.Blend use-I	4.95 bc	2.38 b	4.35 b
T_4.Blend use-II[a]	-	2.47 b	-
T_5.Tubewell water alone	4.76 c	1.87 b	3.83 c

a: Not applied to rice

Table 2: **Effects of cyclic vs. blended use of canal and brackish water on concentrations of various ions (%) in plant grain samples**

Treatment	Na	K	Ca	Cl
	a) Rice Crop (1992)			
T_1. CW	0.035 NS	0.297a	0.254a	0.240 c
T_2. Cyclic	0.037	0.274a	0.233b	0.270 bc
T_3. Blend-I	0.037	0.226b	0.210c	0.310 ab
T_4. Blend-II[a]	-	-	-	-
T_5. TW	0.048	0.222b	0.192d	0.350 a
	b) Wheat Crop (1992-93)			
T_1. CW	0.025 d	0.338a	0.297a	0.177 b
T_2. Cyclic	0.029 c	0.280b	0.266ab	0.197 b
T_3. Blend-I	0.032 b	0.210a	0.233bc	0.253 ab
T_4. Blend-II[a]	0.030 c	0.263c	0.265ab	0.237 a
T_5. TW	0.036 a	0.214a	0.212c	0.287 a
	c) Rice Crop (1993)			
T_1. CW	0.034b	0.322a	0.285a	0.213 d
T_2. Cyclic	0.035b	0.292b	0.280b	0.240 c
T_3. Blend-I	0.040b	0.234c	0.253b	0.296 b
T_4. Blend-II[a]	0.041b	-	-	0.288 b
T_5. TW	0.051a	0.227c	0.225c	0.340 a

Means sharing similar letter(s) are statistically alike at 0.05 probability.

a: Not applied to rice

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Table 3: **Effects of cyclic vs. blended use of canal and brackish water on soil chemical characteristics**

Treatnent	Before rice-I	After rice-I	After wheat-I	After rice-II
		a) EC (dS m^{-1})		
T_1.CW	3.01	2.92 c	3.07 d	3.17 c
T_2.Cyclic	2.97	3.30 b	3.89 c	3.99 bc
T_3.Blend-I	3.19	3.80 b	4.27 b	4.26 b
T_4.Blend-II	-	-	3.72 c	-
T_5.TW	3.75	4.42 a	5.00 a	5.62 a
		b) SAR (mmol $L^{-1})^{1/2}$		
T_1.CW	8.95	8.89 c	8.61 c	9.13 c
T_2.Cyclic	8.94	9.96 c	11.24 b	11.19 b
T_3.Blend-I	9.85	11.23 b	11.61 b	11.24 b
T_4.Blend-II	-	-	10.33 b	-
T_5.TW	9.75	13.39 a	14.10 a	14.63 a
		c) pH		
T_1.CW	8.21	8.18 NS	8.12 c	8.16 c
T_2.Cyclic	8.17	8.19	8.20 c	8.29 c
T_3.Blend-I	8.20	8.24	8.34 b	8.44 b
T_4.Blend-II	-	-	8.31 b	-
T_5.TW	8.19	8.25	8.40 a	8.53 a

T_4 : Not applied to rice

Table 4: **Soil physical characteristics as affected by cyclic vs. blended, canal and brackish water**

Treatment	Soil Bulk Density (Mg m^{-3})			Soil Strength (kPa)		
	After rice-I	After wheat-I	After rice-II	After rice-I	After wheat-I	After rice-II
T_1.CW	1.35 NS	1.35 NS	1.33 NS	396 NS	330 b	326 b
T_2.Cyclic	1.38	1.33	1.36	394	338 b	351 b
T_3.Blend-I	1.41	1.34	1.38	402	392 b	386 b
T_4.Blend-II	-	1.35	-	-	396 b	- a
T_5.TW	1.41	1.40	1.42	437	470 a	511 a

Rice-I = Rice crop (1992)
Rice-II = Rice crop (1993)
Wheat-I = Wheat crop (1992-93)

T_4: Not applied to rice

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Table 5: **Effects of various brackish water management strategies with organic amendments on the grain yield of crops (Average of three repeats)**

Treatment	Rice-I	Wheat-I	Rice-II
	(t ha^{-1})		
T_1.Tubewell water	4.6. c	2.22 c	3.48 c
T_2.TW + GM Sesbania	5.5 ab	2.38 bc	4.08 b
T_3.TW + Pressmud	5.0 b	3.15 a	5.50 a
T_4.TW + PM	5.7 a	5.70 a	2.68 b

Rice-I = Rice crop (1992)
Rice-II = Rice crop (1993)
Wheat-I = Wheat crop (1992-93)

Table 6: **Effects of various brackish water management strategies with organic amendments on the concentrations of various ions (%) in grain samples.**

Treatment	Na	K	Ca	Cl
	a) Rice Crop (1992)			
T_1.Tubewell water	0.040 a	0.133 b	0.156 b	0.401 a
T_2.TW + GM Sesbania	0.034 b	0.187 a	0.193 ab	0.384 ab
T_3.TW + Pressmud	0.031 b	0.175 a	0.200 ab	0.374 b
T_4.TW + PM	0.183 a	0.226 a	0.369 b	
	b) Wheat Crop (1992-93)			
T_1.Tubewell water	0.029 a	0.270 b	0.146 b	0.282 a
T_2.TW + GM Sesbania	0.024 b	0.338 a	0.173 a	0.237 b
T_3.TW + Pressmud	0.019 c	0.351 a	0.200 a	0.256 b
T_4.TW + PM	0.021 bc	0.323 ab	0.186 a	0.252 b
	c) Rice Crop (1993)			
T_1.Tubewell water	0.050 a	0.184 c	0.186 b	0.425 a
T_2.TW + GM Sesbania	0.036 b	0.239 a	0.326 a	0.374 b
T_3.TW + Pressmud	0.036 b	0.247 a	0.360 a	0.367 b
T_4.TW + PM	0.033 c	0.215 b	0.336 a	0.357 b

Means sharing similar letter(s) are statistically alike at 0.05% probability level.

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Table 7: **Effects of brackish water management strategies with organic amendments on siol chemical characteristics.**

Treatment	Before rice-I	After rice-I	After wheat-I	After rice-II
		a) EC (dS m^{-1})		
T_1.TW	3.42	4.18 a	4.23	5.25
T_2.TW + GM Sesbania	3.49	3.14 b	3.55	4.19
T_3.TW + Pressmud	3.40	3.50 b	3.75	4.32
T_4.TW + PM	3.17	3.24 b	3.43	4.08
		b) SAR (mmol $L^{-1})^{1/2}$		
T_1.TW	13.29	16.22 a	17.17 a	18.23 a
T_2.TW + GM Sesbania	13.45	15.35 ab	13.02 b	12.33 b
T_3.TW + Pressmud	13.83	13.36 b	12.96 b	8.22 c
T_4.TW + PM	11.05	11.11 b	11.74 b	8.96 c
		c) pH		
T_1.TW	8.25	8.28^{NS}	8.33^{NS}	8.78 a
T_2.TW + GM Sesbania	8.20	8.13	8.21	8.47 b
T_3.TW + Pressmud	8.94	8.14	8.11	7.98 c
T_4.TW + PM	8.09	8.18	8.21	8.21 bc

Table 8: **Soil physical characteristics as affected by brackish water management strategies with organic amendments (Average of three repeats)**

Treatment	Soil Bulk Density (Mg m^{-3})			Soil Strength (kPa)		
	After rice-I	After wheat-I	After rice-II	After rice-I	After wheat-I	After rice-II
T_1.TW	1.50 a	1.47^{NS}	1.49 a	462 a	488 a	542 a
T_2.TW+GM	1.42 b	1.42	1.36 b	387 b	327 b	250 b
T_3.TW+Pmud	1.40 b	1.39	1.25 c	356 b	314 b	260 b
T_4.TW+PM	1.44 b	1.41	1.33 b	401 a	358 b	259 b

Rice-I = Rice crop (1992)
Rice-II = Rice crop (1993)
Wheat-I = Wheat crop (1992-93)

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Table 9: **Effects of various brackish water management strategies with chemical amendments on the grain yield of crops (Average of three repeats)**

Treatment	Rice-I	Wheat-I	Rice-II
	(t ha^{-1})		
T_1.Tubewell water	4.68 b	1.92 c	2.67 b
T_2.TW + Gypsum	5.43 a	2.55 b	4.50 a
T_3.TW + H_2SO_4	6.10 a	2.94 a	4.67 a
T_4.TW + HCl	5.72 a	2.82 ab	4.16 a

Rice-I = Rice crop (1992)
Rice-II = Rice crop (1993)
Wheat-I = Wheat crop (1992-93)

Table 10: **Effects of various brackish water management strategies with chemical amendments on the concentrations of various ions (%) in grain samples**

Treatment	Na	K	Ca	Cl
	a) Rice Crop (1992)			
T_1.Tubewell water	0.043 a	0.182	0.196	0.388
T_2.TW + Gypsum	0.034 b	0.207	0.290	0.355
T_3.TW + H_2SO_4	0.037 b	0.197	0.270	0.375
T_4.TW + HCl	0.035 b	0.195	0.264	0.394
	b) Wheat Crop (1992-93)			
T_1.Tubewell water	0.031 a	0.277^{NS}	0.200 c	0.287
T_2.TW + Gypsum	0.022 b	0.358	0.275 a	0.239
T_3.TW + H_2SO_4	0.025 b	0.323	0.240 b	0.248
T_4.TW + HCl	0.022 b	0.306	0.253 ab	0.296
	c) Rice Crop (1993)			
T_1.Tubewell water	0.059 a	0.124^{NS}	0.214^{NS}	0.388 b
T_2.TW + Gypsum	0.034 b	0.152	0.306	0.321 c
T_3.TW + H_2SO_4	0.033 b	0.166	0.280	0.333 c
T_4.TW + HCl	0.035 b	0.156	0.240	0.451 a

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Table 11: **Effects of various brackish water managementstrategies with chemical amendments on soil chemical characteristics**

Treatment	Before rice-I	After rice-I	After wheat-I	After rice-II
		a) EC (dS m^{-1})		
T_1.TW	2.72	3.84 a	4.55 a	4.83 a
T_2.TW + Gypsum	2.84	3.04 b	3.71 b	4.24 b
T_3.TW + H_2SO_4	2.82	3.28 b	3.82 b	4.08 bc
T_4.TW + HCl	3.00	3.19 b	3.54 b	3.95 c
		b) SAR (mmol $L^{-1})^{1/2}$		
T_1.TW	10.58	15.37	16.69 a	8.59 b
T_2.TW + Gypsum	10.11	10.70	11.35 c	8.67 b
T_3.TW + H_2SO_4	9.62	10.33	11.33 c	9.88 a
T_4.TW + HCl	9.13	12.86	13.57 b	9.77 a
		c) pH		
T_1.TW	8.23	8.35	8.40 ^{NS}	8.76 a
T_2.TW + Gypsum	8.19	8.28	8.25	8.47 b
T_3.TW + H_2SO_4	8.25	8.14	8.18	8.21 b
T_4.TW + HCl	8.27	8.08	8.21	8.19 b

Table 12: **Soil physical characteristics as affected by brackish water management strategies with chemical amendments (Average of three repeats)**

Treatment	Soil Bulk Density (Mg m^{-3})			Soil Strength (kPa)		
	After rice-I	After wheat-I	After rice-II	After rice-I	After wheat-I	After rice-II
T_1.TW	1.43^{NS}	1.41^{NS}	1.40^{NS}	443	465	492
T_2.TW + Gypsum	1.38	1.35	1.23	383	388	344
T_3.TW + H_2SO_4	1.37	1.30	1.31	365	353	312
T_4.TW + HCl	1.38	1.32	1.30	474	382	357

Rice-I = Rice crop (1992)
Rice-II = Rice crop (1993)
Wheat-I = Wheat crop (1992-93)

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Potassium Release Characteristics of Sand and Silt in Relation to Mineralogy: I. Selected Mollisols, Vertisols, and Inceptisols

M.S. Akhtar, Z.I. Awan, and M. Arshad

ABSTRACT

Seven soil series, representing highly weathered soils of Pakistan, were investigated to document their nonexchangeable potassium (K) pool in sand and silt size fraction in relation to mineralogy. The Biha, Mali, and Murree series were clay loam in texture while the Ushu, Shahi, Kotly, and Jhatpat series were silty clay. The differences in particle size distribution were attributed to the source of parent material. Sand/silt ratio in the Shahi and Ushu series remained unchanged with profile depth suggesting that at respective sites both the soils were developed in uniform parent material.

Sand fraction of the soils were composed of mainly quartz and feldspar with some mica and chlorite except Kotly which was mainly composed of quartz and feldspar. Silt fraction was also composed of mainly quart, mica, and feldspar. The Biha soil contained the higher amount of biotite than Kotly in sand fraction. But in silt size fraction, Kotly profile contained the higher biotite and muscovite than the Biha. The differences in sand and silt mineralogy were attributed to the source of soil parent material and mineral weathering. The soil derived from granite and granodiorite contained higher boiling nitric acid extractable K than the highly weathered alluvial soil. The Kotly sand released the lowest K. The silt fraction in the Kotly and Murree soils yielded 4 to 5 times higher K than the corresponding sand fraction while reverse is true in case of Jhatpat.

Boiling nitric extractable K was strongly correlated with biotite content and correlation with muscovite was significant only in case of alluvial soils while there was no significant correlation with K feldspar content. Addition of biotite and muscovite or biotite, muscovite, and feldspar did not improve the correlation coefficient. Also, boiling nitric acid extractable K was positively correlated with total Al content of the sand fraction where as there was no significant relation with total Mg + Fe. Although, Mg + Fe indicates the trioctahedral nature of the phyllosilicates but probably, the large quartz matrix has masked the effect of nature of mica.

INTRODUCTION

Potassium (K) deficiency to growing crops has been observed as a limiting factor to economical crop production on many soils of Pakistan (Rashid et al., 1992). Nonetheless, crops grown on some light textured soils with rather low soil-test K value

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National Agricultural Research Center, Islamabad 45500

do not respond to K application. Sandy, coarse textured soils sometimes do not show K deficiency. Mica and interstratified mica in the silt and clay fractions has strong correlation with exchangeable K and possibly K released replenishes the soil solution K (Hons et al., 1976). Considering K-uptake and depletion in NH_4OAc-K with plant growth, it was concluded that 43 to 95% of K-uptake came from nonexchangeable K reserves (Rahmatullah et al., 1992). However, Grimme (1974) opined that even sufficient pool of nonexchangeable K in soil would fall short to meat crop K requirement if its release rate from soil matrix does not keep pace with plant uptake.

Potassium reserves are bound within the soil minerals and their availability to plants is directly related to the nature of the K-bearing minerals. The main groups of K bearing minerals are 2:1-type phyllosilicates and K feldspars. In 2:1 layer silicates SiO_4 tetrahedra are linked, at two planes, to Al_2O_6 octahedra forming a complete layer. In case of isomorphous substitution of Al^{3+} for Si^{4+} in tetrahedral sheet or of Mg^{2+} and Fe^{2+} for Al^{3+} in octahedral sheet, the interlayer cation (K) balances the negative charge. Therefore, the layer silicate can be further classified on the basis of location and type of substituting cations in the structure. In trioctahedral series all three octahedral sites are occupied by divalent cations, Mg^{2+} and Fe^{2+} (biotite) and in dioctahedral series two of the three sites are occupied by the trivalent cations, Al^{3+} (muscovite). In contrast to phyllosilicates, K feldspar are tectosilicates composed of a three dimensional framework of SiO_4 and Al_2O_3 tetrahedra with sufficient opening to accommodate K to maintain electroneutrality.

Potassium release characteristics are controlled by the structural variations. Biotite weathers at a faster rate maintaining higher K concentration in the equilibrium solution than muscovite (Martland, 1958; Reed and Scot, 1962; Feigenbaum et al., 1981). The multifold difference in the rate of K release between the di- and trioctahedral mica has been attributed to the hydroxyl (OH) orientation in the octahedral sheet (Basset, 1960). The present study was conducted to determine the relation of mineralogical composition, particularly nature of sand and silt mica, with K release in boiling nitric acid treatment of the respective size fraction.

MATERIALS AND METHODS

Description of the Soil

Seven different soil series representing a range of parent material and profile development were included in the study (Table 1). The Biha soil series is developed in alluvium derived from granite, granodiorites, diorites, norites and some phyllite. Shahi soil is developed in mixed alluvium derived from granite, phyllite, and redeposited loess. The Ushu soil series is developed in alluvium derived from phyllites, micaceous quartzite, and siliceous schists. Mali soil series is derived from granites, granodiorites, and quartz diorites. The soils represent a few of the acidic soils in Pakistan and they occur in subhumid boreal climate.

The Kotly is developed in mixed mineralogy alluvium from the Himalayas derived by the Indus River system. The Jhatpat is developed on piedmont alluvium from Murri-Bughti hills. The soil occur from arid to subhumid climate. The Murree soil is developed from calcareous shale interbeded with sandstone. The Kotly soil is irrigated. The irrigation water brings in unweathered mineral matter.

Biha series has deep (> 1 m thick) non calcareous, moderately well to imperfectly drained, very dark grayish brown mottled loam. It has mollic epipedon, cambic B horizon, 1 to 3% organic matter, and pH ranging from 5.2 to 6.7. Shahi is deep, non-calcareous, well drained, very dark grey, silt loam underlain by yellowish brown silty clay loam with common distinct mottles. It has mollic epipedon and cambic B horizon. Its organic matter upto 60 cm depth ranges from 5 to 9% and pH from 5.6 to 6.2.

Ushu is moderately deep non calcareous, well drained, dark yellowish brown to dark brown, silt loam to silty clay loam, with a mollic epipedon, having organic matter from 1 to 3% and pH from 4.8 to 6.8. The soils occur in subhumid temperate and boreal climate. Mali series occupies 10 to 25% slopes. It is moderately deep, non-calcareous, well drained grayish brown, gravelly loam/sandy loam with a mollic epipedon.

The Kotly soil series occurs in semi-arid to subhumid climate and occupies <1% slope. It is deep non-calcareous, moderately well drained, grayish brown with ochric epipedon and cambic B horizon. Its organic matter content is about 1.1% and pH varies from 7.5 to 7.7.

Soil sampling and fractionation

Bulk soil samples were taken from each genetic horizon, air dried, crushed with a wooden roller, and passed through a 2 mm screen. A 20 g portion of each sample was treated to remove carbonate with 1 N NaOAc buffered to pH 5.0, organic matter with 30% H_2O_2, and iron oxide with citrate-bicarbonate-dithionite (CBD) buffered at pH 7.3 (Jackson, 1979). Treated samples were dispersed in a pH 10 Na_2CO_3 solution by a 15 second sonification treatment with a macrotip. From the soil suspension, sand (2-0.05 mm) was separated by wet sieving through a 0.051 mm nominal pore-size screen and silt (50-2 µm) by five repeated 15 second sonification and centrifugation washing. The remaining total clay (<2 µm) was further fractionated into coarse (2-0.2 µm) and fine (<0.2 µm) clay fractions by similar five-repeated sonification-centrifugation treatments. The sand and silt were oven dried at 105 °C. The coarse and fine clays were made salt free by dialysis and were freeze dried. Particle size distribution was determined on oven dried soil basis.

Mineralogical and Chemical Analyses

For mineralogical analysis each sand and silt fraction was compressed into a wooden holder for randomly oriented X-ray diffraction powder mounts. The sand was ground in acetone before making the powder mounts. Each sample was X-radiated at a

scanning speed of 1° 2Θ per minute with monochromatic Cu K-α radiation.

For total chemical analysis, the sand and silt fractions were digested in HF and aqua regia in pressure bombs and diluted to 100 mL with saturated boric acid solution, and assayed by an atomic absorption. Potassium in the extract was assayed by a flame photometer. Mica percentage in the fractions was calculated assuming 10% K_2O in mica crystals (Jackson, 1979) and assuming that all the K released came from mica.

Microprobe Analysis

For microprobe analysis, 2 soils were selected based on their K release characteristics. The Biha represented acid soils and Kotly weathered alluvial irrigated soils. The sand and silt grains in the upper 3 horizons were mounted on aluminum stubs with carbon tape, and sputter coated with 200 to 300 A gold using Hummer-IV Sputter System run at 10 mA for 2 to 3 min. Microprobe analyses of the sand and silt were done with a JEOL-6400 scanning electron microscope (SEM) equipped with a microprobe at a working distance of 39 at 25 kV accelerating voltage. In each case about 100 grains of 50 to 100 μm sand and 10 to 20 μm size silt of random morphology were analyzed by making 4 to 5 transect over the stubs.

Potassium Release Characteristics

Boiling nitric acid extractable K in the sand and silt was determined by boiling 1 g sample in 25 mL 1N HNO_3 for 10 minutes (Knudsen et al., 1982). Potassium was assayed by flame photometer.

Soil Texture and Degree of Profile Development

The seven soils, the Biha, Mali, and Murree series are light in texture as indicated by their abundant sand fraction. Sand in the Biha soil varied from 45 to 62%, in the Mali 30 to 47%, and in Murree 20 to 55%, except for Cr horizon of Mali where sand fraction was 93%. Silt fraction varied from 27 to 40% in Biha, 37 to 48% in Malli and 40 to 48% in Murree (Table 2). The Ushu, Shahi, Kotly, and Jhatpat series were relatively heavy soils. In these soils, sand varied from 1 to 25%. The silt fraction in Shahi and Ushu varied from 56 to 72%, and in Kotly and Jhatpat 23 to 52% (Table 2). Therefore, the Biha, Mali, and Murree series were clay loam to silt loam and the Ushu, Shahi, and Kotly series were silty clay in texture. The Jhatpat series was a clayey.

The differences in particle size distribution are mainly due to source of parent material. The Biha and Mali soils are developed in colluvium and residuum derived from granite, granodiorite, and quartzdiorite while Ushu is developed in alluvium derived from phyllites, micaceous quartzite, and siliceous schists and Shahi is developed in mixed alluvium derived from granite, phyllite and redeposited loess. The Jhatpat is developed on piedmont alluvium mainly derived from shales and limestone. Therefore, the Ushu Shahi, and Jhatpat soil series have higher clay content than the Biha and Mali series.

Murree is developed in shale interbeded within sandstone, therefore, the sand had been incorporated in the soil matrix.

Sand/silt ratio in the Shahi and Ushu series do not change significantly with profile depth but the change was recognized in the remaining four soils. It is suggested that at respective sites both the soils were developed in a uniform parent material. On the other hand, the Biha and Mali series showed lithologic discontinuity in their profile as indicated by sudden changes in sand/silt ratio. In the Kotly soil there is a textural break at the depth of 132 cm. In the upper part of the profile (0-132 cm) sand varied from 2-3% and in the lower part it is 14 to 24%.

Sand and Silt Mineralogy

Sand fraction from the acidic soil was composed of mainly quartz and feldspar with some mica and chlorite. Selected data X-ray diffraction data is depicted is Figure 1. Mica and feldspar contents in the silt fraction of Biha and Mali soil series slightly increased with depth. Quartz and feldspar peaks were sharp and symmetric while chlorite and mica peaks were relatively wide, suggesting low crystallinity in the later case. Chlorite peak in Mali soil was more intense than the Biha soil. The sand fraction of Kotly, is mainly composed of quartz and feldspar (Fig. 1a). Mica percentage in sand as determined by the total K analysis varied from 6 to 14% in the Kotly and 5 to 20% in Murree (Table 4). Sand size mica in Murree and Kotly increased toward the surface.

Silt fraction was also composed of mainly quart, mica, and feldspar. Mica peak intensity in the silt was higher than the corresponding sand (Fig. 1b). In the X-ray diffractogram of Biha and Malli soils the second order peak was not observed suggesting the trioctahedral nature of the mineral (Moore and Reynold, 1989). As determined by the total K analysis, mica in silt fraction varied from 16 to 24% in Kotly and 7 to 23% in Murree (Table 4). In Murree mica in silt increased with depth but in Kotly it remained unchanged. In the Mali profile silt size mica varied from 13 to 19% and decreased with depth.

Microprobe analysis of the sand from upper three horizons of the two soils suggested that the Biha soil contained the higher amount of biotite than Kotly. The mineral in the sand fraction varied from 3 to 12% in the Biha and 0 to 2% in Kotly (Table 3). There was no biotite in the upper 40 cm of the Kotly profile sand. The Biha soil had no muscovite except about 1% in Bw horizon. In the Biha profile K feldspar varied from 7 to 14 % in sand. The concentration of intermediatory (Ca-Na) feldspar was higher in the Biha soil than the Kotly. In the profile it varied from 30 to 47% with increasing trend toward the surface and replacing quartz. In the Kotly it varied from 5 to 15% increasing with depth.

Microprobe analysis of silt fraction suggested that the Kotly profile contained the higher biotite and muscovite than the Biha. Biotite ranged from 11 to 29% with

increasing trend toward the surface (Table 3). Muscovite was also higher in Kotly than Biha soil. Silt size K-feldspar varied from 8.8 to 20% in Kotly and 7.5 to 11.6% in Biha profile. Again, as in case of sand, silt from the Biha profile contained the higher intermediatory (Ca-Na) feldspar.

Boiling Nitric Acid Extractable Potassium

The soil derived from granite and granodiorite contained higher boiling nitric acid extractable K than the highly weathered alluvial soil (Table 4). In the Shahi HNO_3 K varied from 1340 to 2900 mg/kg in the sand and 1670 to 1990 mg/kg in the silt. In the Biha soil, K content varied from 1200 to 1840 in the sand fraction and 1470 to 1740 mg/kg in the silt fraction. In the Ushu soil, K content ranges from 1090 to 1720 in the sand and 1480 to 1740 mg/kg in the silt fraction. Sand from the Malli soil released 1160 to 1780 mg/kg HNO_3 extractable K and silt 1280 to 2350 mg/kg K. In the Biha and Shahi soils boiling nitric acid extractable K in sand generally increase with profile depth while there was no consistent trend in case of silt from the same soils.

The Kotly sand released the lowest K, it ranged from 110 to 900 mg/kg with increasing trend with depth in the upper 4 horizons. The silt fraction in the Kotly and Murree soils yielded 4 to 5 times higher K than the corresponding sand fraction while reverse is true in case of Jhatpat.

Boiling nitric extractable K was strongly correlated ($p < 0.01$) with the biotite content of the soils. The conclusion was based on only sand and silt fractions taken from the soils of varied parent material and profile development. There was no significant correlation between K feldspar content and HNO_3 extractable K. The correlation between HNO_3 K and muscovite content was significant only in case of alluvial soils (R 0.49, $p < 0.04$). Determining the correlation by adding biotite and muscovite or biotite, muscovite, and feldspar did not improve the correlation coefficient. Also, boiling nitric acid extractable K was positively correlated with total Al content of the sand fraction (R 0.46 $p < 0.01$) but not with total Mg + Fe.

DISCUSSION

The differences in texture may be correlated with soil parent material. The Biha and Mali soils are developed in colluvium and residuum derived from granite, and granodiorite, and quartzdiorite while Ushu is developed in alluvium derived from phyllites and siliceous schists and Shahi is developed in mixed alluvium derived from granite, phyllite and redeposited loess. Therefore, the Ushu and Shahi soil series have higher clay content than the Biha and Mali series. The Jhatpat is developed on subrecent piedmont alluvium derived from Tertiary sandstone, shale, and limestone of Murri-Bughti hills. In this case clayey sediments have been sorted due to slow moving torrents (Soil Survey of Pakistan, 1972). The Murree soil is developed from calcareous shale interbeded with sandstone.

In profile development studies consistency in sand/silt ratio with profile depth is a measure of parent material uniformity. A lithologic discontinuity in the parent material may be recognized when change in sand/silt ratio between the adjacent horizon exceeds 0.08 (Smeck and Wilding, 1980 and Carty et al., 1988). Sand/silt ratio in the Malli soil changed significantly with profile depth. It is suggested that the Malli, Ushu, and Shahi soils are developed in a uniform parent material. In the Kotly soil there is a textural break at the depth of 132 cm.

Mineralogical composition of sand and silt fractions varied with source of parent material and profile weathering stage. The sand fraction of the Kotly was mainly composed of quartz and feldspar is caused by high weathering due to pluvial conditions. Silt fractions was composed of mainly quart, mica, and feldspar. Mica peak intensity in the silt was stronger than corresponding sand indicating dispersion of sand size mica into silt fraction. Total chemical analysis suggested 6 to 14% mica in the sand fraction while XRD did not show the presence of mica. This discrepancy can be explained by (1) in randomly oriented mounts mica has to be >10% to get a detectable XRD peak and (2) mica percentage in the sample was calculated assuming no feldspar was present which is not a valid assumption for these samples.

There was no biotite in the upper 40 cm of the Kotly profile sand which also indicate greater weathering. Biha soil had no muscovite except about 1% in Bw horizon which is due to the nature of the granitic rock. Potassium feldspar in sand was higher in Biha profile than Kotly. But Kotly silt from surface horizon had greater K feldspar where biotite was greater. It may point towards the fact that the irrigation water brings in fresh mineral matter. The concentration of intermediatory (Ca-Na) feldspar in sand and silt was higher in the Biha soil which increased toward the surface and replacing quartz. It is believed that the granite on which the Biha is developed has intermediatory (Ca-Na) feldspar matrix (Martin et al., 1962).

The acid soils have higher hot nitric acid extractable K than the alluvial or loess soils. In the Biha and Shahi soils hot nitric acid extractable K in sand generally increased with profile depth while there was no depth trend in the Ushu and Malli soil which nearly corresponded with lithological discontinuity. In the alluvial soils K extracted followed the degree of profile development. The Kotly sand released the lowest K. The silt fraction in the alluvial soils yielded 4 to 5 times higher K than the corresponding sand fractions. The trend mineralogical composition of sand and silt fractions, particularly the concentration of biotite, matches with K extracted with hot nitric acid from the respective size fraction.

Boiling nitric extractable K was strongly correlated with the biotite content, correlation with muscovite content was significant only in case of alluvial soils, while there was no significant correlation with K feldspar content. Determining the correlation by adding biotite and muscovite or biotite, muscovite, and feldspar did not improve the correlation coefficient. Also, boiling nitric acid extractable K was positively correlated with total Al content of the sand fraction where as there was significant relation with

total Mg + Fe. The conclusions were based on only sand and silt fractions taken from the soils from varied parent material and profile development. Although, Mg + Fe indicates the trioctahedral nature of the phyllosilicates but probably, the large quartz matrix has masked the effect nature of mica. Probably, the sandy matrix has masked the effect of nature of mica.

REFERENCES:

Ahmad, M., Ryan, J. and Paeth, R.C., 1977. Soil development as function of time in the Punjab River plains of Pakistan. Soil Sci. Soc. Am. J. 41:1162-1186.

Basset, W.A. 1960. Role of hydroxy orientation in alteration of mica. Bull. Geo. Soc. Am. 71;449-456.

Carty, D.J., J.B. Dixon, L.P. Wilding, and F.T. Turner. 1988. Characterization of a pimple mound-intermound soil complex in the gulf coast prairie region of Texas. Soil Sci. Soc. Am. J. 52:1715-1721.

Feigenbaum, S., R. Edelstein, and I. Shainberg. 1981. Release rate of potassium and structural cations from mica to ion exchangers in dilute solutions. Soil Sci Soc. Am. J. 45:501-506.

Grimme, H. 1974. Potassium release in relation to crop production. pp.131-136. In Proceedings 10th Cong.IPI held in Budapest.

Hons, F.M , J.B. Dixon, and J.E. Matocha. 1976. Potassium sources and availability in a deep, sandy soil of East Texas. Soil Sci. Soc. Am. J. 40:370-373.

Jackson, M.L., 1979. Soil chemical analysis-advanced course. 2nd ed. Dept. Soil Sci., Univ. Wisconsin, Madison, Wisconsin 53706.

Knudsen, D., G.A. Peterson, and P.F. Pratt. 1982. Lithium, sodium, and potassium.p.237. *In* L.A. Page (ed.) Methods of soil analysis, part 2, Chemical and microbiological properties, 2nd ed. Soil Sci. Soc. Am. Madison, WS 53711. USA.

Martin, N.R., S.F.A. Siddiqui, and B.H. King. 1962. A geological reconnaissance of the region between the lower Swat and Indus river of Pakistan. The Geol. Bull. Punjab University 2:1-15.

Martland, M.M. 1958. Kinetics of potassium release from biotite. Soil Sci. Soc. Am. Proc. 22:503-508.

Moore, D.M. and R.C. Reynolds, Jr. 1989. X-ray diffraction and the identification and analysis of clay minerals. Oxford Uni. Press. Oxford.

Rashid, M., M.I.Bajwa and A. A. Shah 1990. Wheat responses to potash application in some selected areas of Pakistan.In Proceedings of 3rd national Cong.of Soil Sci. pp.131 to 139.

Rahmatullah, M. Salim and B. Z. Sheikh.1990. Release kinetics of nonexchangeable potassium assessed by continuous cropping and chemical extraction in some alkaline calcareous soils. In Proceeding of 3rd National Cong. of Soil Sci. pp.232 to 245.

Reed, M.G. and A.D. Scott. 1962. Kinetics of potassium release from biotite and muscovite in sodium tetraphenylboron solutions. Soil Soc. Am. Proc. 26:437-440.

Smeck, N.E. and L.P. Wilding. 1980. Quantitative evaluation of pedon formation in calcareous glacial deposits in Ohio. Geoderma 24:1-16.

Soil Survey of Pakistan. 1972. Reconnaissance Soil Survey of Jacobabad-Usta Mohammed. Report No 145. Soil Survey of Pakistan. Multan Road, Lahore.

Soil Survey Staff. 1987. Keys to soil taxonomy. SMSS Technical Monograph #6. Cornell University. Ithaca, NY 14853. USA.

Table 1. Classification and location of the soils.

Soil series	Classification*	Location
Ushu	Typic Hapludoll	1 km north west of Kalam, district Swat, NWFP
Biha	Mollic Haplaquept	Thul valley, distric Dir, NWFP
Shahi	Mollic Haplaquept	Shahi valley, district Dir, NWFP
Mali	Typic Ustochrept	North of Pluga village, district Swat, NWFP
Murree	Typic Hapludoll	Jeeka Gali, Murree, Punjab
Jhatpat	Entic Chromustert	Dera Murad Jamali, Baluchistan
Kotly	Entic Chromustert	Daska road Gujranwala, Punjab

* US Soil classification (Soil Survey Staff, 1987).

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Table 2. Particle size distribution in soils.

HORZN	Depth	Sand	Silt	CClay	FClay	SN/SL	CC/FC
	(cm)	----------(%)----------					
Biha, Mollic Haplaquept							
Ap	0-12	55.4	33.6	6.0	1.7	1.65	3.47
Bh	12-30	61.9	26.9	5.8	1.7	2.30	3.39
Bw	30-46	53.7	35.3	6.0	2.1	1.52	2.79
Bg	46-60	44.9	39.7	8.4	3.8	1.13	2.21
Cr	60-73	45.7	38.4	8.4	5.4	1.19	1.55
Shahi, Mollic Haplaquept							
A1B1	0-15	8.8	69.1	15.2	7.3	0.13	2.08
Bw	15-30	.	.	.	.	.	.
Bg1	30-45	5.8	55.6	17.3	8.4	0.10	2.05
Bg2	45-60	2.2	59.3	12.4	9.9	0.04	1.25
BC	60-75	5.9	55.5	18.0	11.2	0.11	1.61
Ushu, Typic Hapludoll							
Ap	0-15	7.2	71.0	11.3	2.5	0.10	4.44
Bw	15-30	6.7	72.2	9.8	5.3	0.09	1.87
B2	30-60	7.7	69.5	11.2	5.8	0.11	1.94
B22	60-100	4.6	72.6	12.5	7.2	0.06	1.74
B2b	100-115	5.7	69.1	13.5	10.4	0.08	1.30
B2b	115-120	4.7	64.6	15.4	10.9	0.07	1.41
Mali, Typic Ustchrept							
Ap	0-12	47.4	36.6	6.6	0.7	1.30	9.92
Bh	12-30	30.5	47.6	8.8	5.6	0.64	1.57
Bg	30-45	38.1	46.3	8.3	5.5	0.82	1.50
Bg	45-60	29.9	48.0	11.5	5.0	0.62	2.29
Cr	60-70	93.6	4.0	1.1	0.3	23.27	3.70
Murree, Typic Hapludoll							
Ae	0-11	26.6	39.2	12.1	8.4	0.68	1.45
Bt1	11-32	25.1	40.4	13.5	6.9	0.62	1.97
Bt2	32-54	26.3	44.6	15.4	12.5	0.59	1.23
2Bt3	54-74	19.7	44.4	18.2	9.0	0.44	2.02
2Bt4	74-92	21.8	44.9	10.3	4.7	0.49	2.20
3Btk	92-113	33.1	46.3	12.4	7.5	0.71	1.67
4BC	113-130	54.4	32.5	7.0	4.5	1.68	1.56
5BC	130-142	17.6	40.6	32.7	9.1	0.43	3.60
5Cr	142-160	10.5	22.9	41.0	11.4	0.46	3.61
Jhatpat, Entic Chromustert							
Ap	0-8	1.1	25.9	26.0	16.2	0.04	1.61
Bw	8-17	2.1	26.0	27.3	22.1	0.08	1.24
Bk1	17-46	1.1	25.3	28.5	23.7	0.04	1.20
Bk2	46-66	1.7	22.3	22.9	20.6	0.08	1.11
Bk3	66-86	2.2	32.3	23.1	26.0	0.07	0.89
Bk4	86-104	5.5	24.1	25.9	14.2	0.23	1.83
BCk	104-125	2.3	37.8	21.8	7.2	0.06	3.03
Ck	125-+	4.8	49.6	14.2	4.7	0.10	3.01
Kotly, Entic Chromustert							
Ap	0-12	3.7	50.0	18.5	22.0	0.07	0.84
Bwc1	12-42	3.8	43.0	18.5	24.6	0.09	0.75
Bwc2	42-75	2.6	51.7	13.5	24.4	0.05	0.55
Bwc3	75-132	2.6	47.3	20.7	24.0	0.06	0.86
BCk2	132-150	14.9	34.7	12.4	.	0.43	.
BCk1	150-178	24.8	42.0	9.6	6.8	0.59	1.41

Legend: HORZN, soil horizon; Sand, sand (2 to 0.05 mm); Silt, silt (50 to 2 μm); Cclay, coarse clay (2 to 0.2 μm); Fclay, fine clay (<0.2 μm); SN/SL, sand silt ratio; and CC/FC, coarse clay fine clay ratio.

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Table 3. Distribution of different mineral phases in the sand and silt as determined by SEM microprobe analysis.

Horzn	Depth (cm)	Qurtz	Chlrt	Biott	Musc	Kfld	Cafd	Nafl	Intd	Tcnt no
		(%)								
Kotly, Entic Chromustert, sand										
Ap	0-12	75.6	0.0	0.0	7.3	9.8	2.4	0.0	4.9	41.0
Bwc1	12-42	77.5	2.8	0.0	1.4	7.0	2.8	0.0	8.5	71.0
Bwc1	42-75	63.0	0.0	1.9	1.9	14.8	1.9	1.9	14.8	54.0
Kotly, Entic Chromustert, silt										
Ap	0-12	40.0	0.0	0.0	25.0	20.0	5.0	5.0	5.0	20.0
Bwc1	12-42	34.1	11.0	9.9	22.0	8.8	0.0	8.8	5.5	91.0
Bwc1	42-75	32.7	5.0	12.9	21.8	13.9	2.0	2.0	9.9	101.0
Biha, Mollic Haplaquept, sand										
Ap	0-12	26.2	1.9	2.9	0.0	10.7	10.7	1.0	46.6	103.0
BA	12-30	15.0	1.9	2.8	0.0	10.3	26.2	1.9	42.1	107.0
Bw	30-46	21.2	5.3	11.5	0.9	14.2	13.3	3.5	30.1	113.0
Biha, Mollic Haplaquept, silt										
Ap	0-12	33.0	7.8	2.9	1.0	10.7	2.9	2.9	38.8	103.0
BA	12-30	26.1	8.1	0.0	5.4	9.9	6.3	12.6	31.5	111.0
Bw	30-46	27.1	4.7	8.4	4.7	7.5	5.6	8.4	33.6	107.0

Legend: HNO3-K, K extracted in 1N boiling HNO3 acid; Qurt, quatrz; Chlr, chlorite; Bott, biotite; Musc, muscovite; Kfld, K feldspar; Cafl, Ca feldspar; Nafl, Na feldspar; Intd, mostly Ca-Na intermediatory feldspar with some unrecognizable minerals; TCnt, total number of grains counted.

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Table 4. Total elemental analysis and boiling HNO3 extractable K in the sand and silt fractions.

Horizn	Depth	Total Al		Total Fe+Mg		Hot HNO3-K		Total K	
		Sand	Silt	Sand	Silt	Sand	Silt	Sand	Silt
	(cm)	---------(%)----------				---mg/kg--		-----%-----	
Biha, Mollic Haplaquept									
Ap	0-12	.	.	.	.	1198	1736	.	.
B1	12-30	6.8	.	2.1	.	1493	1629	1.9	.
B21	30-46	8.7	5.2	3.9	6.2	1577	1712	3.1	1.4
B22	46-60	9.0	5.3	3.5	5.8	1840	1736	1.7	1.4
B3	60-73	5.4	8.7	4.5	11.5	1752	1477	1.5	1.7
Shahi, Mollic Haplaquept									
A1B1	0-15	7.6	0.0	6.0	5.7	1634	1696	1.2	1.1
B21	15-30	8.3	21.5	9.2	5.2	1340	1989	1.8	1.7
B21	30-45	6.4	20.9	12.3	13.2	1407	1858	1.1	1.6
B22	45-60	6.9	21.7	.	4.2	2517	1791	.	1.1
BC	60-75	25.1	21.9	12.6	9.0	1980	1672	0.9	1.1
C1	75-90	.	23.1	.	4.6	2897	1776	.	1.4
Ushu, Typic Hapludoll									
Ap	0-15	9.0	27.4	8.8	4.9	1340	1734	1.4	1.6
BA	15-30	7.4	24.3	7.5	4.9	1339	1480	1.5	1.9
B2	30-60	8.0	28.8	8.0	4.2	1237	1515	1.5	1.6
B22	60-100	.	25.9	.	4.7	1714	1588	.	1.6
B2b	100-115	8.2	23.5	3.8	5.0	1600	1639	1.2	2.3
B2b	115-120	0.0	22.8	.	3.8	1190	1576	.	1.3
Mali, Typic Ustchrept									
Ap	0-12	7.2	22.8	4.1	5.4	1658	1513	2.3	1.6
B2	12-30	9.5	24.0	6.9	5.4	1628	1413	0.7	1.4
B2	30-45	12.1	6.4	7.8	4.4	1774	1758	0.3	1.4
C1	45-60	7.1	20.9	7.2	4.7	1217	1279	0.3	1.3
C2	60-70	6.2	24.4	2.4	4.6	1166	2349	1.9	1.2
Murree, Typic Hapludoll									
Ae	0-11	2.1	2.8	1.1	3.6	178	214	1.8	0.6
Bt1	11-32	1.9	3.9	1.5	3.1	126	311	1.3	0.7
Bt2	32-54	1.4	2.7	1.2	3.5	134	233	0.4	0.7
2Bt3	54-74	1.6	6.4	1.4	3.7	151	324	0.6	1.5
2Bt4	74-92	1.8	7.5	2.3	3.5	139	272	0.5	1.7
3Btk	92-113	1.9	9.9	1.4	4.2	139	272	1.1	1.9
4BC	113-130	1.4	6.4	1.5	3.1	127	362	0.6	1.5
5BC	130-142	2.8	7.8	1.6	4.2	154	511	0.4	1.8
5Cr	142-160	2.0	7.0	2.4	4.5	239	520	0.7	1.8
Kotly, Entic Chromustert									
Ap	0-12	5.8	7.7	1.8	3.1	594	1546	1.2	2.0
Bwc1	12-42	5.7	7.2	3.1	2.9	782	1523	1.0	1.9
Bwc2	42-75	5.4	7.6	5.1	2.5	794	1669	1.1	1.8
Bwc3	75-132	5.5	8.5	6.4	3.4	900	1561	0.7	2.0
BCk1	132-150	3.4	7.3	6.3	4.0	264	1066	0.5	1.5
BCk2	150-178	5.3	7.6	5.6	4.0	110	919	0.8	1.3
Jhatpat, Entic Chromustert									
Ap	0-8		6.9		4.4	1478	455	.	1.7
Bw	8-17		8.4		4.9	2000	549	.	1.5
Bk1	17-46		5.8		3.4	1882	476	.	1.2
Bk2	46-66		6.9		4.2	2750	455	.	1.5
Bk3	66-86		6.8		4.5	2308	283	.	1.6
Bk4	86-104		6.2		4.2	1553	782	.	1.3
BCk	104-125		6.8		4.4	1931	330	.	1.4
Ck	125-		6.0		4.3	455	171	.	1.4

*, Total chemical analysis by digestion in aqua regia and HF in pressure vessel (Jackson, 1979) and #, Boiling nitric acid (Knudsen et al., 1982).

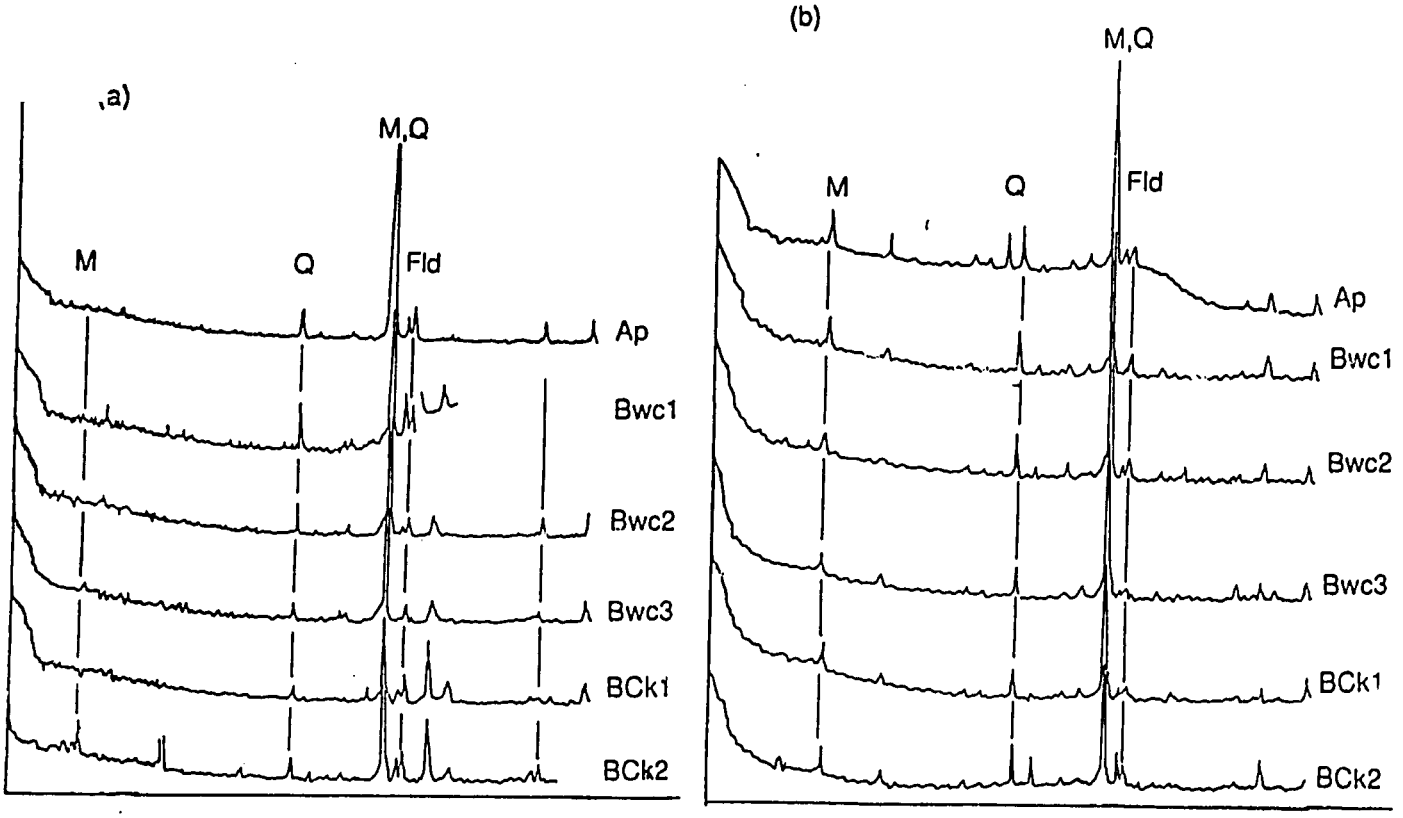

Fig. 1. X-ray diffraction pattern of the Kotly profile: (a) sand (2 to 0.05 mm) and (b) silt (50 to 2 μm). M, mica; Q, quartz; M,Q, mica and quartz; Fld, feldspar.

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Potassium Release Characteristics of Sand and Silt in Relation to Mineralogy: Selected Alfisols and Aridisols

M.S. Akhtar, M. Arshad, and Z.I. Awan

ABSTRACT

Six soil series representing moderately weathered soils of Pakistan were investigated to determine their nonexchangeable potassium (K) pool in sand and silt fraction in relation to mineralogy. The Guliana and Satgra were silt clay and Peshawar and Pacca were silt loam, Wazirabad sandy loam, and Balkassar was loam. Parent material discontinuity as indicated by the variation in sand/silt ratio was recognized in Satgra after 127 cm, in Peshawar after 1 m, and in Pacca after 43 cm. In the Wazirabad and Balkassar sand/silt ratio changed almost with each horizon while in the Guliana sand/silt ratio remained consistent with profile depth.

The sand fractions of the soils were composed of mainly quartz and feldspar with some mica and chlorite. The sand fraction of the Guliana soil contained progressively higher mica content toward lower horizons. This depth trend was not found in alluvial soils. The sand fraction of the Satgra, Wazirabad, and Balkassar was mainly composed of quartz and feldspar. Silt fractions was also composed of mainly quart, mica, and feldspar. Mica in the silt was higher than the corresponding sand. The Satgra silt had mostly dioctahedral mica.

Biotite in sand fraction of Guliana varied from 4 to 6% and in silt 1 to 4%. In both the fractions biotite decreased with profile depth. Muscovite in sand varied from 7 to 10% and in silt 9 to 12% while K-feldspar varied from 6 to 11% in sand and 11 to 12% in silt.

The Pacca sand released the highest K in boiling HNO_3. Boiling nitric acid extractable K from sand ranged from 3070 to 6160 mg/kg in Pacca, 300 to 1360 in Satgra, 300 to 760 in Wazirabad. In the residual soils, the highest K was released by Guliana followed by Peshawar and Balkassar sand. The silt fraction in the Satgra and Wazirabad soils yielded 2 to 5 times higher K than the corresponding sand fraction while in the Pacca soil silt yielded lesser K than the corresponding sand. Potassium released by Guliana sand varied from 1020 to 2130 mg/kg, by Peshawar sand 740 to 1460, and by Balkassar sand 130 to 250 mg/kg. In the Guliana soil boiling nitric acid extractable K varied from 1100 to 1960 mg/kg in the silt fraction. In the Guliana, Balkassar, and Peshawar soils HNO_3 extractable K from sand and silt increased toward the surface as well as to the lower depth.

Boiling nitric acid extractable K was strongly correlated ($p < 0.01$) with the biotite content of the soils. There was no significant correlation between K feldspar content and HNO_3 extractable K. Boiling nitric acid extractable K was positively correlated with total Al content of the sand fraction (R 0.46 $p < 0.01$).

INTRODUCTION

Potassium (K) deficiency to growing crops has been observed as a limiting factor to economical crop production on many soils of Pakistan (Nabhan et al., 1989). However, relationship between soil "available K" and crop response has never been consistent. Crops grown on some light textured soil with rather low soil-test K value do not

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National Agricultural Research Center, Islamabad 45500.

respond to K application vis-a-vis crops on some clay soils with rather high soil-test K faces severe K deficiency. Potassium in soil exists in immediately available and nonexchangeable forms. The nonexchangeable form comprises of fixed and mineral K (Sparks and Huang, 1985).

The two groups of K bearing minerals are 2:1-type phyllosilicates and K feldspars. In 2:1 layer silicates, SiO_4 tetrahedra are linked, at two planes, to Al_2O_6 octahedra forming a complete layer. In case of isomorphous substitution of Al^{3+} for Si^{4+} in tetrahedral sheet or of Mg^{2+} and Fe^{2+} for Al^{3+} in octahedral sheet, the interlayer cation (K) balances the negative charge. This substitution basis for further classification of layer silicates. In trioctahedral series all three octahedral sites are occupied by divalent cations, Mg and Fe^{2+} (biotite) and in dioctahedral series two of the three sites are occupied by the trivalent cations, Al^{3+} (muscovite). In contrast to phyllosilicates, K feldspars are tectosilicates composed of a three dimensional framework of SiO_4 and Al_2O_3 tetrahedra with sufficient opening to accommodate K to maintain electroneutrality.

Mineral K which is major portion of the total K can become available slowly through weathering process but K release is controlled by the structural variations. Release of interlayer K from a trioctahedral mica is 7.5 to 25 times faster than from the dioctahedral mica (Feigenbaum et al., 1981). The multifold difference in the rate of K release between the di- and trioctahedral mica has been attributed to the hydroxyl (OH) orientation in the octahedral sheet (Basset, 1960). Biotite weathers not only at a faster rate but also maintains higher K concentration in the equilibrium solution than muscovite.

Some soils contain weatherable minerals particularly biotite (Akhtar et al., 1989). A trioctahedral mica can maintain solution K^+ upto 1.0 mmole L^{-1} (Rausell-Colon et al., 1965) therefore, a soil containing biotite can replenish the soil solution K causing no response to fertilizer K (Hons et al., 1976). The present study was conducted to document nonexchangeable K in sand and silt fractions and to develop relationship between mineralogical composition, particularly nature of mica and boiling nitric acid extractable K in the soils.

MATERIALS AND METHODS

Description of the Soils

Six soil series representing a range of parent material and profile development were included in the study (Table 1). The Satgra, Wazirabad, and Pacca soil series are developed in subrecent alluvium and occur in semi-arid to subhumid climate. The Peshawar soil is developed from calcareous red shales, the Guliana is developed on loess and the Balkassar is developed on sandstone. The Satgra, Wazirabad, and Pacca soils are irrigated. The irrigation water brings in unweathered mineral matter.

The Satgra soil is deep, moderately calcareous, moderately well drained, light brownish gray to dark brown with ochric epipedon. It has organic matter content upto 1.0% and pH ranging from 9.3 to 10.2 and occupies <1% slope. Satgra has natric B horizon. The Wazirabad soil color varies from yellowish brown to dark brown. Soil pH ranges from 7.0 to 7.7. The soil is non-calcareous with argillic B horizon. The Pacca soil is deep, moderately well drained, strongly calcareous, light brownish gray to pale brown. It has ochric epipedon and cambic and calcic B horizon. Soil texture varies from silty clay in the upper 80 cm to silt loam in the lower portion of the profile.

The Guliana is deep, non-calcareous, well drained silt loam. The soil color is yellowish brown to dark brown. It has ochric epipedon and argillic horizon. The Balkassar soil occupy <1% slope, is deep, brown to dark sandy loam. The Balkassar has ochric epipedon and argillic B horizon with paralithic contact at 145 cm. The Peshawar soil occupy <1% slope, is deep, and strongly calcareous. It is brown to light brown silty clay loam with an ochric epipedon and argillic B horizon.

Soil sampling and fractionation

Bulk soil samples were taken from each genetic horizon, air dried, crushed with a wooden roller, and passed through a 2 mm screen. A 20 g portion of each sample was treated to remove carbonate with 1 N NaOAc buffered to pH 5.0, organic matter with 30% H_2O_2, and iron oxide with citrate-bicarbonate-dithionite (CBD) buffered at pH 7.3 (Jackson, 1979). Treated samples were dispersed in a pH 10 Na_2CO_3 solution by a 15 second sonification treatment with a macrotip. From the soil suspension, sand (2-0.05 mm) was separated by wet sieving through a 0.051 mm nominal pore-size screen and silt (50-2 μm) by five repeated 15 second sonification and centrifugation washes. The remaining total clay (<2 μm) was further fractionated into coarse (2-0.2 μm) and fine (<0.2 μm) clay fractions by similar five-repeated sonification-centrifugation treatments. The sand and silt were oven dried at 105 °C. The coarse and fine clays were made salt free by dialysis and were freeze dried.

Mineralogical and Chemical Analyses

For mineralogical analysis each sand and silt fraction was compressed into a wooden holder for randomly oriented X-ray diffraction powder mounts. The sand was ground in acetone before making the powder mounts. Each sample was X-radiated at a scanning speed of 1° 2Θ per minute with monochromatic Cu K-α radiation.

For total chemical analysis, the sand and silt fractions were digested in HF and aqua regia in pressure bombs and diluted to 100 mL with saturated boric acid solution, and assayed by an atomic absorption. Potassium in the extract was assayed by a flame photometer. Mica percentage in the fractions was calculated assuming 10% K_2O in mica crystals and assuming that all the K released came from mica.

Potassium Release Characteristics

Boiling nitric acid extractable K in the sand and silt was determined by boiling 1 g sample in 25 mL 1N HNO_3 for 10 min. Also, 'plant available K' in the soils was determined by extraction in 1N, pH 7 ammonium acetate solution by shaking 5 g soil in 50 mL solution for 30 min (Knudsen et al., 1982). Potasssium was determined by flame photometer.

RESULTS

Soil Texture and Degree of Profile Development

Wazirabad and Balkassar are light textured as indicated by their abundant sand size fraction. In the Wazirabad sand ranged from 69 to 72% and in Balkassar 24 to 61%. Silt content in Wazirabad ranged from 16 to 22% and Balkassar 24 to 43%. The Guliana, Satgra, Peshawar, and Pacca had high silt size fraction while sand varied from only 1 to 8%. Silt in the Satgra soil varied from 42 to 60%, in the Guliana 61 to 68%, in the Peshawar 54 to 62%, and in Pacca 50 to 71%. Therefore, the Guliana, Satgra, Peshawar and Pacca are silty to silt clay loam. The Wazirabad is sandy loam and Balkassar is loam.

A lithologic discontinuity in the parent material may be recognized when change in sand/silt ratio between the adjacent horizon exceeds 0.08 (Smeck and Wilding, 1980 and Cart et al., 1988). Parent material discontinuity as indicated by the variation in sand/silt ratio can be recognized in Satgra after 127 cm, in Peshawar after 1 m, and in Pacca after 43 cm. In the Wazirabad and Balkassar sand/silt ratio changed almost with each horizon while in the Guliana sand/silt ratio remained consistent with profile depth.

Sand and Silt Mineralogy

X-ray diffraction indicated that the sand fractions of the soils were composed of mainly quartz and feldspar with some mica and chlorite (Fig. 1a). The XRD peaks were sharp and symmetric suggesting high crystallinity. The sand fraction of the loess soil contained progressively higher mica content in lower horizons than the upper horizons while no depth trend was found in alluvial soils. The sand fraction of the Satgra, Wazirabad, and Balkassar was mainly composed of quartz and feldspar.

Silt fraction was also composed of mainly quart, mica, and feldspar. Mica peak intensity in the silt had higher than the corresponding sand (Fig. 1b). In the Satgra silt, particularly, second order mica peak as intense as the first order suggested the dioctahedral nature of the mineral (Moore and Reynold, 1989).

Based on total K analysis, the sand from the alluvial soils contained 5 to 18% mica in the Satgra, 13 to 25% in the Wazirabad, and $$$$$% in Pacca. In the residual soils,

Balkassar sand contained 18 to 22% mica and the Guliana sand contained 12 to 23% mica. Silt from the alluvial soils contained 25 to 35% mica in the Satgra, 17 to 24% in the Wazirabad, and $$$$$% in Pacca. Among the residual soils, Balkassar contained 11 to 24%, the Guliana 19 to 23%, and Peshawar contained 26 to 29% mica in silt size fraction.

Microprobe analysis was carried out on sand and silt fraction of the upper three horizons of the Guliana profile (Table 3). The sand fraction contained 3 to 6% and silt contained 1 to 4% biotite. In both the fractions biotite decreased with profile depth. Muscovite in sand varied from 7 to 10% and in silt 9 to 12% while K-feldspar varied from 6 to 11% in sand and 11 to 12% in silt.

In elemental analysis Mg and Fe(II) indicated the trioctahedral nature of the phyllosilicates. In this case total iron was determined by atomic absorption. The Balkassar and Guliana sand had higher Fe(II) + Mg content than the Satgra and Waziranad and in both the soils these elements increased with profile depth. The silt fraction of Satgra and Wazirabad had higher Fe(II) + Mg content than the silt fraction of Balkassar, Guliana, and Peshawar profiles (Table 4).

Potassium Release in Boiling Nitric Acid

Among the alluvial soils the Pacca sand released the highest K in boiling HNO_3 acid. Boiling nitric acid extractable K from sand ranged from 3070 to 6160 mg/kg in Pacca, 300 to 1360 in Satgra, and 300 to 780 mg/kg in Wazirabad. An increasing trend was observed in Wazirabad and Satgra soils. The silt fraction in the Satgra and Wazirabad soils yielded 2 to 5 times higher K than the corresponding sand fraction while in the Pacca soil silt yielded lesser K than the corresponding sand. Among the residual soils, the highest K was released by Guliana followed by Peshawar and Balkassar sand. Potassium released by Guliana sand varied from 1020 to 2130 mg/kg, by Peshawar sand 740 to 1460, and by Balkassar sand 130 to 250 mg/kg. Boiling nitric acid extractable K from sand and silt was similar in residual soils. In the Guliana soil boiling nitric acid extractable K varied from 1100 to 1960 mg/kg in the silt fraction. In the Guliana, Balkassar, and Peshawar soils HNO_3 extractable K from sand and silt increased toward the surface as well as to the lower depth.

Based on 24 sand and silt fractions taken from Kotly, Biha, Guliana, and Hafizabad soils (data presented in companion papers), it was concluded that boiling nitric acid extractable K was strongly correlated ($p < 0.01$) with the biotite content. There was no significant correlation between K feldspar content and HNO_3 extractable K. The correlation between HNO_3 K and muscovite content was significant only in case of alluvial soils (R^2 0.49, $p < 0.04$). Determining the correlation by adding biotite and muscovite or biotite, muscovite, and feldspar did not improve the correlation coefficient. Boiling nitric acid extractable K was positively correlated with total Al content of the sand fraction (R^2 0.46 $p < 0.01$) where as there was significant relation with total Mg + Fe. In the silt fraction none of the two correlation was significant.

DISCUSSION

The differences in texture may be correlated with soil parent material. The Satgra and Pacca soil series are developed in subrecent clay alluvium of subrecent floodplain while Peshawar soil is developed from calcareous shales and the Guliana is developed on loess which had dominance of silt size particles. The Balkassar is developed on sandstone, therefore, it had abundant sand fraction (Ahmad et al., 1986).

In profile development studies consistency in sand/silt ratio with profile depth is a measure of parent material uniformity. Parent material uniformity is prerequisite to draw conclusions on mineral weathering in a soil profile. Sand/silt ratio in the Balkassar and Wazirabad changed frequently with profile depth, suggesting continuous lithologic break. The data suggested that for the major part the Satgra and Guliana are developed in a uniform parent material as textural break occurred only at the depth of 127 and 170 cm, respectively.

Mineralogical composition of sand and silt fractions varied with source of parent material and profile weathering stage. The sand fraction of the loess soil contained higher mica content than the alluvial soils. The sand fraction of Satgra was mainly composed of quartz and feldspar. Depletion of mica may have occurred even before soil formation.

Silt fraction was also composed of mainly quart, mica, and feldspar. Mica peak intensity in the silt was higher than the corresponding sand. In the Satgra silt, particularly, second order mica peak was as intense as the first order suggested the dioctahedral nature of the mineral (Moore and Reynold, 1989).

Among the alluvial soils the Pacca sand released the highest K in boiling HNO_3 which may be attributed to its early weathering stage. The silt fraction in the Satgra and Wazirabad soils yielded 2 to 5 times higher K than the sand from the same horizon while in the Pacca soil silt yielded lesser K than the sand. The difference may be attributed to the source of parent material. Among the residual soils, the highest K was released by Guliana followed by Peshawar and Balkassar sand. The difference between boiling nitric acid extractable K from sand and silt was not very contrasting in case of the residual soils. In the Guliana, Balkassar, and Peshawar soils HNO_3 K from sand and silt increased toward the surface as well as to the lower depth.

Boiling nitric extractable K was strongly correlated with the biotite content of the soils. Release of interlayer K from trioctahedral mica is 7.5 to 25 times than the dioctahedral mica (Feigenbaum et al., 1981). It has been demonstrated that trioctahedral mica (Biotite) can be completely broken down by H-saturated resin in about 10 days releasing all of its K (Arnold, 1958). This might explain higher correlation between biotite content and boiling nitric acid extractable K. There was no significant correlation between K feldspar content and HNO_3 extractable K. Release of K from a tectosilicate

is more difficult than from the interlayers of mica (Sparks and Huang, 1985). The rate of K release from muscovite and biotite was found 2 and 120 to 190 times higher, respectively, than from microcline (Huang et al., 1968). Determining the correlation by adding biotite and muscovite or biotite, muscovite, and feldspar did not improve the correlation coefficient.

REFERENCES:

Ahmad, M., M. Akram, M. S. Baig, M. Y. Javed and Riaz-ul-Amin 1986. Proceedings of XII Int. Forum on Soil Tax. and Agrotecn. Transfer (Pakistan). 2nd vol.pp.66 to 243.

Ahmad, M., Ryan, J. and Paeth, R.C., 1977. Soil development as function of time in the Punjab River plains of Pakistan. Soil Sci. Soc. Am. J. 41:1162-1186.

Akhtar, M.S., Dixon, J. B. and Wilding, L.P., 1989. Pedogenic changes and mineral weathering in three soils from the Indus river plain of Pakistan.p. 161-168. *In* L. A Douglas (ed.) Soil micromorphology: A basic and applied science. Proc. 8th Working Meeting Soil Micromorphology. Elsevier Sci. Publ. Amsterdam.

Arnold, P.W. 1958. Potassium uptake by cation exchange resins from soils and minerals. Nature (London) 182:1594-1595.

Basset, W.A. 1960. Role of hydroxy orientation in alteration of mica. Bull. Geo. Soc. Am. 71;449-456.

Carty, D.J., J.B. Dixon, L.P. Wilding, and F.T. Turner. 1988. Characterization of a pimple mound-intermound soil complex in the gulf coast prairie region of Texas. Soil Sci. Soc. Am. J. 52:1715-1721.

Feigenbaum, S., R. Edelstein, and I. Shainberg. 1981. Release rate of potassium and structural cations from mica to ion exchangers in dilute solutions. Soil Sci Soc. Am. J. 45:501-506.

Huang, P.M., L.S. Crossan, and D.A. Rennie. 1968. Chemical dynamics of K-release of from potassium bearing minerals common soils. Trans. Int. Congr. Soil Sci., 9th 2:705-712.

Hons, F.M., J.B. Dixon, and J.E. Matocha. 1976. Potassium sources and availability in a deep, sandy soil of East Texas. Soil Sci. Soc. Am. J. 40:370-373.

Jackson, M.L., 1979. Soil chemical analysis-advanced course. 2nd ed. Dept. Soil Sci., Univ. Wisconsin, Madison, Wisconsin 53706.

Knudsen, D., G.A. Peterson, and P.F. Pratt. 1982. Lithium, sodium, and potassium.p.237. *In* L.A. Page (ed.) Methods of soil analysis, part 2, Chemical and microbiological properties, 2nd ed. Soil Sci. Soc. Am. Madison, WS 53711. USA.

Moore, D.M. and R.C. Reynolds, Jr. 1989. X-ray diffraction and the identification and analysis of clay minerals. Oxford Uni. Press. Oxford.

Nabhan, H. c. a.De, M. T. Saleem and D. M. Malik 1989. Crop response to potash fertilizer in Pakistan and some Asian countries. Proceedings of The Workshop on the Role of Potassium in Improving Fertilizer Use Efficiency, pp.41 to 69. Rausell-Colon, J.A., T.R. Sweetman, L.B. Wells, and K.

Norrish 1965. Studies in the artificial weathering of mica.p.40. *In* E.G. Hallsworth and D.V. Crawford (ed.) Experimental Pedology. Butterworth, London.

Smeck, N.E. and L.P. Wilding. 1980. Quantitative evaluation of pedon formation in calcareous glacial deposits in Ohio. Geoderma 24:1-16.

Sparks, D.L. and P.M. Huang. 1985. Physical chemistry of soil potassium.p.201-276. *In* Munson, R.D. (ed.) Potassium in Agriculture. Am. Soc. Soil Sci. Madison. Wisconsin. USA.

Soil Survey Staff. 1987. Keys to soil taxonomy. SMSS Technical Monograph #6. Cornell University. Ithaca, NY 14853. USA.

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Table 1. Classification and location of the soils.

Series	Classification*	Location
Satgra	Typic Natrargid	Lahore, Punjab
Guliana	Udic Haplustalf	Rawalpindi, Punjab
Wazirabad	Udic Haplustalf	Wazirabad, Punjab
Balkassar	Udic Haplustalf	Chakwal, Punjab
Peshawar	Udic Ustochrept	Peshawar, NWFP
Pacca	Ustollic Camborthid	Hyderabad, Sindh

* US Soil classification (Soil Survey Staff, 1987).

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Table 2. Particle size distribution in the Alfisols and Aridisols.

HORZN	Depth	Sand	Silt	Cclay	Fclay	SN/SL	CC/FC
	(cm)	(%)					
Satgra, Typic Natrargid							
Ap	0-18	7.0	60.9	15.2	13.1	0.11	1.16
Bt	18-38	3.3	47.5	28.6	13.7	0.07	2.09
Btkn	38-82	3.1	61.2	15.9	8.0	0.05	1.99
Btkn	82-110	3.0	54.7	20.2	11.2	0.06	1.81
Btkn3	110-127	5.6	50.3	13.5	14.6	0.11	0.93
Bck2	127-150	28.3	42.4	14.2	8.3	0.67	1.71
Wazirabad, Udic Halpustalf							
Ap	0-15	63.2	16.9	6.5	5.2	3.74	1.26
Bt	15-33	69.1	16.1	7.4	7.4	4.30	0.99
Bt2	33-63	63.9	17.7	7.6	7.4	3.61	1.03
BC	63-91	58.9	22.1	7.9	6.9	2.67	1.14
C	91-120	66.3	17.8	6.6	6.3	3.71	1.05
Ck1	120-146	66.5	21.3	5.8	6.2	3.13	0.93
Ck2	146-173	67.4	21.3	3.9	4.1	3.17	0.95
C	173-195	71.9	17.8	2.6	2.9	4.05	0.89
Pacca, Ustollic Camborthid							
Ap	0-10	9.4	55.4	12.3	7.0	0.17	1.76
Bw1	10-23	15.4	54.7	10.3	8.6	0.28	1.20
Bw2	23-43	10.9	61.4	10.2	6.4	0.18	1.60
2BA	43-63	6.9	71.1	5.5	3.4	0.10	1.59
2B1	63-80	3.1	52.0	16.7	10.8	0.06	1.50
2Bw1	80-95	1.0	51.4	20.3	18.5	0.02	1.10
2Bt1	95-111	2.2	49.0	18.5	16.6	0.04	1.12
2Bt2	111-120	2.6	50.4	16.2	16.9	0.05	0.96
2Bt3	120-170	2.5	58.4	10.2	8.0	0.04	1.27
2BC4	170+	17.4	62.5	8.6	6.2	0.28	1.38
Balkassar, Udic Haplustalf							
Ap	0-11	61.3	24.5	5.2	6.9	2.50	0.75
Bt1	11-37	41.7	33.2	9.2	12.2	1.26	0.75
Btk	37-70	26.6	39.2	14.1	16.2	0.68	0.87
BCtk	70-100	23.7	40.1	14.1	13.7	0.59	1.02
C1	100-120	29.2	38.1	8.5	6.4	0.77	1.33
C2	120-143	31.7	39.7	7.1	7.7	0.80	0.92
Cr	143-170	30.9	42.9	7.0	5.7	0.72	1.22
Guliana, Udic Haplustalf							
Ap	0-12	8.3	67.8	8.4	11.2	0.12	0.75
BAtc	12-25	6.0	68.2	10.3	12.7	0.09	0.82
Btc1	25-56	4.4	66.7	9.5	13.5	0.07	0.70
Btc2	56-83	3.6	61.2	11.1	17.0	0.06	0.65
Bctc	83-117	4.0	62.7	14.5	16.8	0.06	0.86
Bctk	117-140	6.1	66.5	10.6	14.0	0.09	0.75
Bck	140-190	5.6	61.8	16.4	7.8	0.09	2.11
Peshawar, Udic Ustochrept							
Ap	0-11	2.0	62.3	12.2	7.9	0.03	1.55
Bt1	11-43	2.2	52.5	11.6	16.8	0.04	0.69
Bt2	43-66	3.5	58.1	12.4	10.7	0.06	1.16
2Btb1	66-98	4.5	54.1	11.3	11.2	0.08	1.01
2Btb2	98-138	6.9	54.3	11.6	11.7	0.13	0.99
2Bk2	138-180	1.2	55.4	12.1	12.5	0.02	0.97

Legend: HORZN, soil horizon; Sand, sand (2 to 0.05 mm); Silt, silt (50 to 2 µm); Cclay, coarse clay (2 to 0.2 µm); Fclay, fine clay (<0.2 µm); SN/SL, sand silt ratio; and CC/FC, coarse clay fine clay ratio.

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Table 3. Distribution of different mineral phases in the Guliana profile as determined by SEM microprobe analysis.

Horzn	Depth	Qurt	Chlr	Bott	Musc	Kfld	Cafl	Nafl	Intd	Tcnt
	(cm)	(%)								number
Sand (2 to 0.05 mm)										
Ap	0-12	65.7	1.0	5.9	6.9	10.8	1.0	0.0	0.0	102.0
BAtc	12-25	61.6	2.7	2.7	9.8	8.0	5.4	3.6	0.0	112.0
Btc1	25-56	65.5	1.8	3.6	7.3	5.5	7.3	5.5	0.0	110.0
Silt (50 to 2 µm)										
Ap	0-12	51.8	2.7	3.6	11.6	11.6	4.5	11.6	0.0	112.0
BAtc	12-25	51.8	2.7	3.6	11.6	11.6	4.5	11.6	0.0	112.0
Btc1	25-56	50.4	8.0	0.9	8.8	10.6	2.7	11.5	0.0	113.0

Legend: HNO3-K, K extracted in 1N boiling HNO3 acid; Qurt, quartz; Chlr, chlorite; Bott, biotite; Musc, muscovite; Kfld, K feldspar; Cafl, Ca feldspar; Nafl, Na feldspar; Intd, mostly Ca-Na intermediatory feldspar with some unrecognizable minerals; Tcnt, total number of grains counted.

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Table 4. Total chemical analysis* and boiling HNO_3 extractable K# in the sand and silt fractions.

Horzn	Depth	Total Al		Total Fe+Mg		Hot HNO3-K		Total K	
		Sand	Silt	Sand	Silt	Sand	Silt	Sand	Silt
	(cm)	(%)				mg/kg		%	
Satgra, Typic Natrargid									
Ap	0-18	4.8	8.2	2.2	3.0	300	2111	1.5	2.1
Bt	18-38	2.8	10.1	2.8	4.3	773	1811	0.8	2.9
BtKn	38-82	4.0	9.0	1.6	4.1	1127	1799	1.0	2.6
Btkn2	82-110	3.4	9.7	2.4	4.8	710	1773	0.4	3.3
BtKn3	110-127	2.6	8.9	3.0	3.8	1358	1639	0.4	2.5
Wazirabad, Udic Halpustalf									
Ap	0-15	4.9	5.0	1.6	3.1	295	1538	1.1	2.0
Bt	15-33	6.8	14.4	2.3	6.5	365	1448	1.2	1.6
Bt2	33-63	6.5	14.1	2.1	6.6	400	992	1.4	1.9
BC	63-91	5.9	11.6	2.1	5.6	544	1159	2.1	1.4
C	91-120	7.7	14.5	2.8	6.6	457	1758	1.4	1.4
Ck1	120-146	6.0	15.0	2.9	6.9	778	1585	1.4	1.6
Pacca, Ustollic Camborthid									
Ap	0-10					3476	1516		
Bw1	10-23					3154	1476		
Bw2	23-43					3774	1599		
BC	43-63					5333	1236		
2B1	63-80					3284	2018		
2Bw1	80-95					6158	2236		
2Bt1	95-111					3632	2017		
2Bt2	111-120					3071	2112		
Balkassar, Udic Haplustalf									
Ap	0-11	43.1	6.2	2.2	2.7	224	275	1.5	2.0
Bt1	11-37	44.2	7.0	3.8	3.2	148	257	1.6	1.8
Btk	37-70	47.4	6.7	3.9	4.0	110	269	1.4	1.9
BCtk	70-100	50.6	6.3	4.1	3.5	255	208	1.8	1.8
C1	100-120	48.2	4.4	3.8	3.2	180	250	1.3	1.1
C2	120-143	40.4	5.2	4.2	3.2	131	210	1.3	1.3
Gulina, Udic Haplustalf									
Ap	0-12	5.9	12.7		2.9	1360	1960	1.1	1.9
BAtc	12-25	5.5	12.6		2.8	1240	1740	1.0	1.8
Btc1	25-56	6.0	11.7	2.3	2.5	1022	1380	1.0	1.7
Btc2	56-83	6.3	12.3	3.2	2.9	1217	1140	1.4	1.6
BCtc	83-117	10.1	12.3	4.1	3.1	2133	1360	0.0	1.7
BCtk	117-140	7.0	13.4	3.8	3.3	2040	1100	1.9	1.6
Peshawar, Udic Ustochrept									
Ap	0-11	.	8.1	.	3.8	1464	647	.	2.2
Bt1	11-43	.	6.9	.	3.6	757	218	.	2.3
Bt2	43-66	.	7.6	.	3.9	794	263	.	2.3
2Btb1	66-98	.	7.3	.	3.8	691	324	.	2.4
2Btb2	98-138	.	6.9	.	3.2	741	171	.	2.2
2Bk2	138-180	.	8.3	.	3.9	1149	269	.	2.4

*, Total chemical analysis by digestion in aqua regia and HF in pressure vessel (Jackson, 1979) and #, Boiling nitric acid (Knudsen et al., 1982).

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Figure

Fig. 1. X-ray diffraction pattern of the Guliana profile: (a) sand (2 to 0.05 mm) and (b) silt (50 to 2 μm). M, mica; Q, quartz; M,Q, mica and quartz; Fld, feldspar.

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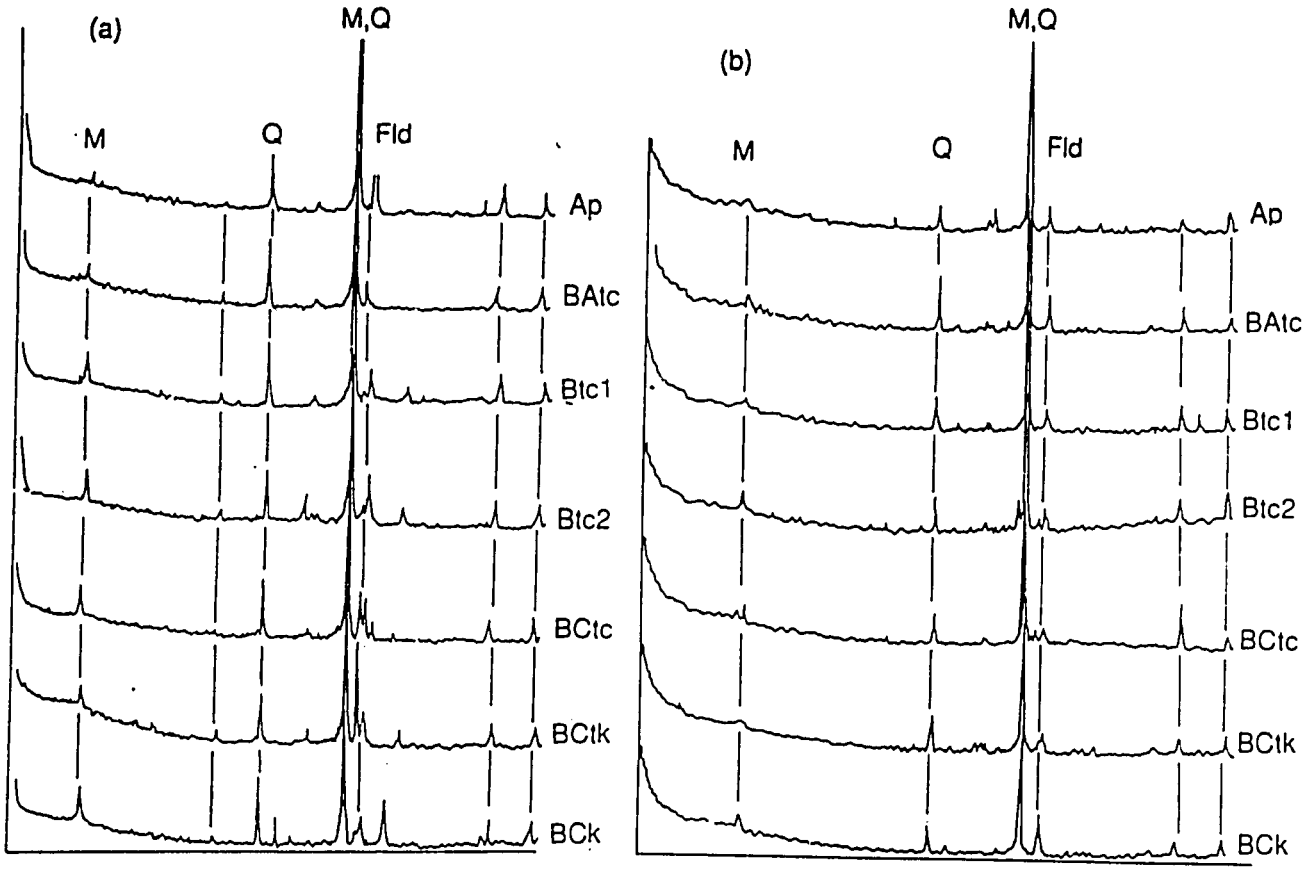
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M,Q
M
Q
Fld
Ap
BAtc
Btc1
Btc2
BCtc
BCtk
BCk
(b)
M,Q
M
Q
Fld
Ap
BAtc
Btc1
Btc2
BCtc
BCtk
BCk

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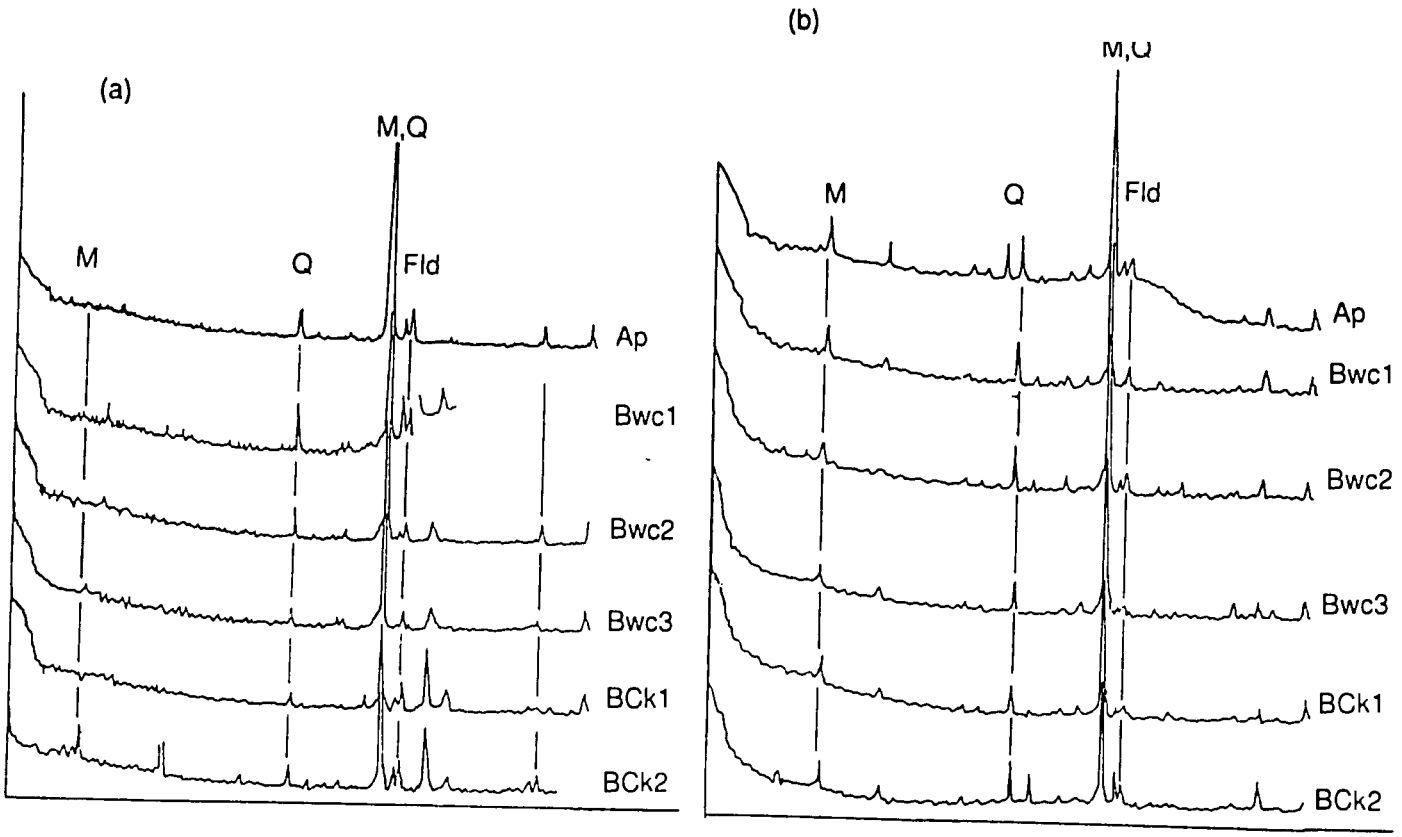
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M,Q
M
Q
Fld
Ap
Bwc1
Bwc2
Bwc3
BCk1
BCk2
(b)
M,Q
M
Q
Fld
Ap
Bwc1
Bwc2
Bwc3
BCk1
BCk2

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USING GEOSTATISTICS IN SOIL FERTILITY RESEARCH ON A FIELD SCALE

By

Aman U. Bhatti
Fida Hussain
Farmanullah
Mohammad Jamal Khan

DEPARTMENT OF SOIL SCIENCE
NWFP AGRICULTURAL UNIVERSITY
PESHAWAR, PAKISTAN

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USING GEOSTATISTICS IN SOIL FERTILITY RESEARCH ON A FIELD SCALE

ABSTRACT

Researchers traditionally conduct fertilizer experiments on small, replicated areas at university or governmental research centers. This approach has two problems: (1) extrapolation of the results to large cropped fields requires taking into account the impacts of spatial variability of soil properties on the optimum amount and type of fertilizer to apply; and (2) farmers have limited access to the research work, particularly in developing countries, which limits the information transfer needed for farmers to adopt the recommended fertilizer techniques. With these problems in mind, field and laboratory investigations were conducted on a farmer's field to study the spatial patterns of some soil properties and wheat yield using geostatistics for determination of fertilizer rates. The field was divided into four parallel transects, and soil samples were collected at 15 m intervals along each transect. These soil samples were analysed for different physical and chemical properties. A uniformity trial was conducted on wheat in the field. Classical statistical and geostatistical techniques were used to describe the variability in measured soil properties and wheat yield. Most of the soil properties and wheat yield had a very high coefficient of variation. Variability in soil properties and wheat yield was best described by a linear model. The potential wheat yield was estimated using the relationship between wheat yield and lime content, and was used in determining N fertilizer rates for different areas. The effects of soil variability on crop growth and profitability of fertilizer use were also demonstrated to the farmers.

INTRODUCTION

Spatial variability of soil properties affects the use efficiency of fertilizers applied at a uniform rate based on the average soil test level of nutrients. Traditional application of a uniform rate of fertilizer on spatially variable soils causes some areas to be overfertilized, while others are underfertilized. Consequently, fertilizer use efficiency is reduced and a problem with underground water pollution may arise because of the downward movement of unused fertilizer in the overfertilized area.

Researchers on government farms often establish replicated research trials on a small scale and extrapolate their results to larger areas. However, large areas may have much spatial variability in soil properties. Thus the results of small scale trials are often not relevent to large fields. Moreover, in replicated field trials, the true treatment effects are sometimes masked by trends in soil fertility (Bhatti et al., 1991), and fertilizer recommendations thus cannot be formulated accurately and economically.

Geostatistical analysis of within-field variations in soil properties or crop yield potential has been used to identify cause-and-effect relationships between a measured property and its environment (Trangmar et al., 1987; Miller et al., 1988; Tabor et al., 1984; Vieira et al., 1983; Perrier and Wilding, 1986; Trangmar et al., 1986; Mulla, 1988; Hamlett

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et al., 1986; Bhatti et al., 1991). Geostatistical approaches involve analysis and modeling of spatial patterns using semivariograms.

Mulla et al. (1992) used spatial patterns of soil fertility to determine fertilizer rates for different landscape positions across an eroded landscape of the Palouse region of eastern Washington. The variable fertilizer rates were compared with a uniform fertilizer rate and no significant difference was found. Bhatti and Mulla (1992) compared various criteria for dividing a field into different management zones and determining fertilizer rates for each zone. Bhatti and Bukhsh (1993) used these techniques to determine gypsum requirements for reclamation of salt-affected soils.

Understanding the nature and magnitude of spatial variability of soil properties is necessary for increasing the efficiency of applied fertilizers. Spatial patterns of soil properties and crop yield can be used to determine the fertilizer rates for different landscape positions on steep eroded lands (Mulla et al., 1992).

Because of increased fertilizer prices, optimization of fertilizer use is needed in the dryland farming areas of the northern part of the North West Frontier Province (NWFP), Pakistan. To achieve this objective, better and modern

techniques are required for assessing and identifying large-scale patterns in soil fertility and crop productivity.

The primary objectives of this research were (1) to use geostatistical techniques to measure quantitatively and assess the nature and extent of spatial variability in soil properties and wheat yields, (2) to divide the landscape into different fertility management zones and determine optimum fertilizer rates for these zones, and (3) to demonstrate the utility of fertilizer usage and variability in crop growth in a uniformity trial.

MATERIALS AND METHODS

Field and laboratory studies involving extensive soil sampling, soil analysis, and measurement of wheat yield using a uniformity trial were conducted on a farmer's field at Thana Swat. The land is situated on a slope (2.32 to 3.57% steepness) in dryland areas of the northern part of NWFP Pakistan. The land has a past history of variable soil erosion.

Site Description

The landscape at the study site (Figure 1) is characterized by steep land, and its slope faces toward a stream. The slope of the field is oriented along the long side of the field from east to west. The relative elevation of the

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sampling locations ranged from 3.14 to 12.82 m, with a coefficient of variation of about 25% (Table 1).

Soil Sampling

The study site was sampled at the end of September 1992. The whole field was divided into four parallel transacts. Two of the transects were 36 m wide, and the other two were 24 m wide, each transect was 200 m long. Each transect was sampled at intervals of 15 m to a depth of 30 cm. Samples were also taken to a depth of 0-90 cm in increments of 30 cm at 30 m spacings. The surface 0-30 cm samples were taken as a composite of five hand auger samples within a 30 cm radius. The surface 0-30 cm samples were analyzed for soil pH, lime content, organic matter, AB-DTPA extractable P and K, total mineral N (Page et al., 1982), and soil texture (Klute, 1984). Available water content was determined for each 30 cm increment to calculate the amount of available water in the 90 cm soil profile.

Uniformity Trial

Wheat (cv. Pirsabak-85) was planted in the third week of October 1992 on the whole field. A uniform rate of fertilizer-- i.e. 120 kg N, 90 kg P_2O_5 and 60 kg K_2O per ha-- was applied to the whole crop. All of the P and K was applied at sowing, while half of the N was applied at sowing and half in the

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month of January. Wheat was harvested from a 1 m^2 area along each transect at spacings of 15 m. Wheat grain yields were measured from the harvested area.

Statistical Procedures

The data collected on measured soil properties and wheat yields were subjected to classical statistical analysis to complete summary statistics and regression equations (Steel and Torrie, 1980). Three-dimensional plots of each measured property were made as a function of position along the transects to observe spatial patterns in relation to landscape position. In most cases, the observed spatial patterns were not random, and exhibited clear relationships to slope position and landscape features.

Geostatistical Procedures

Semivariograms (Bhatti et al, 1991) were developed to determine the structure and magnitude of spatial patterns in various measured soil properties and wheat yield. Semivariance, $\gamma(h)$, as a function of separation distance (lag, h) was computed using the expression:

$$\gamma(h) = \frac{1}{2n(h)} \sum_{i=1}^{n(h)} [Z(x_i) - Z(x_{i+h})]^2 \quad \text{Eq. [1]}$$

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where n(h) is the number of samples separated by a distance h, and Z represents the measured value for a soil or crop property.

The values of semivariance computed in Eq. [1] were interpreted by fitting models to the data using nonlinear least-square methods (Mulla, 1988). The model that gave the best fit to the data in this study was a linear model given by:

$$\gamma(h) = C_0 + mh \qquad \text{Eq [2]}$$

where C_0 is a parameter known as the nugget, and m is simply the slope of the line for a plot of semivariance (γ) versus separation distance (h). Theoretically, $\gamma(0) = 0$. However, many soil properties have nonzero semivariance as h tends to zero. This nonzero variance is called the nugget variance or nugget effect (Journel and Huijbregts, 1978). It represents unexplained or random variance often caused by measurement errors or variability in the measured property that was not detected at the scale of the sampling. With the linear model, spatial correlation of a property exists if the slope is positive.

RESULTS AND DISCUSSION

Variability In Soil Properties And Wheat Yields In Uniformity Trail

Most of the soil properties had a very high coefficient of variation. Clay content had the highest coefficient of variation (about 56%), followed by lime content (about 47%). Next was grain yield, with a coefficient of variation of about 38%. All of the soil samples collected were low in organic matter and deficient in soil phosphorus. Most were also deficient in potassium. This high variability in soil properties and wheat yield was a function of position on the landscape and was not random. Silt content and clay content were higher at upper slope positions, while sand content was higher at lower slope positions (Figures 2-4). Similarly, organic matter was lower at top slope positions as compared with bottom slope positions (Figure 5). These variations in soil properties and wheat yield are the result of historical variable rates of soil erosion and subsequent loss or movement of top soil.

Geostatistical techniques involving semivariogram analysis were used to identify and describe the spatial patterns in soil properties and wheat yield. Semivariograms were developed for each property, and parameters for the best-fitting linear models were estimated (Table 2). Omnidirectional semivariograms with linear models were similar

to the unidirectional semivariograms for the spatially variable soil properties and wheat yield. This indicates that the spatially correlated behavior of various soil properties and wheat yield was in the east-west direction and was controlled by the slope of the land. Moreover, the variability of these measured soil properties and wheat yield is unbounded because the models have no sill.

Identification and description of the spatial patterns of soil properties and wheat yield can help in the fertility management of this land and in ~~in~~ the determination of fertilizer rates for different locations in the field.

Determination Of Fertilizer Rates

As the field was deficient in soil phosphorus and potassium, the desired rates of these nutrients were determined according to the recommendations of the Agriculture Department of NWFP. These rates were 90 kg P_2O_5/ha and 60 kg K_2O/ha for the study area.

Various criteria were used to divide the field into different fertility management zones with significantly different soil properties. When the various criteria were compared, lime content proved better than the others. Lime content was then used to divide the field into different units with significant differences in yield, lime content, soil pH, silt content, and sand content. Lime content was negatively correlated with grain yield, although the value of the

coefficient of correlation was small (r = -0.21). Moreover, lime content was higher at the upper slope positions as compared with the lower slope positions.

Using the relationship between grain yield and lime content, a regression model was developed to predict potential yields at different locations. This model is given by the following equation:

Grain yield (kg/ha) = 4118-120 (lime content-%) Eq. [3]

This equation was used to calculate potential wheat yields at different sampling locations.

A three-dimensional plot of potential yield as a function of landscape position also showed spatial patterns (Figure 6). The semivariogram for potential yield was best described using a linear model (Table 2), which confirmed the existence of spatial variations in potential yield. Spatial patterns in potential yield were used to divide the field into zones with low (<3400 kg/ha), medium (3400-3600 kg/ha), and high productivity (> 3600 kg/ha).

Three different management units were identified based on the potential wheat yields. The potential wheat yield of each zone was used to determine the N fertilizer requirement for that zone using the following expression:

N fertilizer (kg/ha)= [(Potential yield x % N in grain) - total mineral soil N (kg/ha)]/0.5 Eq. [4]

where the factor of 0.5 is the fertilizer use efficiency for wheat (Tomer and Soper, 1981; Shah et al., 1993).

Using this expression, three rates of fertilizer N were determined as shown in Table 3. The proportion of the area for each particular rate was calculated (Table 3). The results showed that nearly 20% of the field had poor productivity, and over 50% required less than the typical rate of fertilizer N.

Use of this approach for fertility management has the advantage that the crop production potential of the land is considered. This approach avoids the overapplication or underapplication of fertilizer, saving resources and reducing pollution hazards.

Variability In Crop Productivity And Profitability Of Fertilizer Use

Despite the uniform rate of fertilizer application to the wheat crop, crop growth in the field was uneven; it was poor at the upper slope positions and good at the other locations. The poor growth at the upper positions was due to higher clay content and a compact surface, which hampered root growth and crop germination. Moreover, higher lime content at these locations may have affected the availability of plant nutrients, especially phosphorus. Lower grain yields were also recorded at the upper slope positions. These effects were shown to the farmers and explained.

The economics of fertilizer use were also calculated. The total cost of fertilizer application to 1 ha was Rs.2222. The total price of wheat (3542 kg/ha @ Rs.4.00/kg) is Rs.14168/ha.

The net profit is Rs.11948/ha, with a cost: benefit ratio of 5.38. Calculating the economics of fertilizer use for the three management zones based on the results of the uniformity trial, it was found that the net profit for the zones 1, 2 and 3 was Rs.9632, 11748 and 12488/ha, respectively, with a cost: benefit ratio of 4.33, 5.28, and 5.62, respectively. It is obvious that the profitability of the use of fertilizer depends upon the crop yield potential of soil within the same field.

CONCLUSION

One advantage of this study was the demonstration of improved fertilizers management techniques to the farmers. Another was the evaluation of new techniques for fertilizer rate determination in spatially variable soils using geostatistical techniques.

REFRENCES

Bhatti, A.U., and A. Bakhsh. 1993. Management of salt affected soils of Paharpur SCARP using variable rates of gypsum. Final Report. Collaborative Research Project. Department of Soil Science NWFP Agri. Uni. Peshawar, Pakistan.

Bhatti, A.U. and D.J. Mulla. 1992. Criteria for managing soil fertility in spatially variable wheat fields. Proceedings of the International Symposium on Nutrient Management for sustained productivity. Vol.II. Deptt: of soils, Punjab Agricultural University Ludhiana, India: 71-72.

Bhatti, A.U., D.J. Mulla, and B.E. Frazier. 1991. Estimation of soil properties and wheat yields on complex eroded hills using geostatistics and thematic mapper images. Remote Sens. Environ. 37: 181-191.

Hamlett, J.W., R. Horton, and N.A.C. Cressie. 1986.Resistant and extrapolatry techniques for use in semivariogram analysis. Soil Sci. Soc. Am. J. 50: 868-875.

Journel, A.G., and C.H. Huijbregts. 1978. Mining geostatitics. Academic Press, New York.

Klute, A. (ed). 1984. Methods of Soil Analysis Part-I. Agronomy No.9. American Society of Agronomy, Madison, Wisconsin, U.S.A.

Miller, M.P., M.J. Singer, and D.R. Nielsen. 1988. Spatial variability of wheat yield and soil properties on complex hills. Soil Sci. Soc. Am. J. 52: 1133-1141.

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Mulla, D.J. 1988. Estimating spatial patterns in water content, matric suction, and hydraulic conductivity. Soil Sci. Soc. Am. J. 52: 1547-1553.

Mulla, D.J., A.U. Bhatti, M.W. Hammond, and J.A. Benson. 1992. A comparison of winter wheat yield and quality under uniform versus spatially variable fertilizer management. Agric. Ecosystems Environ. 38: 301-311.

Page, A.L., R.H.Miller and D.R.Keeney (eds). 1982. Methods of Soil Analysis Part-II. Agronomy No.9, American Society of Agronomy, Medison, Wisconsin, U.S.A.

Perrier, E.R., and L.P. Wilding. 1986. An evaluation of computational methods for field uniformity studies. Adv. Agron. 39: 265-312.

Shah, Z., J.K.Khattak and R.Ali. 1993. Fate of fertilizer nitrogen applied to soil and its effect on maize (Zea mays L.). Sarhad J. Agri. 9(5): 435-446.

Steel, R.G.D. and T.H. Torrie. 1980. Principles and Procedures of Statistics: A biometrical approach. McGraw-Hill, New York, N.y. 2nd ed.

Tabor, J.A., A.W. Warrick, D.A. Pennington and D.E. Myers. 1984. Spatial variability of nitrate in irrigated cotton: I. Petiols. Soil Sci. Soc. Am. J. 48: 602-607.

Tomar, J.S. and R.J.Soper. 1981. Fate of tagged urea N in the field with different methods of N and organic matter placement. Agron. J. 73: 991-995.

BEST AVAILABLE DOCUMENT

Trangmar, B.B., R.S. Yost, and G.Uehara 1986. Application of geostatistics to spatial studies of soil properties. Adv. Agron. 38: 45-94.

Trangmar, B.B., R.S.Yost, M.K.Wade, G.Uehara, and M.Sudjadi. 1987. Spatial variation of soil properties and rice yields on recently cleared land. Soil Sci. Soc. Am. J. 51: 668-674.

Vieira, S.R., J.L. Hatfield, D.R. Nielsen, and J.W. Biggar. 1983. Geostatistical theory and application to variability of some agronomical properties. Hilgardia 51(3): 1-75.

Table 1. Descriptive statistics of soil properties and wheat yield.

SOIL PROPERTY	MEAN	MINIMUM	MAXIMUM	CV(%)
Organic matter (%)	0.32	0.12	0.66	34.66
Lime content (%)	5.34	3.05	14.25	46.98
Soil pH	7.29	6.60	7.90	4.88
AB-DTPA ext.P (mg kg^{-1})	2.68	1.35	3.90	23.78
AB-DTPA ext.K (mg kg^{-1})	47.69	32.00	86.00	24.39
AB-DTPA ext.Zn(mg kg^{-1})	0.51	0.28	0.83	24.55
Relative elevation (m)	8.55	3.14	12.82	25.29
Clay content (%)	2.05	0.40	3.95	55.79
Silt content (%)	25.82	14.20	47.14	34.12
Sand content (%)	72.13	50.76	83.60	12.43
Grain yield (kg ha^{-1})	3542	1000	6500	37.94
Profile available water (cm)	14.19	9.60	17.82	14.60

Table 2. Summary of parameters for semivariogram models[1].

Property	Omnidirectional			Along Rows		
	Nugget	Slope	r^2	Nugget	Slope	r^2
Organic matter (%)	0.0082	0.00004	0.64	0.0078	0.00005	0.95
Lime content (%)	1.2637	0.0519	0.96	0.9431	0.0668	0.98
Soil pH	0.0892	0.0005	0.71	0.1019	0.0005	0.56
AB-DTPA ext.K(mg kg^{-1})	63.35	0.7151	0.71	65.63	0.8922	0.93
Clay content (%)	0.5969	0.0115	0.86	0.7393	0.0119	0.84
Silt content (%)	16.257	0.6317	0.94	14.697	0.8701	0.95
Sand content (%)	23.017	0.5717	0.94	21.186	0.8018	0.94
Grain yield (kg ha^{-1})	1502900	2987	0.47	1510400	3076	0.22

[1] Linear model

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Table 3: Management zones and fertilizer rates.

Management Unit	Fertilizer Rate N kg/ha	Area Proportion
1.	80	0.18
2.	110	0.39
3.	125	0.43

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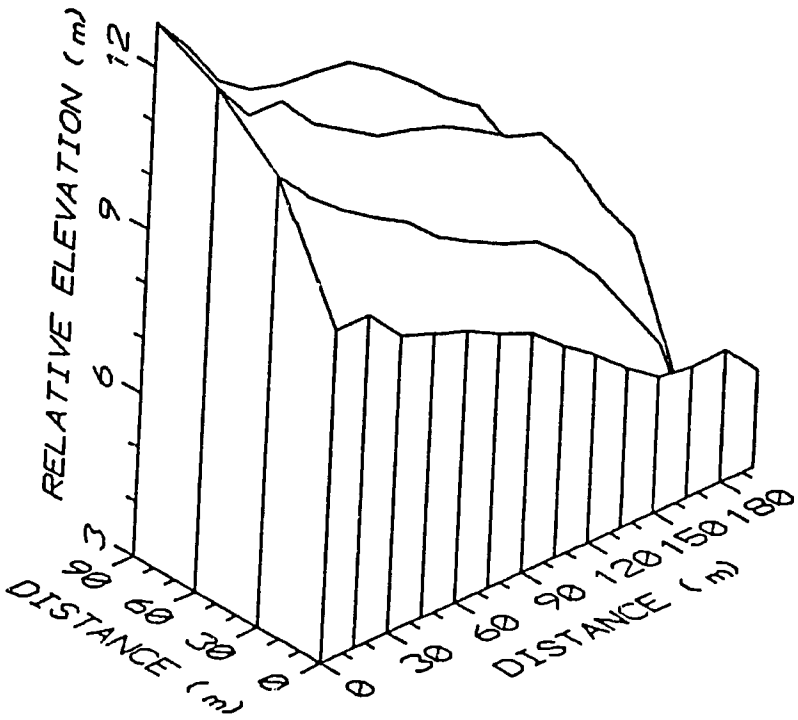
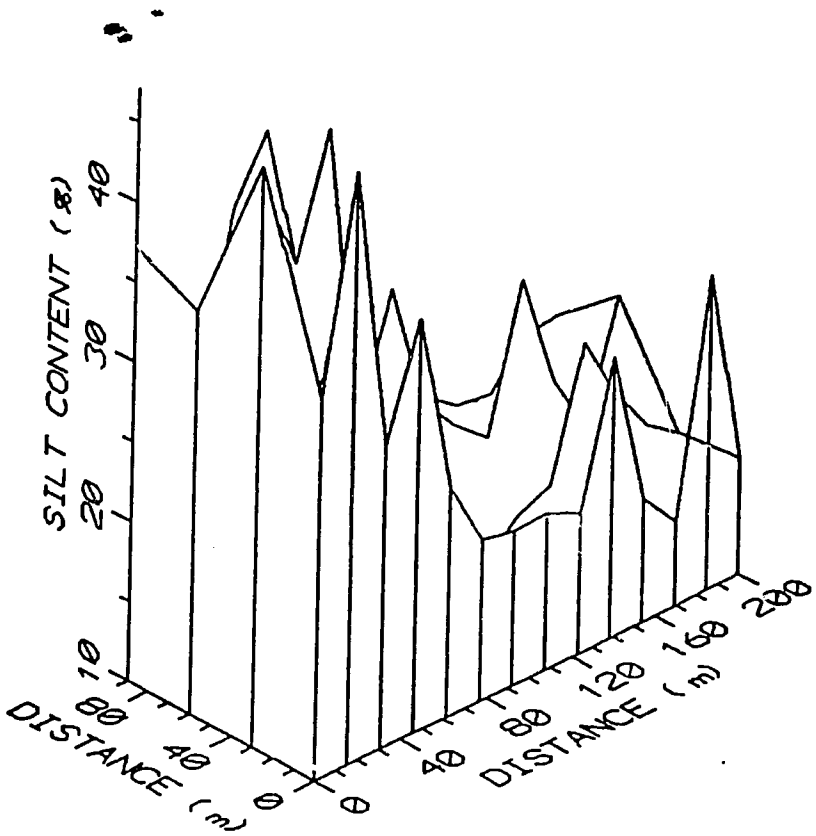


1. Relative elevation of soil sampling locations along the transects.



2. Silt content along the four sampled transects

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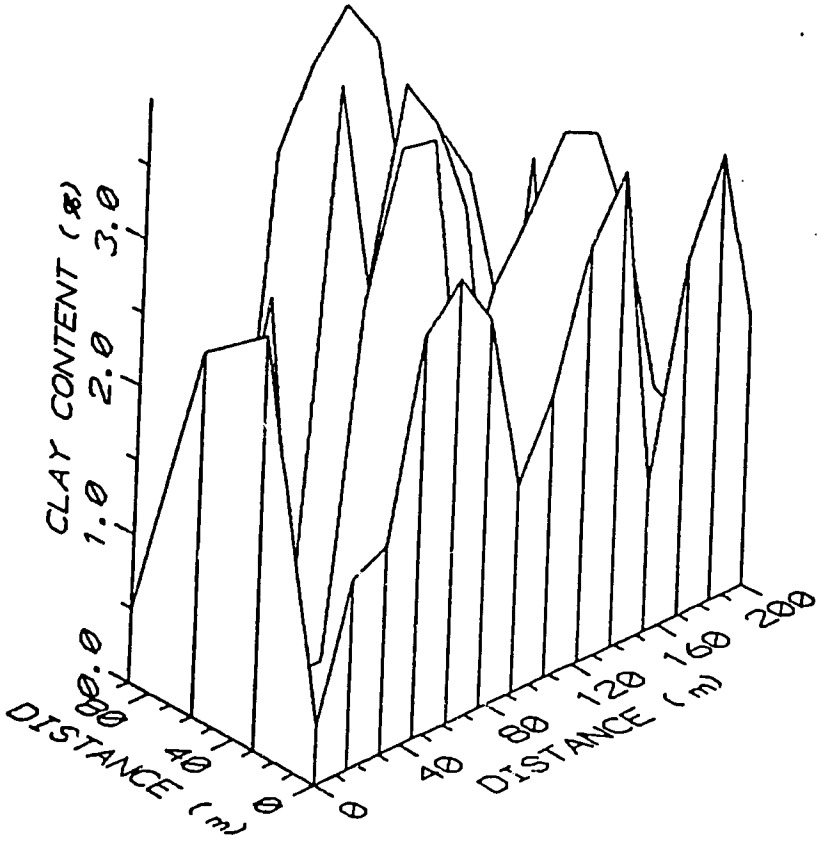

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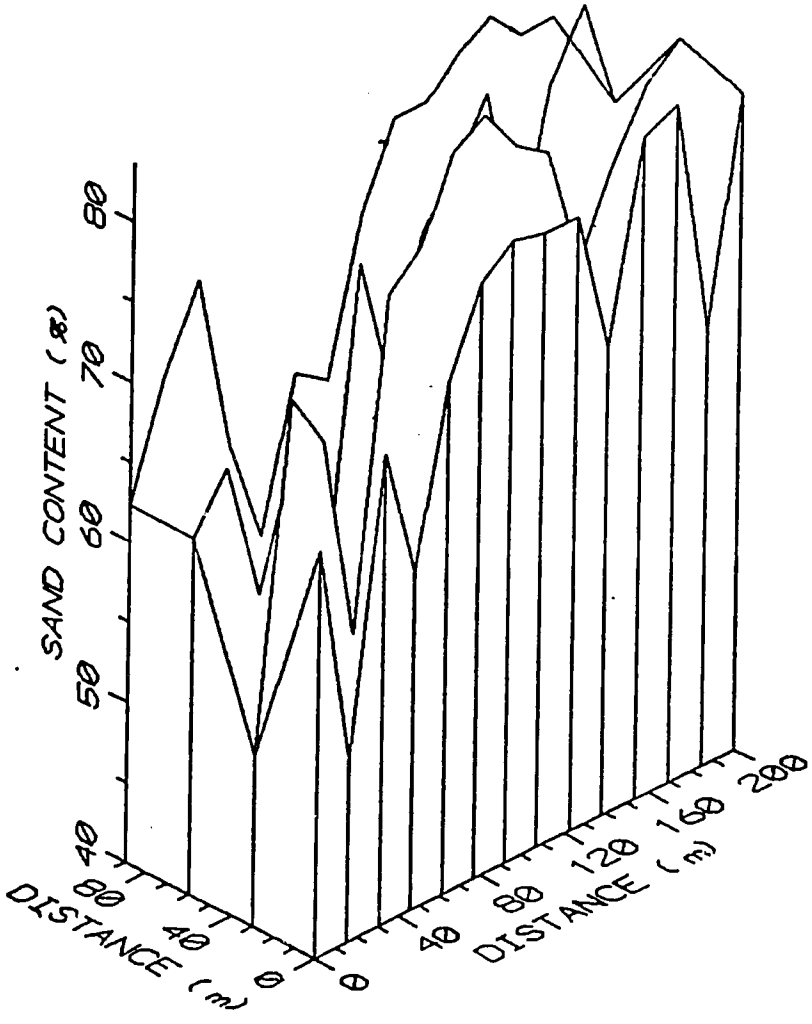

4. Sand content along the four sampled transects.

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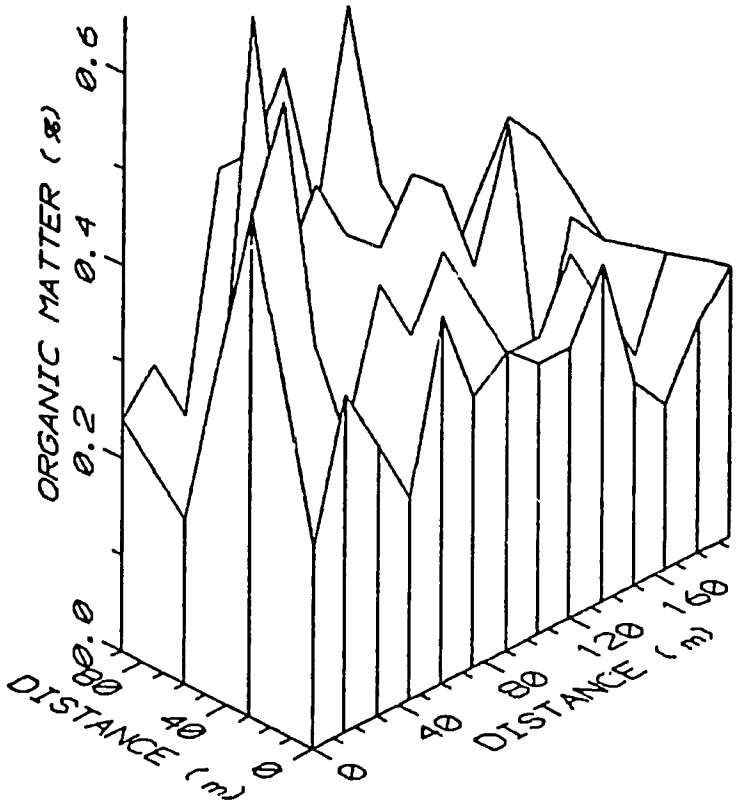
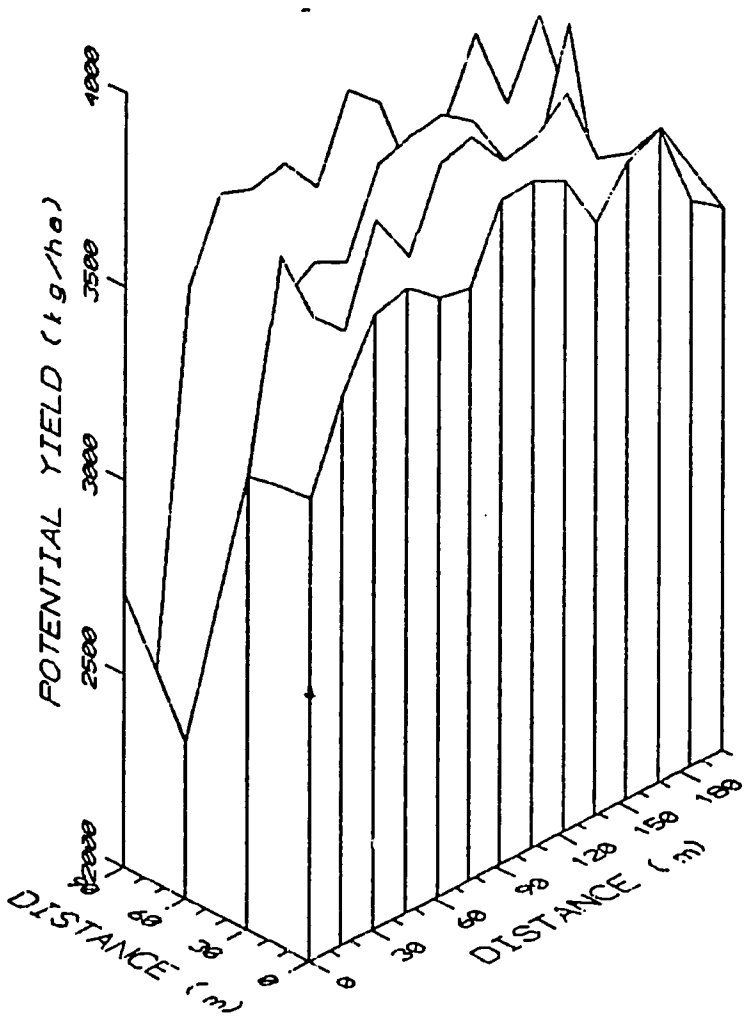


5. Organic matter content along the four sampled transects.



6. Potential wheat yield along the four sampled transects.

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RESPONSE OF WHEAT AND CHICKPEA TO ORGANIC AND INORGANIC MANURES IN RAINFED AGRICULTURE.

M.Hatam, M. Aslam, M. Jamal, M. Sadiq and A.Karim
NWFP. Agricultural University, Peshawar, Pakistan.

ABSTRACT

Wheat (Triticum aestivum L.) and chickpea (Cicer arietinum L.) are the two winter season crops of major economic importance in the rainfed areas of Pakistan. Information on specific management practices to optimize crop production in the rainfed areas is limited. The objective of this study was to determine response of wheat and chickpea to 1) Farm Yard Manure (FYM) and 2) rate and method of NP application in rainfed agriculture. Three field trials with wheat and chickpea each were conducted at three sites: 1) Langer Khel 2) Titer Khel (Bannu) and 3) Agric. Univ. Farm (Peshawar) during the 1992-93 winter season. In one trial Farm Yard Manure (FYM) was applied at the rate of zero or 10 t/ha to wheat as well as chickpea. In the other two trials, main plots were methods of N and P application (Band or Broadcast) and subplots were three levels each of N (0, 23, 46) and P_2O_5 (0, 23, 46) kg/ha in a complete factorial arrangement (3^2) with three replications at each site. Test cultivars for wheat and chickpea were 'Pirsabak-85' and 'CM72', respectively.

This study revealed that application of 10 t FYM/ha depressed wheat grain yield (29%), biological yield (20%) tillers/m^2 (10%) as a result of limited moisture availability at two locations out of three. In chickpea under similar conditions significant increase was noticed in grain yield (25%) and pods per plant (31%) at the same two locations. The number of nodules per plant increased by 84% with 10 t FYM/ha at location 1 and 2. Substantial increase was observed also in dry matter accumulation.

Method of fertilizer application had no significant effect on the wheat traits studied. Wheat grain yields were significantly increased by N and P application at the first two sites. Maximum wheat grain yield (764 kg/ha) was obtained with 46kg/ha N+23kg/ha P_2O_5 and the lowest (285 kg/ha) from the control treatment when combined over these two sites. Highest grain yield (2444 kg/ha), was obtained with 46 kg/ha N + 46 kg/ha P_2O_5 not significantly different from other treatments at Peshawar. Wheat grain yield increased as N rate increased whereas, at Peshawar grain yield tended to decrease. P application affected grain yield at the three sites similarly. The differences in yield due to P were not as great as those due to N. Wheat yield components and biological yields were also affected in similar pattern as grain yield by N and P rates. In the chickpea trial, grain yield was higher with broadcast application, (1458 kg/ha) than band application (1044 kg/ha). Maximum grain yield of 1443 kg/ha was obtained with 46kg/ha N+46kg/ha P_2O_5 as compared to 422 kg/ha for

the control at Langer Khel. Similar response was also observed at Titer Khel. Seed weight and number of pods/plant were also increased by N and P application. However, grains/pod were not affected. This study on wheat and chickpea indicated that application of N and P can enhance crop yields in the marginal rainfed areas of Pakistan. Further study is needed to determine the residual effects of FYM and economic levels of N and P fertilizer for sustainable crop yields.

INTRODUCTION

In Pakistan out of the total cultivated area (20.4 million hectares) about 5.8 million hectares come under rainfed conditions where spring type bread wheat (Triticum aestivum L.) and chickpea (Cicer arietinum L.) are the two most important winter season cultivated crops. The average grain yield of wheat and chickpea are 834 kg/ha and 505 kg/ha respectively, which are very low compared to the average yields obtained throughout the world. Shortage of moisture is one of the major constraints in crop yield. Besides this the soil fertility has been depleted because of continuous wheat and chickpea cultivation without judicious management and application of inputs. The unavailability and ever increasing prices of the fertilizers has made the situation more difficult for the small farmers to meet their requirements. Inspite of the fact that during the last twenty years the use of nitrogen, phosphorus and potassium containing inorganic fertilizers in the country has increased by six, thirteen and nineteen times, yet its application to the non-irrigated crops is limited because of high costs of fertilizers and the greater risk involved due to insufficient and uncertain rainfall during the growing season. In the past most of the research efforts have been directed towards irrigated areas and very little efforts have been made to improve the management practices of rainfed farming system. Low cost domestic organic manures alone are limited to meet the NPK requirements of crops for higher yields. Inorder to enhance crop productivity and soil fertility, through the application of organic and inorganic manures, the following two trials were conducted. 1) Influence of Farm Yard Manure application on rainfed wheat and chickpea and 2) Response of wheat and chickpea to method and rate of NP fertilizer under rainfed conditions.

REIVEW OF LITERATURE

Hu et al (1988) increased the water use efficiency of wheat by 9-24% with 37.5-150t organic manure/ha. However Bocz (1985) suggested that to utilize the available water supply and double the yield of cereals, upto 20 times more mineral fertilizer than the equivalent amount of manure could be applied per hectare. Crop residue increased the availability of native and added P in soil (Murthy et al 1985) and the adverse effect of stubble retention on crop yield was reduced with suitable rates of N

fertilizer application (White et al, 1988). Application of FYM at the rate equivalent to 10 kg N/ha was more effective than inoculation in increasing NPK uptake (Raju et al 1991). Several workers (Annandale, 1984, Singh and De, 1987 Bizhaev, 1988) have reported increases in grain yield of wheat with application of FYM +NPK. Kormilitsyn (1987) believed that in such combination the effect of NP depended on the residual effect of FYM. Farm Yard Manure in combination with NPK increased cereal yields in long and short term rotations (Siegert and Rauhe, 1987, Giardini et al, 1987, Gruev et al 1989). In chickpea the highest increase in yield and P uptake was noticed with residual effects of FYM (Sharma and Sinha, 1989).

Dang et al. (1987) reported that application of 30kg P_2O_5/ha to wheat increased grain yields from 2.21 to 3.18 t/ha. But did not get further increase in grain yields with 60 kg P_2O_5. They also reported that the yields with P drilled at sowing were higher than when P was broadcast applied at sowing or top dressed at the Ist irrigation. Alley (1986) observed that sub surface banding, surface stripping and in-row placement methods were not superior to broadcast treatments of P and K. He reported that fertilization of wheat was based on soil testing for P and K and in dryland areas, residual soil N. Tomar et al. (1983) demonstrated that N application alone and incombination with P increased wheat yield under rainfed conditions. But P alone had little effect on wheat yield. They reported highest net profit from a balance application of 60 kg N 40kg P_2O_5 30 kg K_2O and 25 kg Zn SO4. Chaudhry and Sherif, (1982) found the highest wheat grain and straw yields of 1.62 and 4.30 t/ha, respectively from a combined application of 40 kg N and 60 kg P_2O_5/ha. High rate of NP application increased plant height and number of ears/plant. Monem et al (1990) tested 0, 30, 60 or 90 kg N or p/ha and found that application of 30 or 40 N kg/ha increased wheat grain yield on plots with previous crop of wheat, barley, maize or fallow. While there was no response to N application where previous crop was chickpea. P application did not influence grain yields.

Reddy and Bhardwaj (1984) studied the effects of 0,40,80 and 20 kg N and 0,25 and 50 kg P_2O_5/ha on the growth and grain yield of wheat under limited and adequate irrigation and found that upto 18 kg/ha N increased yields and yield components except grain weight. P application also increased yield and yield components. Ryan et al (1989) applied 0, 40 80 or or 120 kg N/ha with a basal dose of 18 kg P/ha and recommended 40 kg N/ha for shallow soil of semiarid Morocco. They also added that irrespective of soil type or depts, a well managed legume may could sufficient N to the following cereal crop to make N fertilization unnecessary. Spratt and Chowdhry (1978) reported that 40 kg N/ha is the most economical rate in the presence of sufficinet soil P level. Although the response was not as dramatic as for N. Collins and Cresswell (1986) reported that increasing levels of N application from 0-80 kg N/ha did not increase yield of winter wheat under dryland conditions.

Vig and Singh (1983) reported that P application of upto 39 kg/ha significantly increased wheat yields and that drilled application was superior to broad cost method. They reported that P uptake was a linear function of water supply and P uptake beyond a P dressing of 20 kg/ha was limited by soil moisture. Dumbre and Deshmukh (1983) studied management of chickpea under various practices and observed that application of 25 kg N plus 75 kg P_2O_5/ha had not a significant effect on grain yield. Parihar and Tripathi (1989) studied three P-rate of 25, 50 or 75 kg P2O5/ha at 3 irrigation levels and found that total drymatter and grain yield increased with increased P rate, but decreased with increased irrigation due to exessive vegetative growth at the expense of pod formation.

MATERIALS AND METHODS

Inorder to determine the 1) effect of FYM and 2) method and rate of NP application on the grain yield and growth of wheat and chickpea, separate trials were conducted at three locations during the year 1992-93. Two in the main wheat and chickpea growing rainfed area in District Bannu at 1) Langar Khel and 2) Titar Khel and one at the NWFP Agricultural University, Peshawar. Location 1 and 2 are 25km apart and about 250km away from location 3. At location 3 the soil was a clay loam of Peshawar soil series, sub group Typic Ustochrepts according to USDA soil texonomy with .035% N, 3.7 ppm P_2O_5, 141ppm K_2O, 0.73% organic matter and pH 8.2. At the other two locations the soil was a sandy loam of Ghazni Khel series belonging to Typic Torri Psamments according to USDA soil texonomy with 0.032% N, 5.5 ppm P_2O_5, 0.60% organic matter and pH 7.2. For determining, soil nitrogen, phosphorus and potassium, the analysis procedure of Bremer and Mulvancy (1982) Olsen and Sommers (1982) and Knudsen, et al (1982) respectively were adopted. The total annual rainfall at location 3 and locations 1 and 2 was 383mm and 389mm respectively. Out of the total, 66% and 43% of the rainfall occurred during the growing season at location 3 and 1 and 2 respectively. Maximum temperature ranged from 15^{0}C to 32^{0}C at location 3 and 17^{0}C to 34^{0}C during the growing season at the other two locations.

The previous crops at the experimental sites were soybean and wheat at location 3 and locations 1 and 2 respectively. Treated (Vitavax @ 2g/kg of seed) seeds of wheat cultivar Pirsabak-85 and chickpea cultivar CM-72 were planted at the rate of 100 kg and 80 kg per hectare in 30 cm apart rows respectively. At location 3 and 1 and 2 planting was done with hand and camel driven planter respectively. At Peshawar the attack of hairy caterpillar on chickpea from the nearby soybean fields, was successfully controlled with the application of Follidol (monothio phosphate) at the rate of 500 ml/ha. During the dry season up to the end of February, sporadic attack of white ants on wheat was observed at all the locations. Weeds were controlled manually when and where required.

In one trial Farm Yard Manure at the rate of zero and 10 tons per hectare (dry weight basis) was applied uniformly before ploughing. Farm Yard Manure was thoroughly incorporated in to the soil by ploughing crosswise with a cultivator with tines. The Farm Yard Manure used at Peshawar was fresh containing, 0.531% N, 526ppm P_2O_5, 300ppm K_2O while well rotten FYM with a composition of 0.086%N was used at location 1 and 2, in 10 x 20m plots replicated six times in Randomized Complete Block Design. Chickpea was planted on October 14, 9, and 4, 1992 and wheat on November Ist, 9, and 11, 1992 at location 1, 2 and 3 respectively. Number of nodules per plant was determined by carefully uprooting ten chickpea plants with a shovel. The soil was removed from the roots and the number of nodules on the roots and those detached with the soil were counted. Data was collected on several plant characteristics during the growth period and after harvest. Chickpea was harvested on April 23, 24 and 30 1992 and wheat on April 26, 27 and 29,1992 at Langar Khel, Titar Khel and Peshawar respectively. Threshing and cleaning was done manually.

In the other trial, three rates each of N (0, 23, 46) and P_2O_5, (0, 23, 46) kg/ha in a factorial arrangement (3^2) in the form of urea (46% N) and triple superphosphate (46% P_2O_5) were used, respectively. A randomized complete block design was used with three replications at each site. Main plots were alloted to method of application (Band and Broadcast) and subplots were assigned to 9 rates of NP. Wheat and chickpea was planted in November and October 1992 in 5x10m plots.

Data was subjected to analysis of variance according to the appropriate design and means were compared using least significant difference test. Because of similarity in soil type, climate and environment, separate and combined analyses of variance were carried out for the data from Langar Khel and Titar Khel. Data from Peshawar was analysed separately.

RESULTS AND DISCUSSION

Effect of FYM on Wheat

At Peshawar application of 10 t FYM/ha did not affect any of the parameters compared to 0t FYM/ha.(Table 1a). However at the other two locations (combined) application of FYM at the rate of 10 t/ha significantly reduced grain yield (29% %), biological yield (20%), tillers/m^2 (10%), spikes/m^2 (10%) and harvest index % (11%). Plant height, grains/spike and 100 grain weight was not affected. In combined analysis of the two locations none of the location x treatment interactions were found significant. It means the response to the application of manure at both these locations was similar, but different than at Peshawar. Wheat drymatter accumulation during the early stages for manure (10 t/ha) and no manure treatments wre at the rates of 2.6 and 3.2 g/m^2/day at location 1, and 2.3g/m^2/day at location 2 and 0.98 and 1g/m^2/day at location 3 (Table 3a).

Reduction in wheat grain and biological yield, tillers and spikes per m^2 could be because of moisture scarsity. The total annual rainfall at each of these locations was 389 mm. Out of this only 196mm (43%) was received during the growing season from September 1992 to April 1993. The growing period from November to February received only 32.2mm rainfall which resulted in the reduction of tillers/m^2 and ultimately the spikes/m^2. Although good showers were received in March (94.8mm) and April (40.3 mm) yet beneficial effect was not exhibited because of advanced stage of growth. It is evident that low rainfall during the early growing period of wheat did not allow FYM to play its role and afterward the crop approaching maturity was not able to get benefit of the late showers, thus depicting negative effects. Chang et al (1993) demonstrated that manure at any rate of application had a negative effect on yield of non-irrigated barley when moisture was below normal. It is reported that heavy application of manures depressed germination, growth and yield of corn (Murphy et al 1984). The reduction is generally attributed to a build up of soluble salts (McCalla, 1974) and NH_4 toxicity (Hensler et al 1970). The application of FYM neither increased nor decresed yield or yield components at Peshawar. Compared to the other two locations, this could be attributed to the slightly improved condition of moisture availability. Out of the total annual rainfall (383 mm) at this location 66% (285mm) was received during the crop growing season. A major part of it (161mm) was received during the last 30 days of maturity.

Effect of FYM on Chickpea

At Peshawar no significant response was observed for any of the chickpea parameters when FYM was applied at the rate of 10 t/ha. (Table 2a). However combined analysis of the other two locations indicated significant increase in grain yield (25%) and pods/plant (31%). for the 10t FYM/ha treatment. Grains/pod, 100 grain weight, biological yield and harvest index means did not show significant differences. Combined analysis of the two locations did not show significant location x treatment interactions for any of the parameters. Although the application of 10 t/FYM/ha did not icrease the number of nodules/plant at Peshawar but did at Langar Khel 88%, and Titar Khel 80%, (Table 3a). At langar Khel and Titar Khel the number of nodules per plant reduced to almost half when collected in the post blooming period (140 days after sowing). The reason could be that nodulation reaches its peak upto flowering and then degenerate gradually. The number of nodules per plant at Peshawar increased for the same period probably because of depletion of N from the FYM and the better growing conditions.

Application of FYM (10 t/ha) increased the number of nodules per plant in chickpea by 88 and 80% at Langar Khel and Titar Khel respectively. This could be responsible for the 25 and 31% increase in grain yield and pods/plant at these two locations. Since addition of organic matter generally improves the physical condition of the soil, the application of manure might have

provided more favorable environment for nodulation and nitrogen fixation. Also, the well rotten manure may have been very low in available N content. At Peshawar, the grain yield declined with application of FYM and the number of nodules remained the same with or without application of FYM. A higher nitrate content in the fresh FYM may have reduced nodulation until later in the season. It is likely that the soil at Langar Khel and Titar Khel have well established populations of rhizobium due to a long history of chickpea cultivation, while at Peshawar chickpea was grown for the first time. The nodules at this site were mostly in clusters unlike the normal shaped nodules at the other two sites. Gupta and Kaur (1969) believed that the shape and size of nodules might be influenced by the environment, Rhizobium strain and the strain host interaction. Nodule mass is more closely correlated with N-fixation than nodule number per plant (Pate, 1977). At flowering stage the decrease in acetylene reduction activity is generally associated with reduction in nodule mass. (ICRISAT 1982).

It is evident from the results that application of FYM depressed grain as well as several yield components due to limited moisture availability in non-irrigated wheat. However with chickpea, under similar conditions several yield components were improved which resulted in higher grain yield. The most conspecous effect was on the number of nodules per plant. Study of residual effects of FYM and crop rotation are suggested for sound recommendations.

Response of Wheat to NP

As is evident from the data on grain yield (kg/ha) in Table-1, there is no significant difference between the two methods of N and P application. However, the response to fertilizer N and P was significant at the Bannu sites. Maximum grain yield 774 kg/ha was produced by treatment 46N-23 P_2O_5 kg/ha when combined over the Bannu two sites. However, this grain yield was not significantly different from that of treatments 46 N + 46 P_2O_5 and 46 N + 0 P_2O_5 (kg/ha). Consistent with the fact that the soils at these two sites were difficient in P, grain yield increased with increasing rates of P at both Bannu sites. There was no clear response to N and P at the Peshawar site. The data on spikes/m^2 of two sites (Langer Khel and Titer Khel) given in Table-2 indicated that there was no significant effect of method of application. However, the varying rates of N&P had an effect on the number of spike/m^2. No of spikes/m^2 increased as N or P rates were increased. Maximum number of 52 spikes/m^2 at Langer Khel were produced by treatment 46 N+46 P_2O_5 kg/ha, followed by 46 N+23 P_2O_5 and 46N+0P_2O_5 with non significant difference between each other. The response to N&P was similar at both sites.

The data in Table-3 shows that fertilizer rates significantly increased grain weight compared to the control. The maximum grain weight (4.5gm/100 grain) for the combined data was obtained from treatment 46 N+46 P_2O_5 kg/ha followed by 46N+23 P_2O_5,

and 23 N+P_2O_5 kg/ha with non-significant difference from each other. There was no response in the grain weight of N or P at Peshawar. Method of application had no effect on grain weight at any of the sites. The data in Table-4 indicated a trend of higher biological yield for broadcast compared to band application at all the three sites. Also, the rates of N&P affected crop growth. Maximum biological yield (1999 kg/ha) was recorded from a treatment 46 N+46 P_2O_5 kg/ha and lowest was (847 kg/ha) obtained from control treatment when combined over two Bannu sites. However, at Peshawar there was no biological yield response to N or P. Maximum biological yield (5472) was recorded at Peshawar due to greater moisture availability.

There was a significant difference in the harvest index (H.I) (Table-5) as affected by NP fertilizer application. When combined over the two Bannu sites the maximum H.I. (43%) was obtained from treatment 46N+23 P_2O_5 kg/ha; followed by 46 N+ $0P_2O_5$ and 23N+$0P_2O_5$ kg/ha with non-significant difference from each other. Whereas, at Peshawar, maxiumum H.I (40%) was obtained for treatment 46 N+23P_2O_5 kg/ha. These data show a trend for H.I.to be influenced by method of application. Band application obtained higher H.I. values at all 3 sites. As is evident from the data in Table-6, there was a significant increase in plant height with N rates compared to the control at both Bannu sites. Maximum plant height (68cm) was shown by treatment 46N+23 P_2O_5 kg/ha and the lowest (51cm) was obtained from control treatment.

Response of Chickpea to NP

The grain yield data in Table-7 shows broadcast application of fertilizer produced higher grain yield (1458 kg/ha) than that of band application (1044 kg/ha). The rate of application had a significant effect on grain yield. The maximum grain yield (1443 kg/ha) at Langer Khel was produced by treatment 46N + 46 P_2O_5 kg/ha followed by 46N + 23P_2O_5 kg/ha producing 1177 kg/ha. The lowest grain yield (422 kg/ha) was produced by control treatment. With each added increment of N fertilizer there was an incremental and linear increase in grain yield at both Bannu sites. There was a slight negative response to N and P at Peshawar. This site was heavily infested by podborer resulting in very low grain yields. This precludes any interpretation of response to fertilizer at this site. There was a significant increase in 100-grain weight with fertilizer rates (Table 8). Highest grain weight was obtained with NP combinations. Combining of the two Bannu sites shows that the 46 N + 46 P_2O_5 and 46 N + 23 P_2O_5 kg/ha treatments produced the highest grain weights, 16.7 and 16.3g/100 grains compared to only 10.9 g/100 grains produced by the control. At Peshawar, similar to its effect as grain yield, applied fertilizer resulted in a slight reduction in 100 grain weight.

Applied N significantly increased the pod/plant and there appeared to be linear response to N application (Table 9). Maximum pod number 43/plant was produced by treatment 46N+23P_2O_5 across two

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sites (Langer Khel and Titer Khel) followed by treatments 46N+0P_2O_5 (39 pods/plant) and 46N+46P_2O_5 (38 pods/plant) with non significant difference between these two treatments. The lowest number of 20 and 21 pods/plant was obtained from treatment 0N+23P_2O_5 and control respectively. Phosphorus alone had no significant effect on the number of pods per plant. There was no response of grains/pod to either method or rate of N or P application, (Table 10). All pods examined from all treatments contained two pods. Applied N significantly increased plant height Z(Table-11). The data shows a linear postive response with each increment of applied N. There was no apparent effect of P on plant height. Biological yield response to added N and P followed a pattern similar to as that of grain yield (Table-12). Maximum biological yield was (3728 kg/ha) obtained with 46 N + 46P_2O_5 kg/ha at Langer Khel followed by 46 N + 23 P_2O_5 kg/ha (3052 kg/ha). The biological yield response to N&P at Titer Khel was similar.

It is concluded that FYM reduced wheat grain yield but had a positive effect on chickpea grain yield at Bannu sites under limited rainfall conditions. Whereas at Peshawar with relatively better moisture availability FYM resulted in increased crop productivity. There was no response to method of N and P application suggesting that broadcast method being more feasible to farmers may have merit over band method, without any economic loss in yield. The response of wheat and chickpea to N and P fertilizer was positive and need further study to determine economic levels in rainfed agriculture, correlating crop responses to fertilizer rates with soil and meteorological parameters.

REFERENCES

Alley, M. 1986. Fertilization for intensive wheat management. Proceedings NFSA convention (Edited by smith, B.J. 43-53) National Fertilizer Solutioning Association. St. Louis, Mo, USA.

Annandale, J.G; P.S. Hammes; and PC. Nel. 1984. The effect of soil fertility on the vegetative growth, yield and water use of wheat (Triticum aestivum). Crop production. 13, 30-34.

Bizhaev, V.M. 1988. Effect of long term application of fertilizers on soil fertility, nutrient balance and yield of a crop rotation in the Stepple zone of Kabardino Balkar A.S.S.R. Agrokhimiya. 5:37-44.

Bocz, E. 1985. Soil fertility factors for the growth of Hungarian cereal production. Agrokemia-es-talajtan. 34: 20-23.

Bremner, J.M. and C.S. Mulvancy. 1982. Nitrogen - Total. Methods of soil Analysis. Chemical and microbial properties, 2nd ed. In A.R. Page R.H Miller and D.R. Keeney. (ed) Agron. Monogra, Part 2. Am. Soc. of Agron. Madison P. 610-615.

Chaudhry, M.S. and S.M. Sherif. 1982. Dryland wheat production in the Western part of Libya. I. Respone to deep tillage and N-P fertilizers. Libyan Journal of Agriculture 11: 15-22.

Collins, M.C. and C.F. Cresswell. 1986. Influence of the level of nitrogen application on the carbon and nitrogen distribution in selected wheat varieties. Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants (edited by Lambers, H; Neeteson, J.J; and Stulen, I.P. 403-406).

Dang, Y.P., K.S., Verma, B.S.Pannu. 1987. Effect of different rates, methods and times of phosphorus application on wheat. Agricultural Science Digest. India. 7:3, 122-124.

Dumbre, A.D. and R.B. Deshmukh. 1983. Yield of grain varieties as affected by sowing dates, fertilizer application and irrigation. Journal of Maharashtra-Agricultural Universities. 8: 3, 300.

Giardini, L; R.Giovanardi; and M. Borin. 1987. Comparison of four crop rotations carried out for twelve years with different levels of irrigation and fertilization: agronomic and economic aspect. Rivista-di-Agronomia. 21(1): 18-27.

Gruev, T.S; Doncheva; and I. Stoyanova. 1989. Effect of mineral and organic/mineral fertilizers on grain yields and chemicotechnological properties of winter wheat. Rasteniev dni-Nauki. 26(1): 7-14.

Gupta, U.S. and P. Kaur. 1969. First record of abnormally big functional nodules on the roots of gram. Indian Agriculture.

Hensler, r.F., R.J. Olsen, and O.J. Ottoe. 1970. Effect of soil pH and application rate of dairy cattle manure on yield recovery of twelve plant nutrients by corn. Agron. J. 62: 823-830.

Hu, D.Y; A.X. Liu; and W.Q. Liu. 1988. Effect of organic phosphate fertilizers on water utilization and wheat yield in Weibi drylands. Soils. 20(1): 39-41.

ICRISAT (International Crop Research Insitute for the semi-Arid Tropics). 1982. Biological Nitrogen Fixation. In ICRISAT. Annual Report 1982 PP. 115-118 ICRISAT Patancheru. India.

Kormilitsyn, V.F. 1987. Effects of types of fertilizers on soil fertility and yield of crops grown in rotation with irrigation in the Crans-Volga region-Agrokhimiya. (10): 49-60.

Knudsen, D., G.A. Peterson, and P.F. Pratt. 1982. Lithuium, Sodium, and Potassium-Exchargeable and soluble potassium. Methods of soil Analysis. Chemical and microbial properties, 2nd ed. In A.R. Page R.H Miller and D.R. Keeney. (ed) Agron. Monogra, Part 2. Am. Soc. of Agron. Madison P. 228-237.

McCalla, T.M. 1974. Use of animal manure wastes as a soil amendment. J. Soil Water Conserv. 29: 213-216.

Monem, M.A., A. Azzaoui, El. M. Gharous, J. Ryan, & Pour, P.Soltan 1990. Soil test calibration in West Asia and North Africa. (edited by Ryan, J., and Matar, A.) 1990, 52-56. ICARDA Publication NO.167. Aleppo, Syria, ICARDA. Proceedings of the third Regional Workshop, Amman, Jordan, 3-9. Sept. 1988.

Murthy, A.S.P; D.P. Viswanath; and M.N. Sheelavantar, 1985. Soil fertility management in relation to agricultural productivity in vertisols of India. Taronomy and management of vertisols and Aridisols. Procedings of Fifth Insternational Soil Classification Workshop, Sudan. November 2-11, 1982 Part I.285-316 Khartoum Sudan, Soil Survey Administration.

Olsen, S.R. and L.E., Sommers. 1982. Phosphorus-phosphorus soluble in sodium bicarbonate. Methods of soil Analysis. Chemical and microbial properties, 2nd ed. In A.R. Page R.H Miller and D.R. Keeney. (ed) Agron. Monogra, Part 2. Am. Soc. of Agron. Madison. P. 421-422.

Pate, J.S. 1977. Functional biology of dinitrogen fixation by legumes. In A Treatise on Dinitrogen Fixation, Section-III: Biology (Hardy, R.W.F and Silver, W.S. eds) PP. 473-517. Wiley, New York. USA.

Prihar, S.S; and Tripathi, R.S. 1989. Drymatter, nodulation and nutrient uptake in chickpea (Cicer arietinum) as influenced by irrigation and phosphorus. Experimental Agriculture. 25: 3, 349-355.

Raju, M.S; S.C. Varma and N.V. Ramaiah. 1991. Effect of phosphorus in relation to FYM VS Rhizobium inoculation on nutrient uptake by chickpea cultivars under rainfed condition. Ind. J. Agri. Res: 25(1): 43-48.

Reddy, A.S. and R.B.L. Bhardwaj. 1984. Effect of nitrogen and phosphorus on growth and yield of wheat under limited and adequate irrigation. Indian-Journal of Agronomy. 29: 4, 505-509.

Ryan, J. Monem, M.A. and El. Mejabel. 1989. Nitrogen fertilization of hessian fly-resistant "Saada wheat" in a Shallow soil of semi-arid Morocco. Rachis Vol 8(2) 23-26.

Seigert, B; and K. Rauhe. 1987. Modes of action of nitrogen within the soil plant system after 15N-application to irrigated and un-irrigated sugrbeet and the residual effect on spring barley. Archiv-fur-Acker-und-Pflanzenbau-und-Bodenkunde. 31(11): 703-710.

Sharma. U.C; and H. Sinha. 1989. Residual effect of phosphatic sources in rice-gram rotation on acid soil. J. Ind. Soc. Soil. Sci. 37(1): 190-192

Singh, R.K; and R.De.1987. Organic manure and fertilizer management practices for dryland wheat. Fertilizer News.32(7):33-36.

Spratt, E.O. and Chowdhry, S.L. 1978. Improved cropping systems for rainfed agriculture in India. Field Crops Research: 1, 113-126.

Tomar, N.K., A.P., Gupta, S.S. Khanna. 1983. Evaluation of fertilizer needs for wheat under rainfed conditions. Fertilizer News. 28: 4, 35-39.

Vig, A.C. and Singh, N.J. 1983. Yield and P uptake by wheat as affected by P fertilization and soil moisture regime Fertilizer Research. 4:1, 21 - 29.

White, P.J; I. Vallis; and P.G. Saffina. 1986. The effect of stubble management on the availability of 15N-labelled residual fertilizer nitrogen and crop stubble nitrogen in an irrigated black earth. Australian J. Exp. Agric. 26(1): 99-106.

Table-1a. Effect of Farm Yard Manure (FYM) on Plant characteristics of wheat c.v.Pirsabak-85 at three locations. 1992-93.

Parameter	FYM t/ha	Langer Khel	Titter Khel	Combined	Peshawar
Tillers/m^2	0	112 a	125 a	119 a	272 a
	10	108 b	104 a	107 b	286 a
Plant height(cm)	0	53 a	56 a	55 a	98 a
	10	54 a	56 a	55 a	95 a
Spikes/m2	0	109 a	121 a	115 a	270 a
	10	105 a	102 a	103 b	284 a
Grains/spike	0	32 a	32 a	32 a	45 a
	10	30 a	33 a	32 a	46 a
100-Grain wt (g)	0	3.5 a	3.5 a	3.5 a	4.2 a
	10	3.5 a	3.6 a	3.5 a	4.1 a
Biological yield, kg/ha	0	2998 a	3145 a	3072 a	3017 a
	10	2620 a	2295 a	2458 b	2947 a
Grain yield kg/ha	0	1139 a	1234 a	1187 a	939 a
	10	893 b	795 b	844 b	937 a
Harvest index	0	38 a	38 a	38 a	2639 a
	10	34 b	34 b	34 b	30.5 a

Means followed by different letters in a column are significantly different according to LSD at 5%.

Table-2a. Effect of Farm Yard Manure (FYM) on Plant characteristics of Chickpea c.v. CM.72 at three locations. 1992-93.

Parameter	FYM t/ha	Langer Khel	Titter Khel	Combined	Peshawar
Pods/plant	0	40 a	38 a	39 b	92 a
	10	49 a	52 a	51 a	86 a
Grains/pod	0	2 a	2 a	2 a	2 a
	10	2 a	2 a	2 a	2 a
100 Grain wt (g)	0	14 a	14 a	14 a	14 a
	10	14 a	14 a	14 a	14 a
Biological yield, kg/ha	0	2102 a	2010 a	2056 a	2341 a
	10	2204 a	2840 a	2522 a	2545 a
Grain yield kg/ha.	0	892 a	797 b	844 b	599 a
	10	932 a	1185 a	1059 a	467 a
Harvest index	0	44 a	43 a	43 a	26 a
	10	43 a	42 a	42 a	19 a

Means followed by different letters in a column are significantly different according to LSD at 5%.

Table-3a. Effect of Farm Yard Manure (FYM) on Nodules/ Plant and dry matter accumulation in chick pea and wheat. 1992-93.

	Langer Khel		Titter Khel		Peshawar	
Days after sowing.	80	140	80	140	60	120
FYM t/ha			Nodules/plant			
0	10	6	10	5	6	10
10	21	9	20	7	5	11
			Chickpea D.M (g/m^2)			
0	13	160	7	180	52	179
10	24	219	11	261	60	165
			Wheat D.M (g/m^2)			
0	9	169	2	115	-	80
10	10	141	2	154	-	78

Table-1. Grain yield (kg/ha) of wheat cv Pirsabak-85 as affected by methods and levels of N&P application at three Pakistan locations, 1992-93.

Kg/ha N	Kg/ha P	Langer Khel	Titter Khel	Combined	Peshawar
0	0	195 F	376 H	285 E	2333
0	23	266 EF	449 F	357 D	2222
0	46	327 E	425 G	376 D	1722
23	0	439 D	715 D	377 C	2139
23	23	508 CD	685 E	597 BC	1944
23	46	548 BC	707 D	627 B	1889
46	0	597 BC	572 B	734 A	1972
46	23	629 AB	918 A	774 A	1944
46	46	723 A	805 C	764 A	2444
LSD 5%		10.1	1.4	5.0	
Methods					
Band		446	646	546	2197
B.Cast		494	677	582	1938

Means followed by different letters in a column are significantly different according to LSD at 5%.

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Table:-2. Number of Spikes/unit area of wheat cv Pirsabak-85 as affected by methods and levels of NP application at two Pakistan Locations. 1992-93.

Kg/ha N	P	Langer Khel	Titter Khel	Combine
0	0	28 E	52 E	40 F
0	23	33DE	52 E	42 F
0	46	34 DE	72 CD	53 E
23	0	38 CD	72CD	55 C
23	23	42 BC	71 D	56 DE
23	46	43 BC	78 BC	60 CD
46	0	45 ABC	89 A	67 A
46	23	49 AB	75 BCD	62 BC
46	46	52 A	80 B	66 AB
LSD 5%		7.59	6.217	4.74
Methods				
Band		40	71	55
B.Cast		41	71	56

Table:-3. 100 grain weight(gm) of wheat cv Pirsabak-85 as affected by methods and levels of NP application at three Pakistan locations. 1992-93.

Kg/ha N	P	Langer Khel	Titter Khel	Combine	Peshawar
0	0	3.2 D	4.2 B	3.7 E	3.7
0	23	3.4 CD	4.1 BC	3.7 E	3.8
0	46	3.8 BC	4.1 BC	3.9 CDE	3.7
23	0	3.8 BC	4.1	3.9 DE	3.7
23	23	4.3 A	4.3 B	4.3 AB	3.7
23	46	4.1 AB	3.9 C	3.9 CD	3.7
46	0	4.1 AB	4.2 BC	4.4 AB	3.8
46	23	4.2 AB	4.2 B	4.2 BC	3.6
46	46	4.5 A	4.6 A	4.5 A	3.7
LSD 5%		0.466	0.265	0.26	
Method					
Band		3.8	4.2	4.0	3.7
B.cast		4.1	4.2	4.1	3.8

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Table:-4. Biological yield Kg/ha. of wheat cv Pirsabak-85 a: affected by methods and levels of NP application at three Pakistan locations. 1992-93.

Kg/ha N	Kg/ha P	Langer Khel	Titter Khel	Combine	Peshawar
0	0	584 F	1109 H	847 H	5889
0	23	755 E	1282 F	1018 G	5444
0	46	941 D	1240 G	1091 F	6139
23	0	1042 D	1703 E	1372 E	5139
23	23	1243 C	1687 E	1465 D	5555
23	46	1491 B	1920 D	1706 D	5361
46	0	1484 B	2147 A	1815 B	5444
46	23	1426 B	2060 C	1743 C	4477
46	46	1888 A	2111 B	1999 A	5805
LSD 5%		13.53	2.19	6.66	
Method					
Band		1113	1606	1359	5389
B.cast		1299	1785	1542	5555

Table:-5. Harvest index of wheat cv Pirsabak-85 as affected by methods and levels of N&P application at three Pakistan locations. 1992-93.

Kg/ha N	Kg/ha P	Langer Khel	Titter Khel	Combine	Peshawar
0	0	34 D	34 C	34 F	40
0	23	37 BCD	39 ABC	38 CDE	42
0	46	35 CD	36 BC	36 FE	30
23	0	42 AB	42 AB	42 AB	41
23	23	41 AB	38 ABC	40 BCD	35
23	46	37 BCD	38 ABC	37 DEF	38
46	0	40 ABC	43 A	40 ABC	35
46	23	44 A	44 A	43 A	49
46	46	38 ABCD	39 ABC	38 BCDE	41
LSD 5%		5.50	5.15	3.69	
Method					
Band		40	40	40	43
B.cast		37	39	38	36

Table:-6. Plant Height (cm) of wheat cv Pirsabak-85 as affected by methods and levels of NP application at two Pakistan locations. 1992-93.

Kg/ha N	P	Langer Khel	Titter Khel	Combine
0	0	49	55 D	51 C
0	23	52	55 D	54 C
0	46	51	53 E	52 C
23	0	59	64 B	61 B
23	23	64	60 C	62 B
23	46	65	59 C	62 B
46	0	66	66 A	66 A
46	23	68	67 A	68 A
46	46	69	66 A	67 A
LSD 5%		-	1.76	2.34
Method				
Band		60	60	60
B.cast		61	61	61

Table:-7. Grain yield kg/ha of chickpea as affected by methods and levels of NP application at three Pakistan locations. 1992-93.

Kg/ha N	Kg/ha P	Langer Khel	Titter Khel	Combine	Peshawar
0	0	422 F	667 D	544 G	433
0	23	533 E	622 D	578 G	316
0	46	591 E	1033 C	812 F	219
23	0	720 D	1400 B	1060 E	377
23	23	905 C	1666 B	1285 D	233
23	46	872 C	2356 A	1614 C	298
46	0	1082 B	2322 A	1702 BC	317
46	23	1177 B	2533 A	1855 AB	384
46	46	1443 A	2622 A	2033 A	357
LSD 5%		9.87	35.20	17.93	-
Method					
Band		668	1521	1094	282
B.cast		1053	1862	1458	371

Table:-8. 100 grain weight (gm) of chickpea as affected by methods and levels of NP application at three Pakistan locations. 1992-93.

Kg/ha N	Kg/ha P	Langer Khel	Titter Khel	Combine	Peshawar
0	0	10.2 D	11.7 F	10.9 F	13.3
0	23	11.9 C	12.5 E	12.2 E	12.4
0	46	13.2 B	12.8 E	13.0 D	11.5
23	0	13.5 B	13.7 D	13.6 CD	11.7
23	23	14.4 B	14.0 D	14.2 C	13.4
23	46	13.9 B	14.2 D	14.1 C	13.3
46	0	16.0 A	15.2 C	15.6 B	12.5
46	23	16.8 A	15.8 B	16.3 A	11.6
46	46	16.7 A	16.8 A	16.7 A	12.5
LSD 5%		1.33	0.65	0.728	
Method					
Band		13.7	14.2	14.1	12.4
B.cast		14.3	13.9	14.1	12.5

Table:-9. Number of Pods per plant of chickpea as affected by methods and levels of NP application at two Pakistan locations. 1992-93.

Kg/ha N	Kg/ha P	Langer Khel	Titter Khel	Combine Agril.Uni.
0	0	18 E	26 E	21 DE
0	23	19 DE	21 F	20 E
0	46	19 DE	27 E	23 D
23	0	22 CDE	31 D	27 C
23	23	26 ABC	32 D	29 C
23	46	25 BCD	17 G	21 DE
46	0	30 AB	47 B	39 B
46	23	31 A	55 A	43 A
46	46	31 A	45 C	38 B
LSD 5%		5.47	1.97	2.765
Method				
Band		25	37	31
B.cast		24	30	27

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Table:-10. Number of grains per pod of chickpea as affected by methods and levels of NP application at three Pakistan locations. 1992-93.

Kg/ha N	P	Langer Khel	Titter Khel	Combine	Peshawar
0	0	2	2	2	2
0	23	2	2	2	2
0	46	2	2	2	2
23	0	2	2	2	2
23	23	2	2	2	2
23	46	2	2	2	2
46	0	2	2	2	2
46	23	2	2	2	2
46	46	2	2	2	2
LSD 5%		-	-	-	-
Method					
Band		2	2	2	2
B.cast		2	2	2	2

Table:-11. Plant Height (cm) of wheat cv Pirsabak-85 as affected by methods and levels of NP application at two Pakistan locations. 1992-93.

Kg/ha N	P	Langer Khel	Titter Khel	Combine
0	0	49	55 D	51 C
0	23	52	55 D	54 C
0	46	51	53 E	52 C
23	0	59	64 B	61 B
23	23	64	60 C	62 B
23	46	65	59 C	62 B
46	0	66	66 A	66 A
46	23	68	67 A	68 A
46	46	69	66 A	67 A
LSD 5%		-	1.76	2.34
Method				
Band		60	60	60
B.cast		61	61	61

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Table:-12. Biological Yield kg/ha of chickpea as affected by methods and levels of NP application at three Pakistan locations.1992-93.

Kg/ha N	Kg/ha P	Langer Khel	Titter Khel	Combine	Peshawar
0	0	1123 G	1663 G	1393 H	2333
0	23	1434 F	1622 G	1528 H	3033
0	46	1442 F	2621 F	2031 G	2464
23	0	1867 E	3534 E	2700 F	2444
23	23	2362 D	4201 D	3281 E	2478
23	46	2258 D	5853 C	4055 D	2144
46	0	2833 C	6103 B	4468 C	2325
46	23	3052 B	6444 A	4748 B	2778
46	46	3728 A	6580 A	5149 A	2347
LSD 5%		20.32	19.15	13.8	-
Method					
Band		1761	3945	2853	1898
B.cast		2703	4637	3669	3067

Pre-flooding tillage influences on wheat and chickpea production in a semi-arid climate of Pakistan

H.U. Khan, M. Azam, I.U. Awan and M.A. Khan
Faculty of Agriculture, Gomal University
D.I.Khan, NWFP, Pakistan.

ABSTRACT

The Rod-Kohi (flood irrigation/hill torrent) agriculture practiced in the Daman area of the Dera Ismail Khan Division of North West Frontier Province, Pakistan, is unique, bound in tradition and influenced little by modern technology. Water conservation is a major concern because the climate is hot and arid. During the winter growing season, water stored in the soil from a spring or summer flood is the primary source of water. An on-farm research project was started during the 1992 crop season to determine the impacts of alternative tillage practices on crop water availability.

Four preflood tillage practices were evaluated: (1) cultivator, (2) moldboard plough, (3) disc plough, and (4) chisel plough. Two varieties of wheat (Triticum aestivum L.), local and improved variety (Pirsabak-91), and two varieties of chickpea (Cicer arietinum L.), local and improved variety (CM-72), were grown. Each tillage practice was replicated four times in a split plot design. Tillage impacts on soil water content, plant growth, and crop yields were measured.

The availability of water increased with an increase in the depth of tillage: more water was utilized by the wheat plants when the chisel plough was used. Among the four tillage practices, there was no pronounced effect on the characteristics of the wheat and chickpea varieties under study. The improved variety of wheat gave more yield/ha than the local variety, while the improved variety of chickpea gave a slightly better yield than the local one.

INTRODUCTION

The Rod-Kohi (flood irrigation/hill torrent) agriculture practiced in the Daman area of the Dera Ismail Khan Division of North West Frontier Province, Pakistan, is unique, bound in tradition and influenced little by modern technology. Water conservation is a major concern because the climate is hot and arid. During the winter growing season, water stored in the soil from a spring or summer flood is the primary source of water.

Tillage impacts on water availability in the Rod-Kohi irrigated area have not been assessed. However, if water availability could be increased through tillage, the impacts would be very significant since surface water supplies in the area are both limited in amount and erratic in availability. Adopting new crop varieties may be another option for increasing agricultural productivity. Consequently, this study was undertaken to test alternative tillage practices and to test new wheat and chickpea varieties, the commonly grown crops in the area, for the purpose of increasing

agricultural productivity and thereby the living standards of the area's farming communities.

The Daman area is about 0.37 x 10^6 hectares (ha). The farming communities in the area are poor.

The Rod-Kohi irrigation system existed prior to 1905, when it was formally organized under the district administration during the establishment of water rights and land settlements (Bolton, 1990). The Rod-Kohi area is irrigated from semiperennial streams (zams) or from seasonal hill torrents (nullahs). The main sources of irrigation water are Tank Zam, Gomal Zam, Sheikh Haider Zam, Daraban Zam, and Chaudwan Zam (see Figure 1), and miscellaneous hill torrents of District Tank and Tehsil Kulachi. Torrents result from individual rains of 60 to 150 mm, which can occur from mid-February to mid-April and from mid-July to late August. The summer torrents are the major ones, resulting from rains on Sulaiman Range in the west and Marwat Range on the north. The catchment areas of these ranges are barren. Steep slopes exist upstream of the gorges from which the torrents emerge. These torrents have steep gradients and high velocity, and contain large quantities of silt and clay which are deposited on flatter slopes. The torrents often change their course in the piedmont or flood plains and are thus likely to damage lands. The flood water is diverted to fields varying in area (2-10 ha) and surrounded by earthen dikes (bunds) 1-2 m high .

The climate of The Rod-Kohi area is arid to semi-arid, with precipitation occurring in the monsoon (Kharif) and spring (Rabbi) seasons. Annual precipitation varies from 120 to 370 mm. In the Kharif season, rain occurs in the months of July and August, and in the Rabbi season in the months of March and April. June is the warmest month, with a mean maximum temperature of 41 ^{0}C. January is the coldest month, with a mean minimum temperature of 4 ^{0}C. Because of low humidity, the continental location, and a dearth of vegetation, extremes in climate are commonly experienced.

Agricultural Productivity in Daman

The productivity of traditional crops such as wheat, chickpeas, sorghum, millet, and rapeseed/mustard in the Daman area is well below the national average (Ahmad, 1990). This is due to the practice of outmoded and poor methods of agriculture, as well as a lack of soil moisture conservation practices. The fortunes of the Rod-Kohi farmers vary from subsistence to devastation. The latter could be due either to a lack or an overabundance of hill torrents. Soil deposition is large enough to obliterate the soil structure every year.

Only in recent years has any effort been made to evaluate the agricultural production potential of the Rod-Kohi irrigated lands (Amin, 1990; Wadud and Ahmad, 1990; Khan, 1990). The introduction of improved crop varieties and the modification of tillage practices have been identified as potential on-farm practices that could increase agricultural productivity.

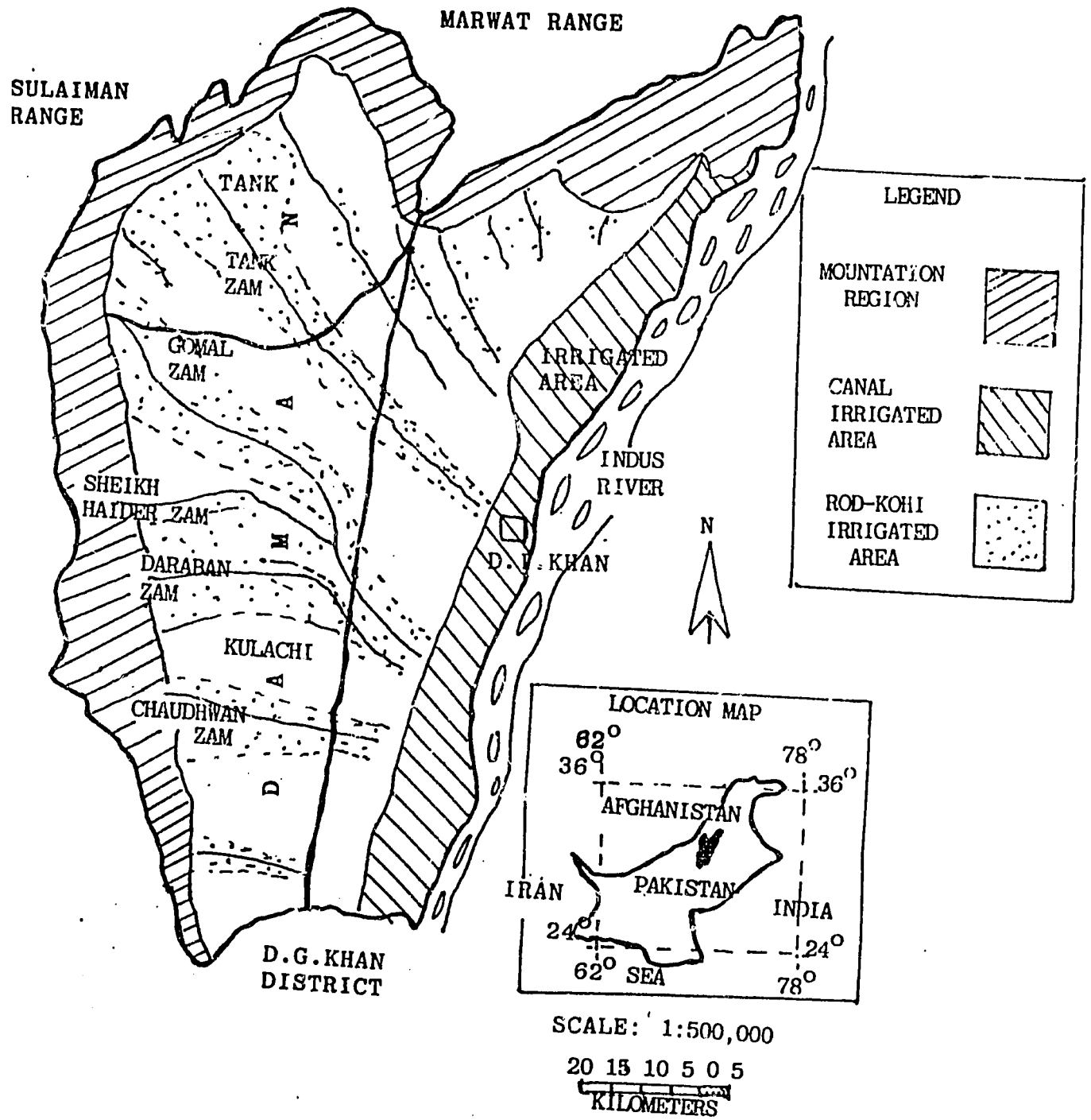

Figure 1. MAP OF DERA ISMAIL KHAN, NORTH WEST FRONTEIR PROVINCE, PAKISTAN

Tillage Practices and Water Availability

According to Unger (1990), water conservation is the net result of the interactions among a series of complex factors and processes. These include climate; the amount, distribution, and type of rainfall; soil physical and hydraulic properties; tillage and residue management practices; weed control; and the crops grown.

Under dryland conditions, especially where growing season precipitation is limited, the amount of plant-available water stored in the soil at planting time has a major effect on the growth and yield of the crop. For example, grain yields of dryland wheat and grain sorghum increased an average of 7.2 and 17.0 kg ha^{-1}, respectively, for each additional millimeter of plant-available water in the soil at planting time (Johnson, 1964; Jones and Hauser, 1975).

Although many factors are involved in the conservation of water in the soil, tillage practices enhance water storage by enabling quick infiltration of stored water and allowing less time for evaporation to take its toll. In arid and semi-arid areas, tillage to depths greater than ordinary ploughing can increase crop yields (Eck and Unger, 1985). Improved germination, tillering, and yield are attributable to a decrease in bulk density and an increase in water infiltration because soil layers that restrict water are disrupted. The water is thus stored in deeper layers (Eck and Taylor, 1969; Singh et al., 1991).

Beneficial, long-term (21 years) effects of soil modification on wheat yields have been documented by Eck (1986). Hardpan or restricting layers below the surface can be removed by ploughing with a chisel or disc plough, which causes a mixing of profile layers (Bradfield and Blanchar, 1977; Burnett and Hauser, 1967; Doty et al., 1975; Eck et al., 1977). The effect of deep tillage is usually greatest when the soil is relatively dry. The benefits usually observed from deep tillage are greater water infiltration and conservation and better root growth, which allows roots to extract water and nutrients from a larger soil volume.

The economics of deep tillage are often unfavourable, except for the production of high-value crops. A fairly recent concept, in which only a portion of the soil restricting zone is affected, is in-row subsoil tillage. In this method, a narrow chisel subsoiler is operated in front of the planter opener. This method is effective in allowing row crop root penetration through a root-restricting compacted tillage zone in coarse-textured soils (Trouse, 1978; Smith et al., 1983).

Tillage affects soil aggregate size distribution and stability, surface roughness, and hence water infiltration. Loosening of the soil with sweep tillage is effective for increasing water infiltration where surface residues are limited. Moldboard ploughing results in lower final infiltration rates than noninverting tillage methods because fewer stable aggregates are deposited on the surface with the latter method (Unger, 1992). However, moldboard ploughing can increase the vegetative growth of

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grasses and some vegetables whose root systems are shallow (Eck and Davis, 1971; Mathers et al., 1971). The most effective tillage methods for increasing infiltration are those that maintain as much organic matter as possible at or near the soil surface, or those that leave the surface rough and more resistant to crusting.

MATERIALS AND METHODS

Study Preparations and Procedures

A general survey of the Rod-Kohi area was made in June 1992. Many villages in the area were visited, and the farmers interviewed and familiarized with the objectives of the project. The main purpose of these efforts was to acquire suitable land for setting up the experiments. Two sites near the village of Shero Kohna about 25 km west of Dera Ismail Khan city were selected, one for wheat and the other for chickpeas. The area of the land at each site was about 2 ha.

The field experiments were conducted during 1992-93. They were carried out in a split plot design with four replications. The plot size was 7.03 x 14.25 m^2.

The land was prepared with four tillage implements--cultivator (0-15 cm depth), moldboard plough (0-30 cm depth), disc plough (0-45 cm depth), and chisel plough (0-60 cm depth)--before preflooding in June 1992. Two varieties were used in each experiment, that is, both local and improved varieties of wheat and chickpeas (Table 1).

TABLE 1

Treatments used in the experiment during 1992-93.

Main Plot	Treatments Tillage Practices
	Cultivator Disc Plough Moldboard Plough Chisel Plough
Sub Plot	**Varieties**
	Local Improved

Soil samples were collected before the crops were sown and analysed for physio-chemical properties per the standard method (Table 2).

The fields were ploughed again with a cultivator in the last week of October and first week of November, 1992, for sowing of the chickpeas and wheat, respectively. The seed rate was 80 kg/ha and 100 kg/ha for the chickpeas and wheat, respectively. Germination trials were run in the laboratory at room temperature to determine

the percentage of germination of both varieties of chickpeas and wheat. (Table 3).

TABLE 2

Physio-chemical characteristics of soils.

Characteristic	Wheat Field	Chickpea Field
Textural class	Sandy loam	Sandy clay loam
pH (1:2.5)	8.71	8.62
NH_4-N (mg/kg)	0.70	0.80
No_3-N (mg/kg)	2.10	2.30
P (µg/g)	17.00	18.00
K (mg/kg)	495.00	551.00

TABLE 3

Germination of seeds.

Name of Variety	Germination (%)
Wheat - Improved (Pirsabak-91)	100
Wheat - Local	98
Chickpea - Improved (CM-72)	100
Chickpea - Local	65

On October 15, 1992, before sowing of crops, soil samples were collected from three places per treatment with an auger to a depth of 20 cm to determine the average water content. The depth of 20 cm was chosen because most of the root system--98 percent in the case of wheat and 90 percent in the case of chickpeas--occurs in the 0-20 cm depth interval. The depth of the soil sampled was increased to 90 cm on May 5, 1993, just after the harvesting of the crops. There had been rains of 2 mm and 62 mm on February 25 and March 3, respectively, adding extra water to the soil. The collected samples were thoroughly mixed in polyethylene bags and stored in a refrigerator until analyzed. The bulk density of the soil under various treatments was also determined. The procedures used for determing average water content at various depths and bulk density were after Hussain and Jabbar (1985) (Tables 4-8).

TABLE 4

Soil water (cm) with different treatments in Wheat crop field, October 15, 1992

Depth (cm)	Cultivator	Moldboard Plough	Disc Plough	Chisel Plough	Mean
0-5	0.13 j	0.15 j	0.13 j	0.29 i	0.17 d
5-10	0.33 hi	0.44 g	0.29 i	0.37 h	0.36 c
10-15	0.58 cde	0.57 de	0.54 ef	0.51 f	0.55 b
15-20	0.61 bcd	0.63 bc	0.66 ab	0.70 a	0.65 a
Mean	0.41 a	0.45 a	0.40 a	0.47 a	

Means followed by same letter(s) are not significantly different at 5% level of significance according to LSD test.

TABLE 5

Soil water (cm) with different treatments in Wheat crop field, May 5, 1993

Depth (cm)	Cultivator	Moldboard Plough	Disc Plough	Chisel Plough	Mean
0-10	0.62 fgh	0.26 lmn	0.15 n	0.15 n	0.30 d
10-20	1.20 b	0.57 fghi	0.57 fghi	0.28 klmn	0.66 abc
20-30	1.44 a	1.02 cd	0.56 fghi	0.22 mn	0.81 a
30-40	1.29 ab	0.63 fg	0.67 f	0.25 lmn	0.71 abc
40-50	1.15 bc	0.35 jklm	0.63 fg	0.38 jklm	0.63 bc
50-60	0.94 de	0.22 mn	0.84 e	0.28 klmn	0.57 c
60-70	1.29 ab	0.35 jklm	0.94 de	0.41 ijkl	0.75 abc
70-80	0.56 fghi	0.42 ijkl	0.98 cde	0.45 ghijkl	0.60 bc
80-90	1.15 bc	0.49 fghij	0.99 cde	0.45 hijk	0.77 ab
Mean	1.07 a	0.48 c	0.70 b	0.32 d	

Means followed by same letter(s) are not significantly different at 5% level of significance according to LSD test.

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TABLE 6

Soil water (cm) with different treatments in Chickpea crop field, October 15, 1992

Depth (cm)	Cultivator	Moldboard Plough	Disc Plough	Chisel Plough	Mean
0-5	0.16 h	0.18 h	0.19 h	0.29 g	0.21 c
5-10	0.30 g	0.36 fg	0.37 f	0.36 fg	0.35 b
10-15	0.42 ef	0.50 bcd	0.48 cde	0.55 bc	0.49 a
15-20	0.47 de	0.57 ab	0.51 bcd	0.65 a	0.55 a
Mean	0.34 a	0.40 a	0.39 a	0.46 a	

Means followed by same letter(s) are not significantly different at 5% level of significance according to LSD test.

TABLE 7

Soil water (cm) with different treatments in Chickpea crop field, May 5, 1993

Depth (cm)	Cultivator	Moldboard Plough	Disc Plough	Chisel Plough	Mean
0-10	0.20 qr	0.32 pq	0.35 pq	0.14 r	0.25 e
10-20	0.66 jklm	0.74 ijk	0.90 hi	0.53 mno	0.71 c
20-30	0.70 jkl	0.74 ijk	0.77 ij	0.41 op	0.66 c
30-40	0.43 nop	0.48 nop	0.36 p	0.42 op	0.42 d
40-50	1.11 efg	0.35 pq	0.59 klmn	0.54 lmno	0.65 c
50-60	1.12 efg	0.67 jklm	1.12 efg	0.80 ij	0.93 b
60-70	1.18 def	0.39 op	1.26 cde	0.98 gh	0.95 b
70-80	1.30 bcd	1.05 fgh	1.34 abc	0.98 gh	1.17 a
80-90	1.44 ab	1.16 def	1.48 a	1.05 fgh	1.29 a
Mean	0.90 a	0.66 a	0.91 a	0.65 a	

Means followed by same letter(s) are not significantly different at 5% level of significance according to LSD test.

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TABLE 8

Effect of different tillage practices on the Bulk Density of soil in the Wheat and Chickpea fields at a depth of 5-15 cm.

Tillage used (up to depth of)	Wheat field (Sandy loam)	Chickpea field (Sandy clay loam)
Cultivator (15 cm)	1.04 g/cm^3	1.18 g/cm^3
Moldboard Plough (30 cm)	1.57 g/cm^3	1.31 g/cm^3
Disc Plough (45 cm)	1.29 g/cm^3	1.20 g/cm^3
Chisel Plough (60 cm)	1.34 g/cm^3	1.34 g/cm^3

Observations

Data on various plant characteristics were recorded following the harvesting of the crops in the first week on May 1993. These data included the following: plant height at maturity, number of tillers/plant, length of spike, number of spikelets/spike, number of grains/spike, weight of 1000 grains, and yield (t/ha).

Plant height at maturity. Ten plants were selected at random from each plot. Their height was measured from the soil surface to the tip of the spike with the help of a meter rod, and average height was calculated (Table 9).

Number of tillers/plant. Ten plants were selected at random from each plot. Their tillers were counted, and the average number of tillers/plant was calculated (Table 9).

Length of spike. Ten plants were selected at random from each plot. The length of the spikes was measured, and the average length of spike was calculated (Table 9).

Number of spikelets/spike. Ten spikes were selected at random from each plot. The spikelets in each spike were counted, and the average number of spikelets/spike was calculated (Table 10).

Number of grains/spike. Ten spikes were selected at random from each plot. The grains were removed from the spikes separately, and the number of grains in each spike was counted. The average number of grains/spike was then calculated (Table 10).

TABLE 9
Effect of different tillage practices on Plant height, Number of Tillers per Plant, and Length of Spikes in Wheat varieties.

Tillage Used (up to depth of)	Average Plant Height (cm)			Average No. of Tillers/Plant			Average Length of Spike (cm)		
	Local Variety	Improved Variety (PS-91)	Mean	Local Variety	Improved Variety (PS-91)	Mean	Local Variety	Improved Variety (PS-91)	Mean
Cultivator (15 cm)	127.00 a	87.83 b	107.42 a	10.00 cd	14.00 ab	12.00 a	8.60 b	10.00 b	9.30 ab
Moldboard Plough (30 cm)	101.00 ab	86.00 b	93.50 a	5.00 e	11.00 bc	8.00 a	6.60 c	10.00 b	8.30 b
Disc Plough (45 cm)	115.00 ab	85.50 b	100.25 a	7.00 de	15.00 a	11.00 a	9.00 b	11.80 a	10.40 a
Chisel Plough (60 cm)	124.00 a	82.00 b	103.00 a	6.00 e	13.00 abc	9.50 a	9.00 b	10.00 b	9.50 ab
Mean	116.75 a	85.33 b		7.00 b	13.25 a		8.30 b	10.45 a	

Means followed by same letter(s) are not significantly different at 5% level of significance according to LSD test.

TABLE 10
Effect of different tillage practices on the Number of Spikelets per Spike, Number of Grains per Spike, Weight of 1000 Grains, and Yield per hectare in Wheat varieties.

Tillage Used (up to depth of)	Average No. of Spikelets/Spike			No. of Grains/Spike			Weight of 1000 Grains (g)			Yield (tones/ha)		
	Local Variety	Improved Variety (PS-91)	Mean	Local Variety	Improved Variety (PS-91)	Mean	Local Variety	Improved Variety (PS-91)	Mean	Local Variety	Improved Variety (PS-91)	Mean
Cultivator (15 cm)	16.80 bc	21.00 ab	18.90 a	34.00 c	62.00 b	48.00 a	50.00 a	46.00 ab	48.00 a	2.63 e	3.15 c	2.89 c
Moldboard Plough (30 cm)	12.00 d	19.00 abc	15.50 b	40.00 c	67.00 ab	53.50 a	45.00 ab	46.00 ab	45.50 ab	2.47 f	3.51 b	2.99 bc
Disc Plough (45 cm)	17.00 bc	21.80 a	19.40 a	36.00 c	68.80 a	52.40 a	42.00 b	50.00 a	46.00 ab	2.35 f	3.82 a	3.10 b
Chisel Plough (60 cm)	14.80 cd	21.00 ab	17.90 a	39.00 c	67.00 ab	53.00 a	40.00 b	44.00 ab	42.00 b	2.78 d	3.86 a	3.32 a
Mean	15.15 b	20.70 a		37.25 b	66.20 a		44.25 a	46.50 a		2.56 b	3.58 a	

Means followed by same letter(s) are not significantly different at 5% level of significance according to LSD test.

Weight of 1000 grains. Weight of 1000 grains replicated three times, was determined after harvesting the crop with a triple beam balance (Tables 10 and 11).

Grain yield. An area of 10 m^2 replicated three times was harvested from each plot. The areas were selected at random, avoiding the border effects. Grain yields, expressed in t/ha, were weighed using a triple beam balance (Tables 10 and 11).

Statistical Analysis

The data were analyzed statistically using Fisher's analysis of variance technique (Steel and Torrie, 1980) and Duncan's multiple range test (Duncan, 1955) to determine the significance of the treatments at 5 percent probability level.

TABLE 11

Effect of different tillage practices on the Weight of 1000 Grains and Yield per hectare in Chickpea varieties.

Tillage Used (up to depth of)	Weight of 1000 Grains (g)			Yield (tones/ha)		
	Local Variety	Improved Variety (CW-72)	Mean	Local Variety	Improved Variety (CW-72)	Mean
Cultivator (15 cm)	143.00 a	147.00 a	145.00 a	4.74 d	5.00 bc	4.87 a
Moldboard Plough (30 cm)	145.00 a	146.00 a	145.50 a	4.94 c	5.08 b	5.01 a
Disc Plough (45 cm)	148.00 a	147.00 a	147.50 a	4.49 f	5.50 a	5.00 a
Chisel Plough (60 cm)	146.00 a	146.00 a	146.00 a	4.64 de	4.61 e	4.63 b
Mean	145.50 a	146.50 a		4.70 b	5.05 a	

Means followed by same letter(s) are not significantly different at 5% level of significance according to LSD test.

RESULTS AND DISCUSSION

The study results are based on a one-year (1992-93). The experiments will be repeated in other Rod-Kohi areas in subsequent years.

Seed Germination Rate

The laboratory testing showed that the improved variety of wheat (Pirsabak-91) had a 100 percent germination rate, while the local variety had a 98 percent germination rate. The improved variety of chickpeas (CM-72) had a 100 percent germination rate, while the local variety had a 65 percent germination rate (Table 3). The seed rate of the local variety (CM-72) was increased appropriately for the experiment to cope with the lower germination rate.

Soil Water

The amount of soil water in the wheat crop field before and after the termination of the experiment is shown in Tables 4 and 5.

All the tillage practices significantly affected the soil water content at various depths of soil in the beginning of the experiment. However, the mean values were nonsignificant, showing the total water content to be almost the same under the four tillage practices (Table 4). The same trend was noted at the end of the experiment. However, the mean values were significantly different in this case (Table 5). Among the treatments, more soil water was retained with the tillage cultivator (1.07 cm), followed by the disc plough (0.70 cm) and the moldboard plough (0.48 cm), and finally the chisel plough (0.32 cm). The lower soil water content with the chisel plough indicates that more water was absorbed/taken up by the plants with this treatment as compared with the others.

The amount of soil water in the chickpea crop field before and after the termination of the experiment is shown in Tables 6 and 7.

As with the wheat crop field, all the tillage practices significantly affected the soil water content at various depths of soil in the beginning of the experiment. However, the mean values were nonsignificant, showing the total water content to be almost the same under the four tillage practices (Table 6). The same trend as with the wheat field was noted at the end of the experiment as well (Table 7). As with the wheat crop field, more soil water was retained with the tillage cultivator (0.90 cm) and the disc plough (0.91 cm), followed by the moldboard plough (0.66 cm) and the chisel plough (0.65 cm). Again the decrease in soil water content with the chisel plough indicates that more water was absorbed/taken up by the plants with this treatment as compared with the others.

Bulk Density

There is marked variation in the values of bulk density under the different tillage practices. These values are lower for the

cultivator as compared with the values obtained with the other tillage practices indicating more compaction of soil in the latter treatments (Table 8).

Plant Characteristics: Wheat Crop

Data on the plant characteristics cited earlier were recorded after the crops were harvested.

Plant Height

The height of the plants of the local variety was significantly greater (116.75 cm) than that of the improved variety (85.33 cm). The height of the plants of the local variety was also affected by the type of tillage: the plants with the cultivator and chisel plough treatments were significantly higher (127 cm and 124 cm, respectively) than the ones with the moldboard and disc ploughs (101 cm and 115 cm, respectively). However, there was no affect of the type of tillage on the height of the plants of the improved variety, the plants being almost of uniform size (Table 9).

Number of Tillers/Plant

The number of tillers/plant was significantly higher (13.25) in the improved than in the local variety (7.0). There was a significant effect of the type of tillage on this characteristic with both varieties. The number of tillers/plant for the local variety was highest with the cultivator (10.0), followed by the disc plough (7.0), chisel plough (6.0) and moldboard plough (5.0). A similar same trend was noted with the improved variety. The number of tillers/plant was highest with the disc plough (15.0), followed by the cultivator (14.0), the chisel plough (13.0), and the moldboard plough (11.0) (Table 9).

Length of Spike

The spikes were significantly shorter (8.30 cm) with the local than with the improved variety (10.45 cm). There was little effect of the type of tillage on this characteristic with either variety, except that with the local variety, the spikes were significantly shorter (6.0 cm) with moldboard plough than with the other treatments (8.0-9.0 cm), while with the improved variety, the spikes were longer (11.8 cm) with the disc plough than with the other treatments (10.0 cm) (Table 9).

Number of Spikelets/Spike

The number of spikelets/spike is directly related to the length of the spike: the longer the spike, more spikelets it will bear. The improved variety bore significantly more spikelete/spike (20.70) than the local one (15.15). There was a partially significant effect of the type of tillage on this characteristic with both varieties.

The number of spikelets/spike were significantly higher with the disc plough (17.0) and cultivator (16.8) than with the chisel plough (14.8) and moldboard plough (12.0) in the local variety. A

similar trend was noted in the improved variety: the number of spikelets/spike was significantly higher with the disc plough (21.8) and cultivator/chisel plough (21.0) than with the moldboard plough (19.0) (Table 10).

Number of Grains/Spike

The number of grains/spike is directly related to both the length of the spike and the number of spikelets/spike: a longer spike with more spikelets will bear more grains. The improved variety had significantly more grains/spike (66.20) than the local one (37.25). The type of tillage had no effect on this characteristic in the local variety. However, it did have an effect in the improved variety: the number of grains was highest with the disc plough (68.80), followed by the moldboard plough/chisel plough (67.0) and the cultivator (62.0) (Table 10).

Weight of 1000 Grains

The difference in the weight of 1000 grains was nonsignificant in the two varieties: the weight of 1000 grains was 44.25 g in the local variety and 46.50 g in the improved variety. There was a partially significant effect of the type of tillage on this characteristic in both the varieties. In the local variety, the weight of 1000 grains was highest with the cultivator (50 g), followed by the moldboard plough (45 g), the disc plough (42 g), and the chisel plough (40 g). In the improved variety, the weight of 1000 grains was highest with the disc plough (50 g), followed by the cultivator/moldboard plough (46 g) and the chisel plough (44 g) (Table 10).

Grain Yield

The grain yield was significantly higher in the improved variety (3.58 t/ha) than in the local one (2.56 t/ha). A similar yield (3.06 t/ha) was reported for an improved variety (in this case Pirsabak-85) in another area of Rod-Kohi, where deep tillage (disc plough only) was used (Khan, 1990).

There was a partially significant effect of the type of tillage on this characteristic in both varieties. In the local variety, significantly more yield was obtained with the chisel plough (2.78 t/ha) than with the cultivator (2.63 t/ha), followed by the moldboard plough (2.47 t/ha) and disc plough (2.35 t/ha). A similar trend was observed in the improved variety: more yield with the chisel plough (3.86 t/ha)/disc plough (3.82 t/ha) than with the moldboard plough (3.51 t/ha) and cultivator (3.15 t/ha) (Table 10).

Plant Characteristics: Chickpea Crop

As with the wheat crop, data on plant characteristics were recorded after the crops were harvested.

Weight of 1000 Grains

The difference in the weight of 1000 grains was nonsignificant in the two varieties: 145.50 g in the local variety and 146.50 g in

the improved variety (CM-72). This characteristic was not affected by the type of tillage with either variety (Table 11).

Grain Yield

The yield of the improved variety was significantly higher (5.05 t/ha) than that of the local variety (4.70 t/ha). There was a significant effect of the type of tillage on yield with both varieties. With the local variety significantly more yield was obtained with the moldboard plough (4.94 t/ha), followed by the cultivator (4.74 t/ha), the chisel plough (4.64 t/ha), and the disc plough (4.49 t/ha). However, with the improved variety, significantly more yield was obtained with the disc plough (5.50 t/ha), followed by the moldboard plough (5.08 t/ha), the cultivator (5.00 t/ha), and the chisel plough (4.61 t/ha) (Table 11). The yield of both the varieties showed an enormous increase (more than double) over that reported previously (Khan, 1990) for another Rod-Kohi area. The tillage used in that study was the disc plough. It is also reported for that study that the chisel plough treatment seemed to give almost the same yield as the disc plough.

GENERAL SUMMARY AND CONCLUSIONS

Rod-Kohi agriculture is practiced in the Daman area of the Dera Ismail Khan Division of North West Fronteir Province, Pakistan. This is a unique traditional system whereby flood water is stored in the fields, serving as the main source of irrigation for growing crops. The yield/ha is very low, and the farmers are living below, the poverty line. The area has an arid to semi-arid climate.

An on-farm project was started in 1992-93 with a view to enhancing agricultural production in these areas. Four preflood tillage practices--cultivator (0-15 cm depth), moldboard plough (0-30 cm depth), disc plough (0-45 cm depth), and chisel plough (0-60 cm depth) were used to determine the degree of water conservation in the soil, and its subsequent effects on various plant characteristics and grain yield in wheat and chickpea varieties. Two varieties of wheat--local and improved (Pirsabak-91)--and two varieties of chickpea--local and improved (CM-72)--with four replications were grown in a split plot design.

The effect of various tillage practices on the conservation of water at various depths of soil was nonsignificant for both crops in the beginning of the experiment. The same trend was noted in the chickpea fields after the harvesting of the crop. However, there was a significant effect of the type of tillage noted in the wheat fields after the harvesting of the crop: significantly more water remained in the soil when the cultivator (1.07 cm) was used, followed by the disc plough (0.70 cm), moldboard plough (0.48 cm), and chisel plough (0.32 cm).

There was no pronounced effect of the type of tillage on the plant characteristics of the four varieties. However, the improved variety of wheat gave more yield/ha than the local one, while the improved variety of chickpea gave a slightly higher yield than the local one.

The project has resulted in many benefits, not only for the farmers of the area, but also for the country at large. The farmers have witnessed for the first time the utility of improved tillage practices, and have started applying these practices in their fields. Thus the project has had an impact even after its first year. It is envisioned that the country's agricultural production of at least traditional crops such as wheat and chickpeas, will increase tremendously after completion of the project as the farmers adopt the improved practices. This in turn will eventually ease the country's burden of importing food grains and save the expenditure of hard-earned foreign exchange.

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REFERENCES

Ahmad, M. 1990. Keynote address. In Rod-Kohi Agriculture. Problems and Prospects Symposium. Pakistan Agricultural Research Council, Islamabad, Pakistan. pp.3-10.

Amin, R. 1990. Improving water management and conservation practices for increased agriculture production in the Arid Zone of D.I.Khan. In Rod-Kohi Agriculture. Problems and Prospects Symposium. Pakistan Agricultural Research Council, Islamabad, Pakistan. pp. 60-64.

Bolton, H, N.1990. A report on irrigation in Dera Ismail Khan. In Rod-Kohi Agriculture. Problems and Prospects Symposium. Pakistan Agricultural Research Council, Islamabad, Pakistan. pp. 16-37.

Bradfield, J. M., and R. W. Blanchar. 1977. Profile modification of a Fragiudalf to increase crop production. Soil Sci. Soc. Am. J. 41:127-131.

Burnett, E., and V. L. Hauser. 1967. Deep tillage and soil-plant-water relationships. In Tillage for Greater Crop Production Conf. Proc, pp. 47-52. Am. Soc. Agric. Eng., St. Joseph, Mich.

Doty, C. W., R. B. Cambell and D. C. Reicosky. 1975. Crop responses to chiseling and irrigation in soils with a compact A2 horizon. Trans. Am. Soc. Agric. Eng. 18:668-672.

Duncan, D.B. 1955. Multiple Range and Multiple F. Test. Biometrics. 11:1-42.

Eck, H. V. 1986. Profile modification and irrigation effects on yield and water use of wheat. Soil Sci. Soc. Am. J. 50:724-729.

Eck, H. V. and R.G. Davis. 1971. Profile modification and root yield, distribution and activity. Agron. J. 63:934-937.

Eck, H. V., and H. M. Taylor. 1969. Profile modification of a slowly permeable soil. Soil Sci. Soc. Am. Proc. 33:779-783.

Eck, H. V., and P. W. Unger. 1985. Soil profile modification for increasing crop production. Adv. Soil Sci. 1:66-100.

Eck, H. V., T. Martinez, and G. C. Wilson. 1977. Alfalfa production on a profile-modified slowly permeable soil. Soil Sci. Soc. Am. J. 41:1181-1186.

Hussain, T. and A. Jabbar. 1985. Soil, Water and Fertilizer Analysis. University of Agriculture, Faisalabad, Pakistan. pp. 19-29.

Johnson, W.C. 1964. Some observations on the contribution of an inch of seeding time soil moisture to wheat yields in the Great Plains. Agron. J. 56:29-35.

Jones. O. R. and V. L. Hauser. 1975. Runoff utilization for grain production. In water Harv. Symp. Proc., Phoenix, Ariz. pp. 277-283. U.S. Dept. Agric. ARS W-22.

Khan, M. 1990. BARD experiences in Rod-Kohi agriculture. In Rod-Kohi Agriculture. Problems and Prospects Symposium. Pakistan Agricultural Research Council, Islamabad, Pakistan. pp. 98-114.

Mathers, A. C., G. C. Wilson, A. D. Schneider, and P. Scott. 1971. Sugarbeet response to deep tillage nitrogen and Phosphorus on Pullman clay loam. Agron. J. 63:474-477.

Singh, Y., S. R. Singh, and K. Singh. 1991. Effect of various degrees of tillage operations on barley yield under dry land conditions. Proc. 12th ISTRO Conf., July 1991, Ibadan, Nigeria.

Smith, E. S., D. H. Vaughn, J. V. Perumpral, and P. H. Reid. 1983. Soybean response to various tillage and planting systems. Am. Soc. Agric. Eng. Paper No.83-1524. Am. Soc. Agric. Eng., St. Joseph Mich.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co. Inc; New York.

Trouse, A. C. Jr. 1978. Physical conditions of soil affecting no-tillage techniques. Proc. First Ann. Southeastern No-Till systems Conf., Univ. Georgia Spec. Publ. No.5, pp. 30-36.

Unger, P. W. 1990. Conservation Tillage systems. Advances in soil Science. Springer-Verlag New York Inc. 13:27-68.

Unger, P. W. 1992. Infiltration of simulated rainfall: Tillage system and crop residue effects. Soil Sci. Soc. Am. J. 56:283-289.

Wadud, A. and B. Ahmad. 1990. Prospects of pulses production in Rod-Kohi area in D.I.Khan. In Rod-Kohi Agriculture. Problems and Prospects Symposium. Pakistan Agricultural Research Council, Islamabad, Pakistan. pp. 79-84.

WATER AND RANGE MANAGEMENT

Guest Editor:

Dr. Dean M. Anderson
Jornada Experimental Range
Agricultural Research Service
U.S. Department of Agriculture
Las Cruces, New Mexico

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REDUCING RISK OF CROP FAILURE BY USING MICRO-CATCHMENT WATER HARVESTING IN ARID AREAS

REDUCTION DU RISQUE DE PERTE DE CULTURE DANS LES ZONES ARIDES PAR COLLECTE D'EAU ET MICRO-INTERCEPTION

Z.A. Qureshi[1] and L.S. Willardson[2]

Abstract

A field study of water harvesting using compacted soil surfaces was conducted from 1986 to 1989 in upland Balochistan Province of Pakistan. Crop yields for ratios of catchment to cropped area of 2:1, 1:1, and control (0:1) were evaluated. An experimental area of 1.44 ha. was divided into four replications of three plots each. Every plot was 20m wide and 60m long.

During the moderate rainfall (205mm) year of 1987/88 total dry matter (TDM) and grain production computed on a total land area basis were 36% and 92% higher in the 1:1 treatment than in the control treatment. Although the 2:1 treatment stored more soil moisture over the three years of study, treatment 1:1 produced 34% more total dry matter and 41% more grain than the 2:1 treatment when yields were computed on a total land area basis.

Résumé

Une étude au champ de la collecte de l'eau par compactage des surfaces de sol a été menée de 1986 à 1989 dans les hautes zones de la province du Balochistan au Pakistan. Des évaluations de rendements de cultures ont été menées pour des rapports surface d'interception sur surface cultivée de 2:1, 1:1, et 0:1 (témoin). Une parcelle expérimentale de 1,44 ha a été divisée en quatre répétitions de 3 unités chacune. Chaque unité était de 20 m de largeur et 60 m de longueur.

Durant l'année 1987/88, année à pluviométrie modérée (205 mm), la production de matière sèche totale (MST) et le rendement grain calculés sur la base de la surface totale étaient de 36% et de 92% plus élevés pour le traitement 1:1 que pour le traitement témoin. Bien que le traitement 2:1 a résulté en un plus grand stock d'eau

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[1] Zahid Ali Qureshi, Agricultural Engineer Arid Zone Research Institute, Quetta, Pakistan

[2] Lyman S. Willardson, Professor, Dept. Bio. & Irrig. Engr. Utah State Univ., Logan, Utah 84322-4105.

dans le sol sur les trois années, le traitement 1:1 a donné 34% de plus en matière sèche totale et 41% de plus en grain que le traitement 2:1 et ceci quand les rendements ont été calculés sur la base de la surface totale.

INTRODUCTION

For thousands of years man has tried to survive in desert regions by managing runoff from rainwater (Boers and Ben-Asher 1982). Runoff farming methods were developed about 4000 years ago in the arid regions of the Middle East, Southern Arabia, and North Africa (Frasier 1980, Gideon and Enthoven 1987, Sharma 1986, Yadav et al 1983).

Micro-catchment water harvesting is defined as the collection of surface runoff water from specially modified surfaces with a flow distance of less than 100 m and storing the water directly in the rootzone for crop production (Boers et al 1986, Boers and Ben-Asher 1982, Obi 1982). A micro-catchment water harvesting system consists of two basic components, the catchment area and the infiltration or cropped area (Cluff 1989, Yadav et al 1983). The size of a micro-catchment could be 5 m^2 for single tree to 1000 m^2 for row crops.

Balochistan, a Province of Pakistan, is situated between 24° N to 32° N latitude and 60° E to 72° E longitude and has an arid and semi-arid climate (Kidd et al 1988). Farmers in the arid areas of Balochistan have practiced rainwater collection and soil moisture conservation techniques for many years. Construction of ridges on the soil surface (Kamra et al 1986) and diversion of ephemeral stream from mountains onto the cropped fields are very

common in Balochistan. Although farmers use these rainwater collection techniques, they often experience winter crop failure. In winter farmers usually plant wheat on only the small portion of their fields which may receive water from ephemeral streams. The farmers face crop failures in five to seven years out of ten. Even in good years (rainfall >280mm), yield expectations are only 400 kg/ha (Rees et al 1991). Winter wheat is grown as a dual purpose crop, grain is used for human consumption and the straw is used for animal feed (Buzdar et al 1989).

The weather system which determines the climate of Balochistan is divided into two growing seasons Winter (Oct-May), and Summer (June-Sept). The average annual rainfall is 250mm with an exceedance probability of 30% (Table 1). Average precipitation during the Winter season is 180mm with an exceedance probability of 50% for 200mm rainfall.

TABLE 1. Seasonal Rainfall (mm) Probability Distribution at Quetta Experimental Station.

	EXCEEDANCE PROBABILITY									
	90%	80%	75%	70%	60%	50%	40%	30%	20%	10%
Annual	136	162	173	183	202	221	241	264	293	336
Winter	92	123	135	146	169	191	213	244	281	337
Summer	1	4	6	8	12	16	22	29	39	56

Due to the variations in elevation from sea level to 3000m, and the mountains, which act as barriers to movement of air, Balochistan has great variations of temperature. Minimum temperature is frequently below freezing from the middle of November to the end of February (Sharma et al 1986). The

experimental site can expect an average of 95 days below freezing ranging from 72 days to 125 days (Kidd et al 1988).

In this high risk environment agricultural inputs are very low. Animal traction is the main source of power but 60% of the farmers are now using tractors for agricultural activities. Balochistan is more than 40% of the total area of Pakistan, but the population is less than 10% of the total for Pakistan. Most of the lands have been unused for hundreds of years due to aridity and the unavailability of labor. The land use intensity is only 20%. The principal agricultural activity in Balochistan is small ruminant production with rangeland grazing as the main source of animal feed. During winter there are shortages of natural grazing and farmers use the remnants of previous grain crops. The economic value of such remnants can equal or exceed that of the seed yield (Rees et al 1991).

MATERIALS AND METHOD

The experiment location and the experiment design are shown in Figures 1 and 2, respectively. A single factor randomized complete block design with four replications was used. Each replication had three treatments with catchment area to cropped area ratios of 2:1, (two-thirds of the area as catchment and one-third as cropped area), 1:1, half of area as catchment and half as cropped area, and a control (0:1), which has all of the area planted. The research area (240m X 60m) was divided into 12 plots each 60-m long and 20 m wide (Fig. 2). Catchments were established with a natural slope of 1% by clearing rocks and weeds from the soil surface (Evenari

1968), plowing by a moldboard plow. The plowed soil surfaces were then pulverized, smoothed, and compacted (Boers and Ben-Asher 1982, Sharma 1986) using a heavy wooden plank mounted on the three point hitch of a tractor. A soil crust formed on the pulverized and compacted silty clay loam soil surface, which had a clay content of 40-50%, (Sharma 1986), after the first winter rain. Soil bunds (raised soil dikes) were built 0.5m to 1.0m in height around every plot (Fig. 2), to prevent the entrance of water from adjacent areas into the experimental area. A camel drawn plow-cum-drill which constructs ridges on the soil surface and at the same time places seeds deep into moist soil was used to plant a wheat crop at a seeding rate of 90 kg/ha in the three treatments.

Rainfall and ambient air temperature data were measured near the experimental site (Table 2). Soil moisture data were collected using a calibrated neutron probe. Crop yield were obtained by harvesting 1.0m X 0.40m areas randomly in the different plots.

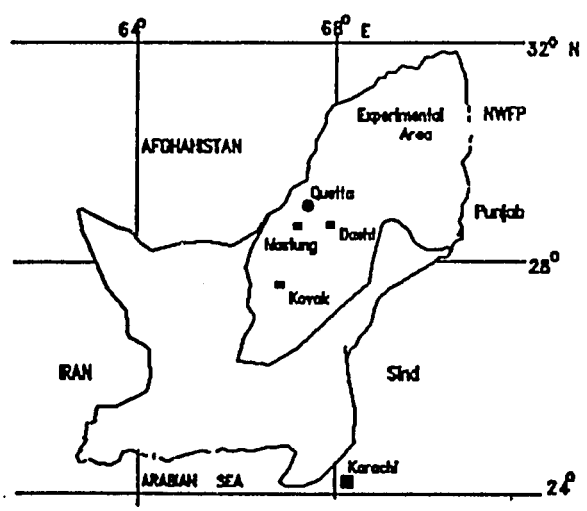

FIGURE 1. Location of Trial Site in Balochistan (Pakistan).

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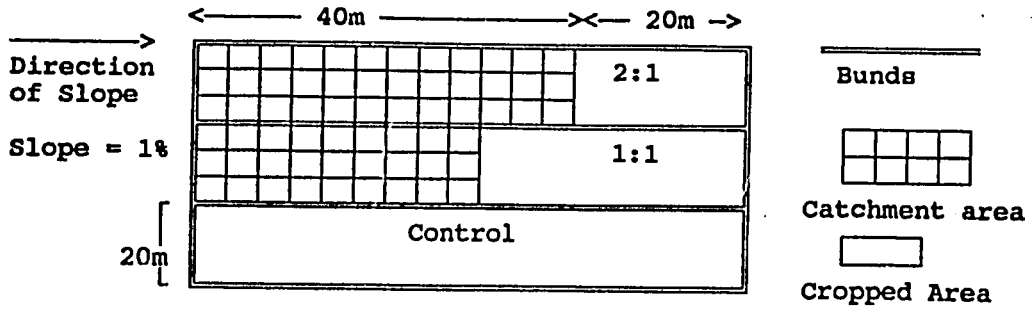

FIGURE 2. One Replicate of the Field Layout used in the study.

TABLE 2. Monthly Rainfall, Minimum and Maximum Temperatures and Number of Days below 0° C in the three Cropping Seasons.

Month	1986/87 Rain mm	Min °C	Max °C	Days <0°C	1987/88 Rain mm	Min °C	Max °C	Days <0°C	1988/89 Rain mm	Min °C	Max °C	Days <0°C
JUL	2.0	18.0	37.0	0	0.0	18.3	38.0	0	18.3	20.1	39.1	0
AUG	73.2	17.0	38.0	0	6.5	15.5	44.0	0	0.0	16.3	37.4	0
SEP	0.0	11.6	32.2	0	0.0	6.0	37.0	0	0.0	6.0	10.4	0
OCT	0.0	-0.6	31.4	3	0.0	-3.1	32.4	4	0.0	6.4	27.7	0
NOV	6.9	-5.0	25.6	15	0.0	-5.0	25.6	15	0.0	-2.4	28.4	7
DEC	11.4	-4.0	23.0	7	50.5	-4.0	23.0	7	13.9	-3.2	20.7	7
JAN	28.2	-6.1	15.6	23	9.1	-9.0	18.9	19	88.3	-6.1	15.5	24
FEB	64.2	-10.0	20.0	4	17.8	-7.0	25.0	10	22.4	-6.8	17.3	16
MAR	106.0	4.0	23.0	0	117.0	-0.3	26.7	1	42.2	-3.1	22.7	2
APR	11.8	7.0	33.0	0	4.3	3.9	31.5	0	8.6	3.9	31.5	0
MAY	9.4	4.4	35.5	0	0.0	13.1	35.5	0	5.8	2.6	35.9	0
JUN	28.2	12.3	38.0	0	0.0	9.0	40.6	0	1.6	16.2	36.7	0
TOT	341.3			52	205.2			56	201.1			56

RESULTS

Soil Moisture

Accumulated Soil moisture is one factor, besides crop yield that quantifies the difference between treatments. The 1986/87 cropping season was exceptionally wet and the soil in all the treatments had adequate moisture for germination at planting time in October 1986 due to 73mm of rainfall that occurred in August. The cropped area in 2:1 treatment had a 48% higher soil moisture content in October than did the control treatment. The higher water

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content improved the early germination of seeds in the 2:1 treatment. A total of 200mm of rainfall fell during January, February, and March in 1986/87 (Table 2) adding to the soil moisture storage. Due to the continuous large amounts of rain, excess water collected at the down slope end in all the plots. Recharge of water in April 1987 was 81% higher in the 2:1 treatment, and 62% higher in the 1:1 treatment than in the control.

The 1987/88 season had moderate rainfall with a total of 205mm. The Summer season (July-Sept) did not receive any rainfall therefore, soil moisture sufficient for germination was not available at the time of planting. However, a of total 194mm rain (Table 2), occurred during December to March 1987/88, and was the only source for soil moisture in the cropping season.

Figure 3, shows that soil moisture increased in April and May 1988 following a 117mm rainfall (Table 3) in March. The 2:1 treatment stored 50% more water than the control, which was more than the potential crop evapotranspiration requirement at that time and 1:1 treatment stored 20% more soil moisture than the control treatment in the middle of April 1988. Soil moisture was more than double in 2:1 treatment and was 95% higher in the 1:1 treatment than in the control treatment at the beginning of May 1988.

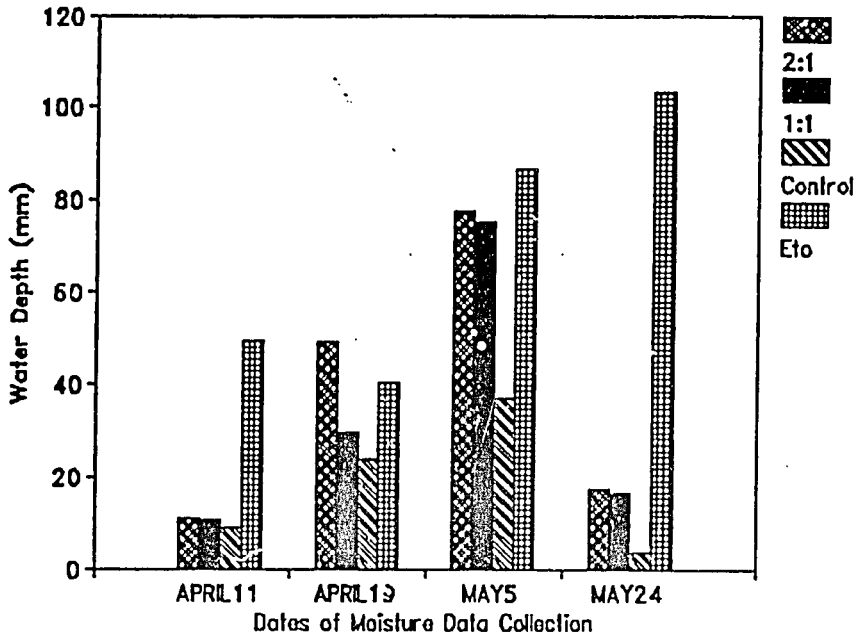

FIGURE 3. Average Soil Moisture in Three Treatments after 117 mm of Rainfall in March 1988 and Potential Evapotranspiration During Same Period.

TABLE 3. Total Dry Matter TDM and grain Yield (kg/ha) During Three Cropping Seasons 1986/87, 1987/88, and 1988/89.

Treat	Cropped Area Basis 86/87	87/88	88/89	Total Land Area Basis 86/87	87/88	88/89
			Total Dry Matter			
Control	2093	137	588	2093	137	588
1:1	3220	348	806	1840	186	403
2:1	3035	382	1099	1301	147	366
Mean	2783	289	831	1744	157	452
s.e	616	55	104	319	23	37
			Grain Yield			
Control	562	25	196	562	25	196
1:1	962	92	223	593	48	111
2:1	926	78	317	397	30	107
Mean	817	65	245	517	35	138
s.e	213	15	45	127	7	16

The 1988/89 season received the least rainfall (201mm) among the three cropping seasons. Soil moisture for germination was not available at planting time due to no rainfall in August and September 1988 (Table 2). Soil moisture at the end of April 1989 showed 27% more soil moisture in 2:1 treatment and 14% more moisture in 1:1 treatment than in the control from a 166mm rainfall that occurred during December 1989 to March 1989.

Crop Yield

Crop yields (kg/ha), for the three treatments on a actual cropped area basis and a total land area basis are summarized in Table 3. The 1986-87 yields of total dry matter (TDM), was 45% higher on an actual cropped area basis in the 2:1 treatment and was 54% higher in the 1:1 treatment than in the control treatment. In 1987/88 cropping season TDM in the 2:1 and 1:1 treatments were more than double that in the control treatment. During 1988/89 Season, TDM produced was 87% higher in the 2:1 and 37% higher in the 1:1 treatments than in the control treatment, for yields measured on an actual cropped area basis.

When yields were computed on a total land area basis (yield is divided by the cropped area plus the watershed area), the control treatment produced 14% higher TDM than the 1:1 and 61% more than the 2:1 treatments in 1986/87.

During the 1987/88 season, the yields of TDM and grains were 36% and 92% higher, respectively, in the 1:1 treatment than in the control treatment (Table 3). The 2:1 treatment performed better than the control, but TDM was only 7% more and the grain yield was only 20% higher than in the control. During the 1988/89 cropping

season, the 2:1 and 1:1 treatments produced lower TDM and grains than the control treatment when calculated on a total land area basis (Table 3), because of the rainfall pattern and amount.

A comparison of total dry matter (TDM), and grain produced for the three-cropping-seasons, calculated on a cropped area basis, is presented in Figure 4. TDM results on a cropped area basis show 60% higher yields in 2:1 treatment and 55% higher yields in 1:1 treatment than in the control treatment. Grain yields are 69% higher in the 2:1 and 63% higher in the 1:1 treatment than in the control treatment.

When yields are computed on a total land area basis, the control showed a 16% higher TDM and a 4% higher grain yield than the 1:1 treatment.

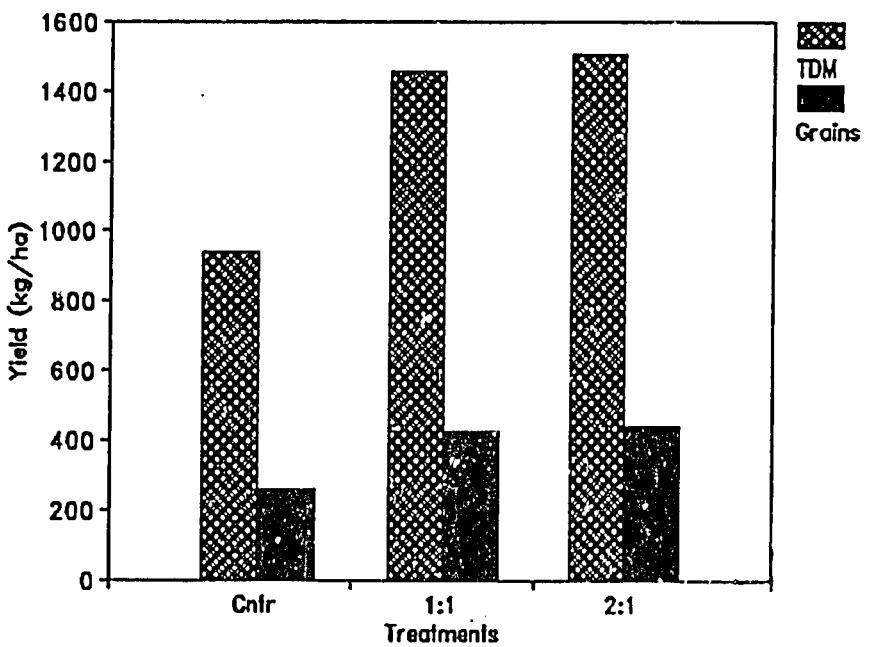

FIGURE 4. Comparison of the Yields of Total Dry Matter (TDM), and Grains Averaged over the Three Cropping Seasons 1986/87, 1987/88, and 1988/89 on Cropped Area Basis.

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DISCUSSION

The 1986/87 season was exceptional due to the 73 mm of rain that occurred in August 1986; therefore, sufficient soil moisture was available for germination at the normal planting time in October 1986, which helped in early germination of seeds in all the treatments. It appears that 300mm of annual rainfall will make the control treatment yields (Watt 1948), equal to the micro-catchments.

Yields were expected to be higher in the 2:1 treatment than in the 1:1 and control treatments because of the greater expected amount of runoff from the bigger catchment area (Gurmal et al 1981). Due to the 341mm rainfall (Probability < 20%) in 1986/87, excess water from runoff collected at the downslope end in 2:1 treatment. The ponding of water near the downslope bund destroyed a portion of the crop in the 2:1 treatment due to waterlogging. Yield loss due to excess water is evidenced by comparing 1986/87 yields with the results of low rainfall years of 1987/88 and 1988/89 when the crop yields were higher in the 2:1 treatment than in other two treatments.

The 1987/88 cropping season received only 205mm rainfall which is insufficient to grow a good wheat crop. Although yields were higher in the catchment treatments than in the control, production in the 1:1 treatment only recovered the seed that was used as input. There could be two reasons for the very low crop yields observed in the 1987/88 cropping year. First, the crop was planted in dry soil in October 1987 because of no rainfall in summer 1987.

Germination of the seeds were delayed until the end of February, because from November to February, air temperatures (Table 2), were not high enough to allow seed germination. Second, no rainfall occurred in April, May, and June 1988, which affected soil moisture storage at crop production growth stages. The crop suffered water stress at the critical stages of crop development and seed maturity; therefore, even though the TDM was more than 340 kg/ha in the water harvesting treatments, the grain yield was very low.

The 1988/89 cropping period received a total of 201mm of rainfall, which was similar to the total rainfall in 1987/88, and during the summer season (July-Sept) of 1988 only 18.3mm of rainfall occurred. The crop was planted in dry soil in October as was done in 1987/88; however, TDM yields were three times higher in 1988/89 than in the 1987/88 in the three treatments. Higher yields in 1988/89 than in 1987/88 resulted from two factors: first, while both years received approximately the same total amount of rainfall, Table 2 shows a better rainfall distribution during the 1988/89 cropping season. Rainfall started in December and every month received some rainfall until the end of the cropping season in June. Second, air temperatures were also higher in the beginning of 1988/89 than in 1987/88. However, both seasons had a total of 56 days with less than or equal to 0° C air temperature. Table 2 shows that most of the cold days were during January and February and that in 1988/89 these two months also received a total 110mm rainfall that was stored in the soil profile, due to a low evaporative demand, and which also helped in the seed development process.

Although, crop yield results from the micro-catchment treatments calculated on a total land area basis (Table 3), do not look as favorable as the control, micro-catchments may have many advantages over the control treatment. First, risk of crop failure is reduced by using micro-catchment water harvesting techniques because of higher moisture storage in the soil profile. Second, inputs for plowing, planting, seeds, fertilizer and weed control will be required on only 50% or less of the area when using micro-catchment water harvesting rather than cultivating the whole land area. Because farmers can save half of their inputs by using the micro-catchment water harvesting techniques, they can use the other 50% of their resources to bring more unused land, which usually remains fallow, under cultivation.

Conclusion

1. During the moderate rainfall year 1987/88 when winter rainfall was 205mm, both 1:1 and 2:1 treatments produced three times higher TDM and grain on a cropped area basis than the control treatment. Total dry matter (TDM) and grains on total land area basis were 36% and 92% higher in the 1:1 and 2:1 treatments respectively, than in the control.

2. Over the three cropping years 1986-1989, the 1:1 treatment produced 55% more total dry matter and 63% more grain, and the 2:1 treatment produced 60% higher TDM and 69% higher grain yield than the control on a cropped area basis. Yields of TDM and grain in the 1:1 treatment were 16% and 4% less respectively, than the control when yields were computed on a total land area basis.

3. Results for 1987/88 showed that runoff contributing area requirements for good yields are reduced to zero for average winter rainfalls greater than or equal to 300 mm.

4. Although the 2:1 treatment, as expected, stored more soil moisture over the three years of study, the 1:1 treatment produced 34% more total dry matter and 41% more grains than the 2:1 treatment when yields were computed on a total land area basis. Therefore, farmers who use the 1:1 treatment to grow winter wheat, can get better yields by putting the inputs on half the land and using the other half of the land as a water catchment area.

REFERENCES

Boers, Th.M., Zondervan, K., and J.Ben Asher. 1986. "Micro-Catchment Water Harvesting (MCWH) for Arid Zone Development", Agricultural Water Management. no. 12, pp 21-39.

Boers, T.M., and J. Ben-Asher. 1982. "A Review of Rainwater harvesting", Agricultural Water Management. no. 5, pp 145-58.

Buzdar, N., Sabir, G.F., Nagy, J.G., and Keating, J.D.H. 1989. Rainfed Agriculture in Highland Balochistan: a Farming System Perspective. Quetta AZRI/ICARDA Research Report 34.

Cuff, C. B. 1989. "Water Harvesting systems in Arid lands", Desalination. no. 72(1/2), pp 149-159.

Evenari, M., Shanan, L., and N.H. Tadmor. 1968. " 'Runoff Farming' in the Desert. I. Experimental Layout", Agronomy Journal. no. 60, pp 29-32.

Fink. D. H., Frasier, G.W., and K.R. Cooley. 1980. "Water Harvesting by wax-Treated soil surfaces: Progress, Problems, and Potential", Agricultural Water Management. no. 3, pp 125-134.

Frasier, G.W. 1980. "Harvesting water for agricultural, Wildlife, and domestic uses", Journal of Soil and Water Conservation. no. 35(3), pp 125-128.

Gideon, O., and Gerda Enthoven. 1987. "Stochastic consideration in optimal design of a microcatchment layout of runoff water harvesting", Water Resource Research. no. 23(7), pp 1131-1138.

Gideon, O., Ben-Asher, ., A. Issar. 1983. "Economic evaluation of water harvesting in microcatchments", Water Resource Research". no. 19(5), pp 1099-1105.

Gurmal, S., Koranne, K.D., and Bhushan, L.S. 1981."Advances in Rainfed Farming", Central Soil & Water Conservation Research & Training Institute Dehradun, India. pp 83-88.

Hargreaves, G.H., and Zohrab, A.S., 1985. "Reference Crop Evapotranspiration from Temperature", Soil and Water Div., ASCE,". no. 1(2), pp 96-99.

Kamra, S.K., Narayana, V.V.D., and K.V.G.K. Rao. 1986. "Water harvesting for reclaiming alkali soils", Agricultural Water Management. no. 11(2), pp 127-135.

Keating, J.D.H., and Rees, D.J. 1988. "An Analysis of Precipitation and Air Temperature Records in the Quetta Valley, Pakistan. The Implications for Potential Improvement in Agricultural Productivity", The AZRI/ICARDA Research Report, 19, Arid Zone Research Institute, Quetta, Pakistan.

Kidd, C.H.R, Rees, D.J., Keatinge, J.D.H, Rehman, F., Samiullah, A., and Raza, S.H., 1988. "Meteorological Data Analysis of Balochistan", The AZRI/ICARDA Research Report, 19, Arid Zone Research Institute, Quetta, Pakistan.

Obi, M.E. 1982. "Runoff and Soil loss from an Oxisol in South-Eastern Nigeria under various management practices, "Agricultural water management", no. 5, pp 193-203.

Sharma, K.D., Pareek, O.P., and P. Singh. 1986. "Microcatchment water harvesting for raising Jujube orchards in an arid climate", ASAE no. 29(1), pp 112-118.

Sharma, K.D. 1986. "Runoff behavior of water harvesting microcatchments", Agricultural Water Management. no. 11(2), pp 137-144.

Singh, S.D. 1985. "Potentials of water harvesting in the dry regions", Annls of Arid Zone. no. 24(1), pp 9- 20.

Watt, R.D. 1948. "The Problems and Possibilities of Growing Wheat in Australia", Emp.J.Exp.Agric. no. 16, pp 187-194.

Yadav, R.C., Kumar, V., and V.B. Ogunlela. 1983. "Biological water harvesting: a method of enabling dryland crops to endure periods of drought", Journal of Arid Environment. no. 6(2), pp 115-117.

Assessing the fattening potential of male Balochi and Harnai lambs fed different protein and energy rations under confinement

InamUl Haq, Shahid Rafique, Arshad Ali, Sohail Zia and A.S.Alvi
Arid Zone Research Institute, PARC, P.O. Box 63,
Brewery Road, Quetta, 87300 Pakistan.

ABSTRACT

Two trials were conducted to study the fattening potential of male Balochi and Harnai lambs. The first trial was a five week feeding trial conducted between April and May 1993 at the Animal Nutrition Research unit(ANRU), Arid Zone Research Institute(AZRI) Quetta, Pakistan using sixteen male Balochi lambs 8 to 10 months of age each weighing 18.4 to 33.2 kg. The lambs were randomly divided into eight groups having similar live weights. Each pair was randomly assigned to one of eight fattening pens. Two iso-caloric rations viz., A and B were prepared. The base ration (A) contained 12 % Crude Protein(C.P.). The basal ration was supplemented with urea (B) to raise the crude protein level to 17 percent. Data on feed offered and refused was recorded daily while live weight data were taken weekly. Mean daily gain was 189±29 and 245±29 grams by the lambs fed on rations A and B, respectively. A non-significant difference was observed among both the groups. Significantly more ($P<0.05$) feed consumption was observed in the lambs fed on ration B as compared to those fed on ration A. However, a Non-Significant difference was observed in feed efficiency values of the lambs fed on ration A and B. The results of study suggest that the Balochi lambs 8 to 10 months of age are equally good in gaining weight when fed on ration containing 12% or 17% Crude Protein.

The second trial involved 16 Balochi and 16 Harnai male lambs about four to five months old at ANRU, AZRI, Quetta, Pakistan, during September to December 1993. The lambs were divided into eight groups in such a way that there were four lambs of a breed in one group. These groups of lambs were assigned to eight different lamb fattening pens randomly. The lambs were fed on two iso-nitrogenous(CP 14%) rations A and B having 2300 and 2700 Kcal of Metabolizable Energy per Kg(ME) respectively. The experimental rations were also assigned randomly in such a way that there were two pens (8 lambs of Harnai breed) on ration A and two pens (8 lambs of Harnai breed) on ration B. Similarly the two pens (8 lambs of Balochi breed) were assigned to ration A and two pens (8 lambs of Balochi breed) to ration B. Data on feed offered and feed refused was collected on daily basis. While data on live body weights were collected on weekly basis. The trial continued for a period of twelve weeks. Significant difference in overall average daily weight gain was observed. The lambs fed on ration B gained significantly more weight than those fed on ration A. Daily weight gain values for the Balochi and Harnai lambs fed on rations A and

B were 172±12 and 127±12 grams respectively. Balochi lambs gained significantly ($p < 0.05$) more weight as compared to Harnai lambs. Balochi lambs consumed significantly ($p < 0.10$) more feed as compared to Harnai lambs. Significantly more feed was consumed by the lambs fed on ration A than the lambs fed on ration B. The results of the study indicated that the Balochi lambs performed better in terms of weight gain than the Harnai lambs. Moreover the lambs fed on high energy ration A(2700 Kcal/Kg of ME) gained more weight as compared to the lambs fed on low energy ration B(2300 Kcal/Kg of ME).

INTRODUCTION

The Balochistan province has unique importance in the arid and semi-arid regions of Pakistan. Ninety-three percent out of 34.7 million ha, is considered rangeland, and 60 percent of this rangeland i.e., 21 m ha is used for grazing (FAO, 1983). About 11.7 of the 20.9 million ha of rangeland is classified as poor and provides only 30-50 kg/ha of dry matter per year while the better quality rangelands (2.9 million ha) provide 250-280 kg/DM/ha per year (FAO, 1983). The existing rangelands are being depleted rapidly due to the degradation of vegetation cover, soil erosion and cutting of woody vegetation for fuel wood. The native range plants usually have a short growing season and their nutritive quality deteriorates quickly with advancing stage of maturity. Protein, energy and mineral deficiencies during the greater part of the year have been found to be the limiting factor affecting animal production in this region.

The main occupation of the people of Balochistan is raising animals specifically the small ruminants including sheep and goats. These animals are grazed over large areas of the region principally by nomads, who frequently move to take advantage of ephemeral sources of water and vegetation. This type of management makes it difficult to collect accurate data and consolidate information about the potential productivity of the animal resource. The small ruminant population of the province is estimated to be 11.1 and 7.3 million head of sheep and goats, respectively (GOP, 1986). Larger ruminants including cattle and camels are estimated to be 1.16 and 0.35 million head, respectively (GOP 1986). The livestock sector has been estimated to contribute 25 percent to the Gross Agricultural Production of Balochistan (FAO, 1983: GOB, 1989).

The sheep and goat population of Balochistan has increased rapidly during the past 30 years from 1.9 million head in 1955 to 18.4 million head in 1986 (Masood et al., 1988). However, most of these small ruminants are underfed and have poor health due to the rapid increase in animal population and decrease in output from the ranges. Animal production is subject to great fluctuations, because of the nomadic system of management that predominates in the region which is not based on intensive forage production and the production of feed reserves. The foraging animals basically depend

upon poorly managed rangelands and sparsely available crop residues. This poor underlying nutrition makes it extremely difficult to carry animals through critical periods even without expecting increased productivity per animal.

Being able to wean lambs at 3-4 months of age and fatten them using intensive feedlot techniques has the potential of not only relieving grazing pressure on some of the most degraded ranges during one of the most critical periods of the year but also reducing the nutrient needs of the dam for milk production. The weaned lambs could be either fattened by farmers themselves, or sent to commercial feedlots at other locations. In a similar study it was found that the feed cost and amount of TDN requirement per unit edible meat production increased gradually as the fattening period increased beyond 60 days and fattening after 120 days seemed to be un-economical(Khan and Pasha 1990). The present study was therefore conducted to asses the fattening potential of Balochi and Harnai lambs under intensive feed lot conditions.

MATERIALS AND METHODS

TRIAL I

Assessing the fattening potential of male Balochi lambs fed 12 and 17 percent protein rations.

A five week feeding trial was conducted between April and May 1993 at the ANRU, AZRI, Quetta, Pakistan using sixteen balochi lambs born under free-ranging conditions 8 to 10 months of age each and weighing 18.4 to 33.2 kg. The lambs were randomly divided into eight groups in such a way that two lambs having similar live weights were put in one group. Each pair was randomly assigned to one of eight fattening pens. The eight lamb fattening pens(1.5m x 2.4m) Were constructed adjacent to each other using welded net wire fabric panels such that line of sight was open among all eight pens. The feeding pens were located inside a well ventilated masonry building.

Necessary cleaning and disinfection of all the experimental pens was done before the start of trial with lime and BHC (Benzene Hexa Chloride). Drenching of all the experimental lambs was done at the start of experiment with Systamex for the control of internal parasites. lambs were put in to the fattening pens 12 days before the feeding trial began and shifted slowly from routine feed to the experimental rations A and B so the lambs could adopt to the confinement and experimental rations.

Two experimental rations having similar ME values but differing in CP levels were fed at 08.00 hrs., daily. Two lambs in a pen were fed <u>ad libitum</u> on the experimental rations together to serve as one experimental unit. Quantity of feed offered was assessed from the previous day refusal. About 10% additional

quantity of feed was added more to the previous day feed consumption. Four randomly selected pens served as a control and received the basal ration un supplemented with urea (A); it contained 12 % CP. Urea was added to increase the crude protein level of the basal ration to 17 percent in the supplemented group B (Table 2). The rations were composed of Barley, Peaseeds, Cotton seed cake, Rice polishings (comes of the rice as a result of it being polished), Sorghum, Wheat bran, Molasses and Vitamin mineral premix. The daily feed refused was collected separately before offering the fresh feed. The feed that was not eaten was discarded and never reintroduced at the next feeding. Water was available ad libitum. Daily record of feed offered and refused was recorded to the nearest 0.2 g. The lambs were weighed weekly on thursday in the morning before feeding and watering. The study began on 29.04 93 And continued uninterrupted for five weeks.

Representative samples of the feed ingredients were collected and analysed (Table 1) according to the methods and procedures described by AOAC(1975). The data were analyzed using analysis of variance technique in a Randomized complete Block design according to the methods and procedures described by Steel and Torrie (1980) using the statistical package PCSAS (SAS, 1985).

TRIAL II

Assessing the fattening potential of male Balochi and Harnai and Harnai lambs fed different levels of energy rations under confinement.

This trial was conducted on 16 Balochi and 16 Harnai lambs 4 to 5 months old at ANRU, AZRI, Quetta Pakistan during September-November 1993. Experimental lambs used in the study were having body weights ranging from 11.6 Kg to 17.0 Kg in case of Harnai and 15.2 to 18.8 Kg in case of Balochi breed. Lambs of each breed were divided randomly into four groups having four lambs in each group.The eight groups of lambs were put randomly into eight lamb fattening pens (1.5 m x 2.4 m) in such a way that there were four pens of each breed. Experimental pens were randomly assigned to the eight different groups of lambs.

Necessary cleaning and disinfection of all the experimental pens was done with lime and BHC (Benzine Hexa Chloride) before the start of trial. Drenching of all the experimental lambs was done with Systamex for the control of internal parasites at the beginning of the trial. All the lambs were put in the lamb fattening pens 20 days prior to the start the of actual experimental period so that the lambs may get adjusted to the experimental pens and rations. Two iso notrogenous (CP 14%) experimental rations having 2300 and 2700 Kcal ME were prepared manually. The composition of the experimental rations is given in table 5. The two experimental rations were randomly assigned to the different groups of lambs in such a way that there were four pens

(Two pens with Balochi and two pens with Harnai lambs) on each ration.

Feed was offered ad libitum at 08.00 hrs daily. Water was made available round the clock. Daily record of feed offered and refused was maintained. The daily feed refused was collected separately and reintroduced in the feed offered. All the lambs were weighed weekly in the morning before feeding . Daily record of minimum and maximum temperature in the experimental shed was maintained with the help of thermometer.The study continued for a period of twelve weeks. The data collected during the study was compiled and analyzed statistically as Completely Randomized Design according to the methods and procedures as described by Steel and Torrie, 1980 and using the statistical package PCSAS (SAS, 1985).

RESULTS AND DISCUSSION

Trial I

Maximum daily weight gain was recorded during the 2nd week for the group fed on ration A (Fig. 1). While the maximum weekly and daily weight gain values for the group fed ration B were recorded during the 4th week (table 3). One explanation for this difference could be that the lambs were grown enough at the initiation of the experiment and after making the compensatory growth reached maximum at 2nd and 4th week of the experiment period. The lambs showed a slight decline in the weight gain afterwards. However, there was a non-significant difference in the weight gain of lambs fed on ration A and B during different weeks except at 4th week of the experimental period. But overall difference in weight gain of lambs on high and low protein rations was found to be non-significant. Indicating thereby that there is no need to feed Balochi male lambs on high protein rations at the age of 8 to 10 months.

Mean live weights across the four pens within a treatment varied 33.2 and 20.2 Kg; 32.0 and 18.4 kg for treatments A and B, respectively. Therefore, each treatment was evaluated as a high (average initial body weight 30.6 Kg) and low (average initial body weight 23.5 Kg) body weight subgroups. The data for the lambs fed according to different body weights are presented in table 4. The data indicates that the daily live weight gain was better in the lower body weight group than the lambs in the higher body weight group at 3rd week of the experimental period. However, there was a non-significant difference in the overall weight gain values of both the groups. It indicates that the male Balochi lambs of 8 to 10 months of age but of different body weights gain almost the same daily weight.

The data indicated that Balochi male lambs fed on ration containing 17 % CP (B) gained almost the same weight when compared to the lambs fed on ration A containing 12 % CP. These data agree

with the studies conducted by Spelman and Collions (1964). They reported some sheep breeds can gain nearly one pound per day, while others only gain 1/3 of a pound daily. Similar conclusions have been reported by Khalil and Maqsood (1987). These researchers conducted a trial on young Afghan male and female sheep having mean daily live weight gains of 239 and 209 g, respectively.

The mean daily feed consumption values for the lambs fed on rations A and B are presented in table 3. The data indicates that lambs fed on ration B consumed more feed as compared to the lambs fed on ration A. Feed consumption by the lambs fed on ration A was maximum during 2nd week and minimum during 5th week of the experiment. The maximum feed consumption for the lambs fed on ration B was during 4th week while the minimum values for feed consumption were recorded to be during 1st week of the experimental period. The overall mean weekly feed consumption values for Balochi lambs fed on rations A and B indicated that lambs fed on ration B containing 17 % CP consumed significantly more feed than the lambs fed on ration A. The lambs in the higher body weight group consumed significantly ($P< 0.01$) more feed as compared to lower body weight group. The mean daily feed consumption was highest during 4th week and minimum during the 5th by the higher body weight (B1) group (table 4). Highest mean daily feed consumption was observed at 3rd week by the lower body weight group (B2). Minimum feed consumption values were observed at 1st week. Statistical analysis of the data revealed significant difference in overall daily feed consumption by the lambs in high and low body weight groups. The increase in feed consumption by the lambs fed on ration B may be due to addition of urea and increased level of CP. The results of the study are in agreement with those reported by Behrane et al. (1982). These workers reported daily feed intake of 1.67 kg/head in growing lambs. The feed consumption values are also similar to those reported by Khalil and Manzoor (1987). They found that the mean feed consumption/head/day was 1.73 kg in Lohi and 1.54 kg in Sipli males. While the values are lower than those reported by Khalil and Maqsood (1987) . They found that the mean concentrate ration consumption/head/day was 2.19 and 2.48 Kg in Afghan male and female young sheep, respectively. The higher values reported may be due to the different composition of rations and breeds involved in the study.

A non-significant difference was observed in the feed efficiency values of the lambs fed on rations A and B except during 4th week of the experiment (table 3). Significant difference in the feed efficiency was observed between the lower and higher body weight groups during 3rd and 4th week of experiment. However there was an overall non-significant difference in the feed efficiency between both the body weight groups. Non-Significant difference was also observed in the overall feed efficiency values of the lambs having low and high body weights (Table 4).

Economics of the lamb fattening trial mainly depends on feed

cost. But it is also influenced by weight of lambs, method of feeding, length of feeding period, rate of gain, kind of lambs and kind and price of feed ingredients. The economics of these trial can not be worked out directly, because the study was conducted for a research trial under controlled conditions. According to the cost-benefit estimates presented in table 8, the profit of Rs. 11.94 and Rs. 12.19 per day was estimated on each animal fed on ration A and B respectively.

TRIAL II

The twelve weeks experimental period was divided into three different periods viz., Period I (01-28 days), Period II (29-56 days) and Period III (57-84 days) for the sake of analysis and interpretation of research results. The maximum and minimum temperatures recorded during differents periods were 31, 16; 26, 10 and 23, 8 C for the periods I, II and III respectively.

Lambs fed on both the rations gained comparatively more weight during the first 28 days of the experimental period as compared to the rest of the periods (table 6). Rate of increase in body weight of lambs kept on decreasing during rest of the periods (fig.2). Possible reason may be that lambs used in the study were being raised under range conditions and not receiving any supplement feed to fulfi their body requirements for optimum growth before the start of trial. When these lambs were put on experimental rations and allowed to consume rations ad libitum, they made the compensatory growth and attained the maximum growth rate during initial period. Since the lambs were well fed during initial period of study, could not gain as much body weight during rest of the experimental period. Significantly better ($P< 0.05$) weight gain (167.3±12.00 g) was observed in the lambs fed on high energy ration (2700 Kcal) as compared to the lambs fed on ration B (131.3±12.0 g). Lambs gained more weight on ration B due to the higher energy content of the feed as compared to feed A. Significant difference in the weight gain of Balochi and Harnai breed was observed (table 7). Balochi lambs gained significantly ($P< 0.05$) more weight (171.6±12.0 g) as compared to Harnai lambs (127.4±12.0 g). It indicates that male lambs of Balochi breed has better potential for weight gain as compared to Harnai lambs.

It can be concluded from the above observations that lambs fed or ration B gained significantly more weight than the lambs fed on ration A. It can also be concluded from the above studies that Balochi male lambs of 4 to 5 months age gained more weight as compared to Harnai male lambs.

The weight gain values recorded in the study were similar to the values recorded by Jankowski et al., 1985. They reported daily weight gain values of 160 grams for the lambs fattened in industrial lamb fattening units when fed ad libitum with all mash concentrate. The results of the study were also in accordance with

those reported by Daniel, P., (1986). He observed highest daily live weight gain in lambs as 204 grams per day in 1983 and 147 grams per day in 1984, fed on silage mixtures. The weight gain values are also in accordance with those recorded by Urbaniak, M., et al., (1991). They reported body weight gain of 152 to 168 grams per day in the crossbred lambs fed on iso-nitrogenous and iso-energetic diets. The body weight gain values recorded in the study were higher than the values recorded by Pojan et al., (1990). They observed daily weight gain values of 73.13, 111.52 and 131.15 grams per day in the lambs fed on rations containing 85 percent Cassava chips at 200, 400 and 600 grams per day respectively. The daily weight gain values were also higher than those reported by Jia et al (1990). They recorded daily weight gain values of -15, 20, 78 and 88 grams per day in the lambs given concentrate at the rate of 0, 0.2, 0.4 or 0.6 Kg per head daily. The higher weight gain values recorded in the study than recorded by the above researchers may be due to the differences in rations fed and the experimental conditions. However the daily weight gain values recorded in this study were lower than those recorded by Webster and Povey (1990). They observed a growth rate of 350 to 400 grams per head per day in the lambs fed intensively on cereal based diets. The reasons of higher values recorded in the above referred study may be due to the difference in the use of experimental rations and the experimental conditions.

The feed utilized by the lambs fed on rations A and B during different periods of the trial are presented in table 6. Maximum amount of both the rations was consumed during 29 to 56 days of the experiment. While a decline in feed consumption was observed in the following period. One of the reasons for lower feed consumption in the last period of the study may be sudden drop in environmental temperature. The maximum and minimum temperature recorded during the last week of the experimental period was 20 and 4 C respectively. Significantly ($p < 0.10$) more feed was consumed by the lambs fed on feed A (1071.3±17.7 g) than the lambs fed on ration B (1057.7±17.7 g). One of the reasons for more consumption of feed A may be the lower energy content of the feed. Feed consumed by Balochi and Harnai lambs fed on different rations is presented in table 7. The data indicated significant difference in the feed consumed by both the breeds. Balochi lambs consumed significantly ($p < 0.10$) more feed (1214.7±17.7 g) than the Harnai lambs (1016.4±17.7 g). More utilization of feed by the Balochi lambs resulted in better weight gain as explained earlier.

It can be concluded from the above results that the lower energy ration was consumed more by the lambs as compared to high energy ration. Male Balochi lambs consumed more feed as compared to male Harnai lambs of 4 to 5 months of age under confinement.

The average feed consumption values recorded in the study were in accordance with the values reported by Khan and Pasha (1990). They observed average daily feed consumption in thalli lambs from

1.16 to 1.57 Kg up to 60 days of fattening. The average feed consumption recorded in the study was however lower than those reported by Burgkart, et al. (1988). They observed the average daily intake of 1.56, 1.12, 1.54 and 2.75 Kg per animal for the lambs fed with four maize products. The feed consumption values were also lower than those observed by Khalil and Maqsood (1987) in Afghan lambs. The higher feed consumption values recorded by some of the researchers as compared to the feed consumed by the lambs in this study may be due to the differences in the composition of experimental rations, experimental conditions and breed of the experimental lambs.

Since it was a research trial conducted under different controlled conditions, economics of the trial can not be worked out directly. Economics based on the partial budget analysis is presented in Table 9. The daily profit on each of Balochi and Harnai male lamb was found to Rs. 3.7 and Rs. 1.50 respectively.

MANAGEMENT IMPLICATIONS

Male lambs of Balochi breed, 8 to 10 months of age with body weight below 23.5 Kg may be preferred for intensive fattening and may be fed on rations containing 12% Crude Protein.

Male lambs of Balochi and Harnai breeds, 4 to 5 months old may be fed on high energy rations (2700 Kcal) for better gains.

Whenever there is a choice between Balochi and Harnai Breeds, Balochi male lambs may be preferred for rearing under intensive feedlot for better body weight gain.

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REFERENCES

AOAC, 1975. Official Methods of Analyses. 12th ed. Association of Official Analytical Chemists, Washington, DC.

Berhane Kiflewahid, Gordon R. Potts and Robert M. Drysdale 1982. By-product utilization for Animal Production. Proceedings of a workshop on applied research held in Nairobi, Kenya, 26-30 September 1982. 12 p. International Development Research Center, Canada.

Burgkart, M., Mendel, G., Sattes, R. and Wallner, F., 1988. Conserved maize products in lamb fattening. Wirtschaftseigene futter, 34(1), 38-47.

Daniel, P., 1986. Studies on the utilization of silage mixtures of whole plant maize and vicia faba for lamb fattening. Landwirtschaftliche forschung, 39(3), 230-236.

FAO (Food and Agriculture Organization) 1983. Report of the assistance to rangeland and livestock development survey of Balochistan. FAO Technical Cooperation program report TCP/PAK/007, FAO Islamabad, Pakistan, pp33.

GOB (Government of Balochistan), 1989. Agricultural statistics of Balochistan. Statistics wing, Directorate General of Agriculture Department, Balochistan, Quetta, Pakistan, 136 pp.

GOP (Government of Pakistan) 1986. Pakistan Census of Livestock. Vol. I, Agricultural Census Organization, Statistics Division, Government of Pakistan, Lahore, Pakistan.

Jankowski, S., Pokora, K., Tyszka, Z. and Niznikowski, R., 1985.Principles and methods of industrial lamb fattening. Annals of Warsa Agricultural University SGGW AR., Animal Science (Poland), No. 18, P., 43-51.

Jia, D.J., FU, -YK., Li -GL, Wen, -SW, 1990. Effect of grazing on sainfoin pasture and supplementary feeding with concentrates on lamb fattening. Pratacultural Science, 7(6): 18-21.

Khalil, A. and Manzoor, A. 1987. Fattening potential of Lohi and Sipli sheep. 8th ann.rep. Directorate, Livestock Production Research Institute, Bahadurnagar, Okara, Pakistan, 84-87 pp.

Khalil, A. and Maqsood, A. 1987. Study on feed efficiency in

Afghan sheep at Livestock Production Research Institute, Bahadurnagar, Pakistan, 80 p.

Khan, H.A. and Pasha, T.S., 1990. Fattening potential of different indigenous breed of sheep/goats for mutton production. Final report of PARC project, 30 p.

Masood, M.A., Afzal, M., Nagy J.G. and Khan, S.M. (1988) Agricultural and related statistics of upland Balochistan. MART/AZR Res. Rep. 20, ICARDA, Quetta, Pakistan.

Pojun, S., Pojun, P., Rojanastid, S., 1990. Using Cassava chip as basal feed in concentrates for lamb fattening. Kasetsart Journal, Natural Sciences., 24:(3) 278-286.

SAS, 1985. SAS Users Guide:Statistics. Version 5 Edition. SAS Institute, Cary NC.

Spelman B., and Collions, B.S., 1964. Profitable sheep. 4th ed. Macmillian Co. New York. 159 pp.

Steel, R.G.D. and Torrie, J.H., 1980. Principles and procedures of statistics. 2nd Ed. Mc Graw-Hill Book Co. Inc. N.Y.

Urbaniak, M., Potkanski, A., Krol, R. and Przybecki, T., 1991. Effect of substituting soybean oilmeal by leguminous seeds on results of lamb fattening and tissue carcass composition. Roczniki Akademii Rolniczej w Pozaniu, Zootechnika., No. 41, 27-35.

Webster, GM., and Povey, GM., 1990. Nutrition of the finishing lambs.Occasional Publication, British Society of Animal Production, No. 14; 71-82.

TABLE 1
Composition of the feed ingredients used in the experimental rations.

NAME OF THE FEED INGREDIENTS	D.M.[a] (%)	C.P.[b] (%)	C.F.[c] (%)	T.D.N.[d] (%)
Barley grains	89.0	11.0	2.0	80.0
Cotton seed cake	88.0	23.0	11.0	70.0
Pea seeds	90.0	21.0	6.0	77.0
Rice polishings	90.0	11.2	21.10	80.0
Sorghum grains	89.0	12.0	2.0	78.0
Wheat bran	89.0	12.0	11.0	70.0
Wheat straw	90.0	4.0	41.1	44.0
Molasses	78.0	0.0	0.0	70.0
Urea	98.0	287.5	0.0	0.0

a Dry Matter
b Crude Protein
c Crude Fiber
d Total Digestible Nutrients

TABLE 2
Composition of the two experimental rations fed to the Balochi lambs.

INGREDIENTS	RATIONS	
	A	B
	----------%	----------
Barley	25.00	25.00
Cotton seed cake	06.00	21.50
Pea seeds	05.00	06.00
Rice polishings	21.50	13.00
Sorghum	15.00	15.00
Wheat bran	22.00	13.00
Molasses	05.00	05.00
Urea[1]	00.00	01.00
Premix	00.50	00.50
Crude protein	12.00	17.00
Metabolizable energy (K.cal)	2600	2600

[1] The basal ration (A) contained 12% Crude Protein. Urea was added to the basal ration to increase the CP TO 17% in ration (B).

TABLE 3
Average daily gain, feed intake and feed efficiency of Balochi lambs fed on rations A and B.

PARTICULARS	Treatments			
	RATION A	RATION B	S.E.	S.E.M.[a]
Average daily gain (g/d)				
Week 1 (01-07 d)	171.4	282.1	b	49.40
Week II (08-14 d)	289.3	253.6	N.S.	25.20
Week III(15-21 d)	139.3	185.7	N.S.	31.30
Week IV (22-28 d)	192.9	357.1	d	28.1
Week V (29-35 d)	153.6	146.4	N.S.	73.4
Overall (01-35 d)	189.3	245.0	N.S.	28.70
Average daily feed consumption (g/d)				
Week I (01-07 d)	1421.1	1672.1	b	109.80
Week II (08-14 d)	1535.0	1721.2	b	84.90
Week III (15-21 d)	1531.4	1700.0	N.S.	98.30
Week IV (22-28 d)	1411.2	1860.7	d	59.70
Week V (29-35 d)	1330.0	1738.9	c	103.10
Overall (01-35 d)	1445.7	1738.6	c	80.40
Feed efficiency (g/g)				
Week I (01-07 d)	12.2	6.2	N.S.	4.53
Week II (08-14 d)	5.5	6.8	N.S.	0.81
Week III(15-21 d)	12.1	11.0	N.S.	3.25
Week IV (22-28 d)	8.3	5.4	d	0.52
Week V (29-35 d)	18.0	15.6	N.S.	13.00
Overall (01-84 d)	7.9	7.2	N.S.	1.39

a Standard Error of the Mean
b Significantly Different (P<0.10)
c Significantly Different (P<0.05)
d Significantly Different (P<0.01)
N.S. Non-Significant
S.E. Statistical Effects

TABLE 4
Mean daily gain, feed intake and feed efficiency of light(Mean live weight (B2)=23.5 Kg) and Heavy (Mean live weight(B1)=30.6 Kg) Balochi male lambs fed on two different rations for five weeks.

PARTICULARS	Body Weight Groups B1	B2	S.E.	S.E.M.[a]
Average daily gain (g/d)				
Week 1 (01-07 d)	214.3	239.3	N.S.	49.40
Week II (08-14 d)	275.0	267.9	N.S.	25.20
Week III(15-21 d)	128.6	196.4	b	31.30
Week IV (22-28 d)	360.7	189.3	d	28.1
Week V (29-35 d)	135.7	164.3	N.S.	73.4
Overall (01-35 d)	222.9	211.4	N.S.	28.70
Average daily feed consumption (g/d)				
Week I (01-07 d)	1754.3	1338.9	c	109.80
Week II (08-14 d)	1833.2	1423.0	d	84.90
Week III(15-21 d)	1796.4	1435.0	c	98.30
Week IV (22-28 d)	1860.4	1411.5	d	59.70
Week V (29-35 d)	1679.6	1389.3	c	103.10
Overall (01-35 d)	1784.8	1399.5	d	80.40
Feed efficiency (g/g)				
Week I (01-07 d)	12.6	5.8	N.S.	4.53
Week II (08-14 d)	6.7	5.6	N.S.	0.81
Week III(15-21 d)	15.2	8.0	b	3.25
Week IV (22-28 d)	5.4	8.3	d	0.52
Week V (29-35 d)	21.6	12.1	N.S.	13.00
Overall (01-84 d)	8.4	6.7	N.S.	1.39

a Standard Error of the Mean
b Significantly Different at P<0.10
c Significantly Different at P<0.05
d Significantly Different at P<0.01
N.S. Non-Significant
S.E. Statistical Effects

TABLE 5
Composition of experimental rations fed to the Balochi and Harnai male lambs

INGREDIENTS	RATION A (%)	RATION B
Barley	16.00	37.50
Cotton seed cake	06.00	20.00
Pea seeds	12.00	03.00
Rice polishing	06.00	15.00
Sorghum	25.00	01.00
Wheat bran	15.00	01.00
Molasses	03.00	15.50
Wheat straw	14.00	04.00
Urea	01.00	01.00
Di Calcium Phosphate	01.50	01.50
Premix	00.50	00.50
COMPOSITION		
Crude protein	14.00	14.00
Metabolizable energy (K.cal)	2300	2700

TABLE 6
Average daily gain and Feed intake of experimental lambs fed on rations A and B.

PARTICULARS	Treatments			
	RATION A	RATION B	S.E.	S.E.M.[a]
Average daily gain (g/d)				
Period 1 (01-28 d)	226.3	218.7	N.S.	12.20
Period II (29-56 d)	146.4	203.6	b	232.20
Period III(57-84 d)	21.0	28.4	N.S.	31.80
Overall (01-84 d)	131.2	167.2	c	12.00
Average daily feed consumption (g/d)				
Period I (01-28 d)	1068.7	1045.0	N.S.	46.00
Period II (29-56 d)	1078.7	1278.1	N.S.	58.30
Period III(57-84 d)	966.6	1056.0	b	75.40
Overall (01-84 d)	1071.3	1059.7	b	17.70

a Standard Error of the Mean
b Significantly Different at P<0.10
c Significantly Different at P<0.05
N.S. Non-Significant
S.E. Statistical Effects

TABLE 7
Average daily gain and Feed intake of Balochi and Harnai lambs fed on two different rations

PARTICULARS	Breeds			
	BALOCHI	HARNAI	S.E.	S.E.M.[a]
Average daily gain (g/d)				
Period 1 (01-28 d)	260.3	184.8	b	12.20
Period II (29-56 d)	194.2	155.8	N.S.	23.20
Period III(57-84 d)	60.3	41.5	N.S.	31.80
Overall (01-84 d)	171.6	127.4	c	12.00
Average daily feed consumption (g/d)				
Period I (01-28 d)	1164.2	949.4	b	46.00
Period II (29-56 d)	1347.1	1109.7	c	58.30
Period III(57-84 d)	1132.6	990.0	N.S.	75.40
Overall (01-84 d)	1214.6	1016.4	b	17.70

a Standard Error of the Mean
b Significantly Different at P<0.10
c Significantly Different at P<0.05
N.S. Non-Significant
S.E. Statistical Effects

Table 8
Cost-Benefit estimates of the 16 lambs fed on rations A (12% CP) and B (17% CP) for five weeks under feedlot fattening conditions.

A. Expenditures (Rs.)		Ration A	Ration B
Animal cost	(400/Animal)	3200.00	3200.00
Feed cost	3.95 & 4.36/Kg	1603.70	2124.20
labour cost	1000/Month	1000.00	1000.00
Medicines		250.00	250.00
Miscelaneous		100.00	100.00
Total A		6153.70	6674.20
B. Income (Rs.)			
Animal sale	35/Kg Live Wt.	9415.00	10003.0
Manure sale	0.25/d/animal	84.00	84.00
Total B		9499.00	10083.00
C. Net Income	(B-A)	3345.3	3412.8
D. Profit	Daily/Animal	11.94	12.19

1 US $ = Rs. 30.00

Table 9
Cost-Benfit estimates of the 16 Balochi and 16 Harnai male lambs fed on rations for 12 weeks under feedlot fattening conditions.

A. Expenditures (Rs.)		Balochi	Harnai
Animal cost	(200/Animal)	3200.00	3200.00
Feed cost	4.30/Kg	6935.04	6703.87
labour cost	1000/Month	3000.00	3000.00
Medicines		200.00	200.00
Miscelaneous		100.00	100.00
Total A		13435.04	13203.87
B. Income (Rs.)			
Animal sale	35/Kg Live Wt.	18067.00	14903.0
Manure sale	0.25/d/animal	336.00	336.00
Total B		18403.00	15239.00
C. Net Income	(B-A)	4967.96	2035.13
D. Profit	Daily/Animal	3.70	1.51

1 US $ = Rs. 30.00

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Fig.1.Mean dily gain and feedintake of Balochi lambs fed on rations A and B.

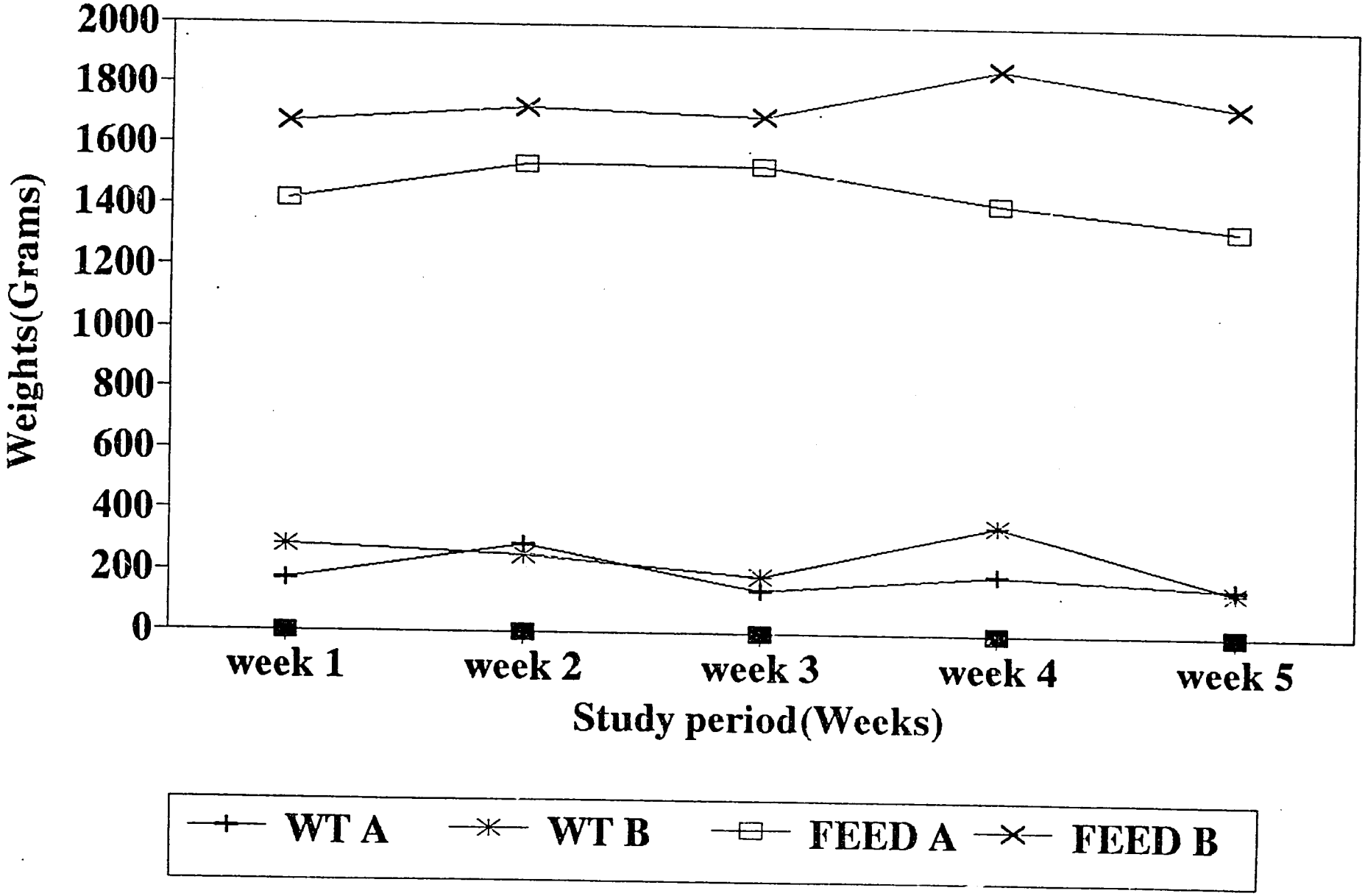
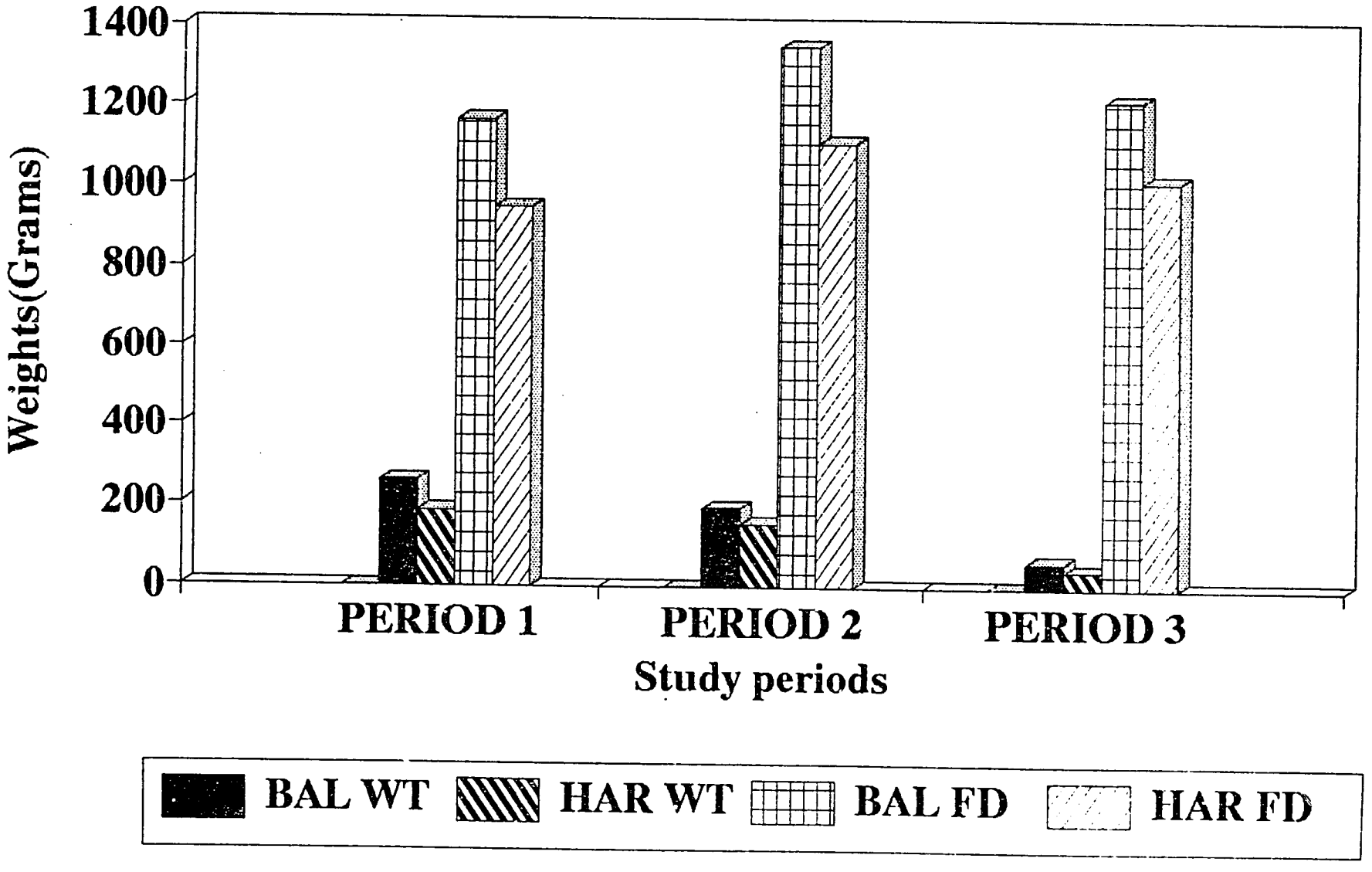


Fig.2.Mean dily gain and feedintake of Balochi and Harnai lambs



REDUCING WATER SEEPAGE FROM EARTHEN PONDS

Shahid Ahmad*, M. Aslam and M. Shafiq***

Water Resources Research Institute, National Agricultural Research Centre, Islamabad, Pakistan.

ABSTRACT

Small earthen reservoirs have proved a promising answer to the problem of storing water on arid-land farms. However, these little ponds or tanks can lose very large amounts of water through seepage. To reduce such losses is a vital need. In the present experiments, chemical, physical, and biological methods for reducing seepage were evaluated in small ponds created in a permeable, calcareous, silt-loam soil. The chemical method involved treating the top 10 cm of soil with sodium carbonate (Na_2CO_3). The physical method involved compacting the soil. And the biological method (the so-called "bio-plastic sandwich") consisted of successive layers of soil, manure, vegetative material and soil. The chemical treatment proved less efficient than the other two. Compared to the untreated soil, the physical and biological methods reduced the mean cumulative seepage rates (measured 350 days after the initial wetting) by 72 percent and 67 percent, respectively. Both of these treatments seem to be cost-effective and ready to be applied to the farm ponds of Pakistan.

KEYWORDS: Pakistan, water harvesting, arid lands, seepage control, bio-plastic

INTRODUCTION

In the drylands that constitute 75 percent of Pakistan, the efficient collection and storage of water is critical. With increasing pressures on the lands, the capture of more rainfall and the efficient storage and use of that water is even becoming a national necessity.

In most of Pakistan farmers depend entirely on rainfall. They also depend on storing water because the rains are far from uniform from month to month (not to mention from year to year). About 60 percent of the rain falls during the July through September monsoon, the rest during February and March (for details, see Siddiqi, 1992). This means that the water collected from the last rains of each wet period must be conserved for about 3 months. It is especially important to keep water on hand to cover the months of April, May and June.

Previous work has indicated that the traditional small earthen pond is a good method for storing incident rainfall. Moreover, the potential for greater use of these so-called "tanks" is high (Ahmad et al., 1984). There are, however, two big problems. For one thing, these small farm ponds are normally shallow and this means that their high surface area (in relation to their volume) fosters excessive evaporation. More importantly, however, they are usually constructed in soils that are permeable and not conducive to holding water for months at a time. The dual losses of evaporation from the top and seepage from the bottom and sides is a main reason why the ponds often go dry just at the time when water is most needed to keep crops and livestock alive. This forces people to migrate out of the rural areas, causing vast dislocations to themselves and to the country (Ahmad, 1993).

Seepage is especially high in calcareous soils, which are common throughout Pakistan. The calcium in these soils reacts with clay in a way that causes the clay to shrink, thereby opening up pores through which water can readily drain away (National Academy of Sciences, 1974). Various ways to reduce this have been tried. These can be classified roughly into chemical, physical and biological approaches.

Chemical

If the clays are of an appropriate type, certain sodium salts can reduce seepage in earthen ponds (Decker, 1963). Sodium ions cause clay particles to swell (floculate) rather than shrink, and they thereby reduce or plug water-conducting pores in the soil. Reginato et al. (1973) determined the type and amount of sodium salt most suitable. In their field experiments they tested sodium chloride, tetra-sodium pyrophosphate, sodium hexametaphosphate and sodium carbonate. Sodium carbonate performed the best, both for reducing initial seepage and for subsequently maintaining low seepage rates.

Narayana and Kamra (1980) recommended that seepage losses be reduced by mixing sodium carbonate with locally available soil (1 percent by weight) and applying the mixture by sedimentation (i.e. letting it settle out through the water column). They recommended applying 750 kg of sodium carbonate per hectare. These authors found that samples of sodic soils, which are naturally high in sodium salts, will also do the job. Although sodic soils are only too plentiful in Pakistan, transportation costs are obviously a limiting factor. Narayana and Kamra found that it took 75 tonnes of sodic soil to treat one hectare.

Physical

The soil in ponds can be physically compacted. This is done with either manual- or tractor-mounted compactors. Sometimes merely walking cattle or buffaloes over the area will help. The amount of compaction achieved depends on the load applied (Rasmursen, 1985) and the dampness of the soil (Hillel, 1982). The soil's physical and chemical properties are also important (Hillel, 1982; Soomro et al., 1983). The level of compaction can be assessed by measuring the soil's bulk density (Cassel, 1982; Canarache et al., 1984).

Biological

For at least 20 years there have been indications that layers of organic materials that are rich in colloids (mucilaginous compounds that swell in water) can reduce percolation losses (National Academy of Sciences, 1974). One form of this is the so-called "bio-plastic," a sandwich made up of successive layers of soil, manure (from pigs, cattle or other livestock), vegetative materials and soil. This creates an underground barrier to seepage. Kale et al. (1986) obtained a seepage reduction of approximately 9 percent by using a mixture of cow dung, paddy husk and soil.

METHODS AND MATERIALS

The objective of this study was to evaluate the effectiveness and economics of various chemical, physical and biological treatments to reduce seepage losses from small earthen ponds constructed in the calcareous soils of Islamabad, Pakistan.

Site Preparation

Sixteen ponds were constructed at the Field Station of the Water Resources Research Institute, National Agricultural Research Centre, Islamabad. The control and the three treatments: sodium carbonate, mechanical compaction and bio-plastic were laid out in a randomized complete block design, each with four replications. The ponds were established in a silt loam, having a 17% clay content. Its physical and chemical characteristics are given in Table 1.

Each pond had a volume of 1.6 m^3 and was surrounded with a wall constructed of bricks and plastered with cement mortar to prevent lateral seepage. The walls were 1.6 m high but extended only 30 cm into the soil therefore, lateral water movement below each treatment was neither controlled nor accounted for. A water-depth of 1 m was held throughout the study period by adjusting the level in each pond daily. About 30 cm of unfilled capacity was always maintained to provide for any rainfall. A small earthen channel around the ponds was filled with a constant head of water to reduce any influence from the surroundings (the so-called "oasis" effect).

The coefficient of variation was calculated at specific intervals to determine variability among the replicates.

Treatments

The following methods were employed for each of the three approaches.

Chemical. Commercial grade sodium carbonate was applied at a rate of 2.5 tonne per hectare to the soil in four ponds. This amounted to 250 g per pond and it was thoroughly

mixed into the top 10 cm of the soil. This layer was then saturated with water for 24 hours so as to let the sodium ions and soil particles equilibrate before the pond was filled. The sodium application rate was determined using the following formula (Reginato et al., 1973):

$$Na_2CO_3 = 0.00771\ DA\ (0.15\ CEC - ES)$$

where

Na_2CO_3 = Sodium carbonate, kg
D = depth of soil to be treated, cm
A = area to be treated, m^2
CEC = cation exchange capacity, cmol/kg
ES = exchangeable sodium, cmol(+)/kg.

Physical. The bottoms and sides of four ponds were soaked with water until their moisture contents were close to field capacity. Each was then compacted using a manual compactor of 10 kg weight. Using a core sampler, the soil density was measured to a depth of 10 cm. The mean bulk density prior to compaction was 1.49 g cm^{-3}; the mean bulk density after compaction was 1.79 g cm^{-3}.

Biological. To construct a bio-plastic sandwich, a layer of cow-dung 7 cm deep was placed on the bottom of the four remaining ponds. This was then covered with a layer of paper-mulberry leaves and clippings from local grass species. This in turn was covered with 10 cm of soil, which was subsequently compacted to a density of 1.49 g cm^{-3} (its original density, see above).

Data Collection

The daily losses from each pond were measured using two independent methods. In the first method, the volume of water needed to restore the 1-m head was recorded using a meter capable of measuring the amount delivered with an accuracy of $\pm$ 0.00005 m^3. In the second method, the depth of each pond was checked with a J Hook measuring gauge having an accuracy of $\pm$ 1 mm. The levels were recorded each morning, before and after filling each of the ponds.

The evaporation rates at the site were determined daily, using US standard screened pans. This so-called "pan evaporation" was taken to equal the evaporation from each pond. It ranged between 1.5 and 9.5 mm d^{-1}. The day's evaporation figure was then subtracted from the amount of water-loss measured in each pond. In this way, the amount of water lost from each of the 16 ponds by seepage alone was arrived at.

Measurements were begun in the second week of February, 1993. However, after 10 days one of the ponds that had been given the compaction treatment showed a suspiciously high (and increasing) rate of water loss, and it was found that rats had tunneled into it. This required rehabilitation, which took 60 days. For consistency, all the ponds were dried down during that period. They were then all refilled together.

As a result of this mishap, actual data collection began 70 days after the initial wetting. To overcome the variability in daily seepage rates, the mean cumulative seepage rates since initial wetting were compared.

RESULTS AND DISCUSSION

The experiments are still ongoing, but at the end of one year, the following results can be reported.

Seepage Rate

The mean daily seepage rates for the control and each of the three treatments are compared in Table 2. The rates showed a large reduction 70 to 120 days after the initial wetting. During this early period, the control showed a decrease in seepage rate of 20 percent, presumably due to natural swelling of the soil particles. The chemical treatment almost doubled the decrease, to 38 percent. However, the compaction and bio-plastic treatments each decreased seepage to almost 50 percent. After 120 days, the mean cumulative seepage rates for the chemical and physical treatments continued to decline slightly, whereas the rates for both the control and bio-plastic treatments appeared to be stabilized.

The coefficient of variation was lowest for the control and the bio-plastic method, and highest for compaction treatment (Table 3). The high coefficient of variation for the compaction treatment was due to extreme variation in the replication that was beset by the rodent problems (as well as to unknown causes, which continued even after the rats had been eliminated).

After 350 days, the physical and biological treatments performed better than the chemical treatment. The mean cumulative seepage rates were 9.0 cm d^{-1} for the control, 7.1 for Na_2CO_3, 2.5 for compaction and 3.0 for bio-plastic. Thus, through compaction or the

use of bio-plastic about 72 and 67 percent reduction in seepage over the control was possible, respectively.

CONCLUSION

Based on the above seepage rate of 9 cm d^{-1} in the untreated silt loam, it can be said that seepage is stealing about 8 m of water from the farm ponds of Pakistan just during the 3 month period that is critical to the farmers' overall success. If the daily loss is reduced to 2.5 cm d^{-1} by using the compaction method, then only 2.25 m of water are lost to seepage during that crucial 90-day rainless period between the seasons. This is a truly significant benefit. For comparison, the pond's evaporation losses would be just over 70 cm during the period.

Thus it can be seen that the compaction or bio-plastic methods may dramatically help reduce water loss due to seepage, thereby providing water during dry spells. Furthermore, compacting the soil and making the bio-plastic sandwich is cheaper than the chemical treatment -- at least where farmers can find appropriate machinery or suitable organic materials.

However, there is a limit to the practical use of this technology. For drier regions than Islamabad (notably those receiving rainfall of less than 350 mm per annum), there is a need to reduce the seepage losses even beyond the levels achieved in these experiments. In such areas evaporation also becomes more acute, and may even exceed 2.5 m per

annum. This is today difficult to control economically. Perhaps by using various covers and plastic pond-linings this might be overcome in the future.

For use in cases of extreme water shortage, ponds can be created with a small deep area as a special reserve. During this research, such a method was tested. The reserve area was only 0.5-1 m deeper than the rest of the pond, but this was enough to maintain some water for 15-30 extra days.

Management Implications

Both the compaction and bio-plastic treatments are deemed practical and cost-effective for many areas of Pakistan. Compaction in ponds can be done using locally available tractors. The major technical requirement is that the soil moisture content must be optimal for compaction. Technical advisors could help the farmers identify the times when the soil is just damp enough to compact well. They could also provide details on the pond depth necessary to assure adequate water volume during dry spells in the given area. Most farmers own cows or buffaloes so that manure is readily available for use in the bio-plastic sandwich.

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REFERENCES

Ahmad, S. 1993. Integrated agricultural production and resource management systems. Integrated APARMS Handbook, Pakistan Agricultural Research Council and Food and Agriculture Organization of the United nations, 48 p.

Ahmad, S., M. Shafiq and M.Z. Ikram. 1984. Rainfall intensity-runoff relationship for small catchments in Pothwar plateau. J. of Eng. & Appl. Sci., 3(2):37-44.

Canarache, A., D. Colibas, I. Horobeanu, V. Patru, H. Simota and T. Trandafirescu. 1984. Effect of induced compaction by wheel traffic on soil physical properties and yield of maize in Romania. Soil and Tillage Research, 4:199-213.

Cassel, D.K. 1982. Tillage effects on soil bulk density and mechanical impedence. p. 45-67. In P.W. Unger and D.M. Van Doren (ed.) Predicting tillage effects on soil physical properties and processes. Spec. Pub. 44. Am. Soc. Agron. Madison, WI, USA.

Decker, R.S. 1963. Sealing small reservoirs with chemical soil dispersants. In Proceedings Seepage Symposium. ARS 41-90. U.S. Dept. Agr., Washington, D.C. pp. 112-129.

Hillel, D. 1982. Introduction to Soil Physics. p. 196-199, Academic Press, Inc. Orlando, FL, USA.

Kale, S.R., J.R. Ramteke, S.B. Kadrekar and P.S. Charpe. 1986. Effect of various sealent material on seepage losses in tanks in lateritic soil. Indian J. of Soil Conservation, 14(2):58-59.

Narayana, V.V.D. and S.K. Kamra. 1980. Studies on use of alkali soil and sodium carbonate as sealants for controlling seepage losses. Indian J. of Soil Conservation, 8(1):23-28.

National Academy of Sciences. 1974. More water for arid lands. Promising technologies and research opportunities. Washington, D.C. pp. 81-85, 111-115, 81-86.

Rasmursen, K.J. 1985. Soil compaction with different surface pressure. Tidsski Planteaul. 89:31-46. (Bio. Abstr. 80 AB-957).

Reginato, R.J., F.S. Nakayama and J. B. Miller. 1973. Reducing seepage from stock tanks with uncompacted, sodium-treated soils. J. of Soil and Water conservation, 28(5):214-215.

Soomro, M.S., A.M.H. Kango and I.D.M. Koondher. 1983. Tractor tyre compaction. A drawback in mechanized farming. J. Eng. and Appl. Sci., 2:43-53.

Siddiqi, M.J. 1992. Analysis of daily rainfall data to know the best planting dates of summer and winter season crops in Islamabad. J. of Eng. and Appl. Sci, Vol. 11, No 1, January-June, 1992.

TABLE 1

Initial chemical and physical composition of soil and cow dung used for seepage study at National Agricultural Research Centre, Islamabad.

Type	Soil*	Cow Dung**
pH	7.79	7.67
ECe (dS/M^2)	0.23	7.8
Na (cmol(+)/kg)	1.22	6.60
Ca (cmol(++)/kg)	3.11	1.95
Mg (cmol(++)/kg)	0.79	--
SAR	0.88	--
ES (cmol(+)/kg)	0.008	--
ESP	0.04	--
CEC (cmol/kg)	21.00	--
Clay (%)	17.00	--
Silt (%)	21.00	--
Sand (%)	62.00	--
Textural class	Silt loam	--
Bulk Density (Mg/M^3)	1.49	--

* Mean across all pond.
** Fresh cow dung.
*** NaCl is normally fed to buffaloes in big lumps.

TABLE 2

Mean cumulative seepage rate (cm d^{-1}) in silt loam soil of Islamabad.

Days since initial wetting	Treatments			
	Bio-plastic	Compaction	Chemical	Control
01	3.11	3.23	11.16	11.53
10	7.05	10.60	15.77	14.07
70	6.42	9.93	17.31	14.75
80	4.69	6.88	14.80	13.22
90	4.17	5.76	13.57	12.48
110	3.47	4.46	11.25	12.13
120	3.34	4.27	10.56	11.96
160	3.20	4.14	10.00	11.79
170	3.17	4.08	10.11	11.93
230	3.11	3.65	9.96	12.16
240	3.11	3.50	9.54	12.23
250	3.14	3.38	9.23	12.23
260	3.23	3.23	8.82	12.13
280	3.24	3.13	8.54	11.90
290	3.21	2.99	8.25	11.47
300	3.19	2.88	8.04	10.93
310	3.16	2.80	7.87	10.54
330	3.09	2.65	7.50	9.76
350	3.01	2.49	7.13	9.00

TABLE 3

Treatment coefficients of variation (%) in silt loam soil of Islamabad.

Days since initial wetting	Treatments			
	Bio-plastic*	Compaction	Chemical	Control
01	30.03	104.43	65.78	23.75
10	67.98	57.07	44.36	31.37
70	65.60	64.49	53.72	34.40
80	53.75	73.56	60.50	31.09
90	46.07	77.75	61.53	27.93
110	36.80	92.82	61.43	23.27
120	36.15	96.42	61.13	22.38
160	35.56	106.04	61.26	17.22
170	34.94	109.07	64.32	15.22
230	32.45	117.47	75.36	20.54
240	31.82	120.77	77.91	25.94
250	32.43	122.50	79.81	29.25
260	34.08	124.50	81.28	32.68
280	34.97	125.28	82.28	34.09
290	35.47	126.77	84.20	36.08
300	35.65	127.80	86.02	36.04
310	35.71	127.89	87.15	35.98
330	35.84	129.58	89.25	35.74
350	35.97	131.05	91.18	35.35

* Bio-plastic is a sandwich of soil + cow dung + vegetative material + soil.

** The ponds were kept dried for 60 days starting 10 days after the initial wetting.

EFFECT OF SALINITY ON GROWTH OF *Rhizobium* sp., NODULATION AND HEIGHT OF *Prosopis* SPECIES

Durr-e-Habib Roomi, A. Ghaffar, R. Ahmad and S. Ismail
Biosaline Research Laboratories,
Department of Botany,
University of Karachi,
Karachi, Pakistan.

ABSTRACT

The effect of salinity on the growth of *Rhizobium* sp. isolated from *Prosopis glandulosa* and *P. juliflora* was studied *in vitro*. Growth of rhizobium isolated from both *Prosopis* species increased at all salinity levels as compared with the control (0.01%) except at 1% NaCl level. *Rhizobium* sp. isolated from *P. glandulosa* showed a gradual decline as salinity increased from 0.3 to 1.0%; that isolated from *P. juliflora* showed a decline as salinity increased from 0.2 to 1.0%. Increasing salinity caused a gradual reduction in seed germination, height of the plant, number of nodules per plant and nodule size.

INTRODUCTION

Increasing soil salinity has greatly reduced agricultural productivity in various parts of the world. The effects on leguminous crops appear to be greater presumably because of a direct influence on the nitrogen-fixing ability of root nodule bacteria (Munns et al., 1977). Increasing levels of NaCl have been found to cause reduction both in nodulation and nitrogen-fixing ability in peas and mungbeans (Steinborn and Roughley, 1975). Mesquite (*Prosopis* spp.), a nitrogen-fixing tree legume, grows luxuriantly under various salinity and aridity regimes. The plant has been found to survive and even flourish in soil made saline with 12,800 mg/l NaCl, and its roots can extract water from soil that is salinized with more than 17,900 mg/l (Jarrell and Virginia, 1984). *Prosopis* seeds collected from widely divergent saline areas of Africa, Argentina, Mexico, and the United States have been grown in sand culture up to 3.3% NaCl concentration (Rhodes and Felker, 1988). Nodulation has been reported in 12 *Prosopis* species with different rhizobial strains (Felker and Clark, 1980).

The present work was carried out to study the effects of different levels of NaCl salinity on the growth of rhizobium species isolated from *P. glandulosa* and *P. juliflora*. The study also investigated the effects of different NaCl concentrations on seed germination, height of the plant, and the number and size of nodules of *P. juliflora* after inoculation with *Rhizobium* sp.

MATERIALS AND METHODS

Isolation of *Rhizobium* sp.

P. glandulosa and *P. juliflora* roots collected from fields of Sujawal, Pakistan, were used. After washing in running tap water, the nodules were separated and surface sterilized in 0.01% HgCl for 5 minutes, followed by washing in sterilized distilled water. The nodules were dipped in 70% ethyl alcohol for 3 minutes, and after washing in sterilized distilled water, were crushed with a sterilized glass rod in a petri dish and streaked on YEM agar containing Congo red (Subba Rao, 1977).

Effect of Salinity on Growth of *Rhizobium in vitro*

Yeast extract mannitol broth (yeast extract, 1.0 g; mannitol, 10 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; distilled water, 1 L) was poured @ 25 ml/flask and sterilized at 15 psi for 15 minutes. NaCl was used to adjust the salinity of the YEM broth to 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, and 1.0% concentrations. Flasks with 0.01% NaCl served as the control. Three-day-old rhizobial cultures isolated from *Prosopis* sp. were inoculated in YEM broth @ 1 ml/flask. Five replicates of each treatment were incubated for 72 hours at room temperature (30 - 35 ^{o}C) on a rotary shaker. The growth of rhizobia in culture was determined at every transfer of suspension by determining the number of colony-forming units (cfu) of the broth culture. Using serial dilution of the broth culture, one ml from 10^{-6} to 10^{-9} dilutions was transferred aseptically in sterile petri dishes, and was then mixed with 15 ml. melted YEMA. Each of these treatments was replicated three times. Petri dishes were incubated at 30 ^{o}C for 3 days, and the number of colonies (cfu) in each plate was then

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[illegible] (Somasegaran and Hoben, 1985). The population of rhizobia was estimated using the formula:

Colony-forming unit (cfu) of rhizobia = No. of colonies x dilution factor.

Effect of Rhizobial Inoculation Under Saline Conditions on Seed Germination, Height of the Plant and Nodulation

Seeds of *P. juliflora* collected from Sujawal, were used. Rhizobial strains isolated from nodules of *P. juliflora* were grown on yeast extract mannitol agar. The rhizobia were transferred serially from the culture of lower to higher salt concentration and maintained at 0.01, 0.2, 0.4, 0.6, 0.8, and 1.0% NaCl salts. The seeds were scarified in concentrated H_2SO_4 and thoroughly washed with distilled water, then inoculated with the appropriate suspension of *Rhizobium* using 1% gum arabic as sticker. Loamy sand soil collected from Karachi University experimental plots was autoclaved at 15 psi and transferred into 8 cm. diameter plastic pots without any drainage, 250 g in each. The soil was moistened to field capacity with respective saline solutions to give an EC_e equivalent to 0.01 to 1% NaCl solutions. Seeds inoculated with rhizobium from the different salinity treatments were sown in the pots in soil with the same adjusted salinity of 0, 0.2, 0.4, 0.6, 0.8 and 1.0% NaCl. The pots were watered twice a week with tap water. In a second set of experiments, uninoculated seeds were grown in sterilized soil. When the seedlings were 10 days old, the soil was drenched with rhizobial suspension of acclimatized salinities. In a third set of experiments, seed inoculation treatment, was followed by soil drench. There were three replicates of each treatment and the pots were randomized on a greenhouse bench. The soil was kept at 50% water-holding capacity by daily watering the plants. The plants were harvested after 6 week and height of the plant and number and size of root nodules were recorded.

RESULTS

The Effect of Salinity on growth of Rhizobia *in vitro*

The growth of *Rhizobium* sp. isolated from *P. glandulosa* showed an increase from

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32×10^6 to 252×10^6 cfu at 0.1 and 0.2% NaCl salinity, respectively, followed by a decline to 125×10^6 cfu at 0.3% and 29×10^6 cfu at 1% salinity, as compared with the control at 0.01% salinity (Figure 1a). *Rhizobium sp.* isolated from *P. juliflora* (Figure 1b) showed a gradual decline from 208×10^6 cfu at 0.1% NaCl salinity to 35×10^6 cfu at 1% salinity as compared with the control. Rhizobium grown at different salinity levels showed no change in cell size and morphology.

Effect of Rhizobial Inoculation Under Saline Conditions on Seed Germination, Height of Plant, and Nodulation

Seeds of *P. juliflora* inoculated with rhizobium acclimatized at different salinity levels and sown in soil adjusted to different NaCl salinity levels showed 62, 25, 20, 16 and 6.6% germination, respectively, in 0.2, 0.4, 0.6, 0.8 and 1.0% salinity, as compared with 84% germination in the control (Figure 2). With the gradual increase in soil salinity, there was a gradual reduction in height of the plants as compared with the control. There was a significant ($P < 0.05$) decrease in number of nodules at 1.0% NaCl salinity as compared with the control (Table 1). With an increase in salinity, a significant decrease ($P < 0.05$) in diameter of nodules was observed. When seedlings of *P. juliflora* were drenched with rhizobial suspension, a gradual reduction in size of plant, number of nodules/plant, and size of nodules was observed in soil adjusted at 0.2 to 1.0% NaCl salinity as compared with the control (Table 2). The plants were taller and nodule more numerous than those grown in salinized soil. In the third set of experiments, where seed inoculation was applied followed by a soil drench with *Rhizobium*, no significant ($P > 0.05$) difference in height of the plants was observed. A significanct ($P < 0.05$) increase in number and size of nodules was observed at 0.2% NaCl salinity; however, a gradual decrease in height of plants, number of nodules per plant and size of nodules was recorded from 0.4 to 1.0% NaCl treatment as compared with the control (Table 3).

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DISCUSSION

The growth and multiplication of the rhizobium isolated from *P. glandulosa* and *P. juliflora* declined rapidly as the NaCl concentration increased from 0.2 to 1.0%. A wide variation in salt tolerance has been reported among *Rhizobium* species. *Rhizobium japonicum* isolated from *Glycine javanica* was found tolerant to 0.5% (Wilson & Norris, 1970); *R. trifolii* isolated from *Trifolium* sp., to 0.50-0.72% (Pillai and Sen, 1966); and *R. meliloti* isolated from *Melilotus* sp., to 3% NaCl salinity (Graham and Parker, 1964; Subba Rao et al., 1972). The growth and multiplication of rhizobia from soybean declined rapidly when the NaCl concentration was increased from 0.2 to 0.8% (Tu, 1981). Similarly the growth of *R. leguminosarum* declined rapidly as the salinity increased from 0.01-2.0% (Rai, 1983).

Salinity reduced seed germination in *P. juliflora*. Inoculation with *Rhizobium* did not appear to be advantageous at the germination stage. Higher soil salinity has been reported to delay seed germination in wheat (Ansari et al., 1980) and in tropical legumes (Keating et al., 1986). In the present work, the presence of a high NaCl concentration in the soil decreased the growth and multiplication of rhizobia and reduced the number and size of the nodules of *P. juliflora*. Similar observations have been made with other legumes (Tu, 1981; Haq and Larher, 1983). Conditions that promote growth of root hairs, rhizobial growth and multiplication are considered favourable for nodulation (Nutman, 1958). This would suggest that high salinity adversely affects nodulation. Of the three methods used for inoculation, soil drench appear to have been the most suitable methods of inducing nodule formation. Better growth performance of the plant was also observed.

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REFERENCES

Ansari, R., Naqvi, S.M. and Ala, S.A. 1980. Response of wheat cultivars to the presence of sodium salts at germination and early development. Biologia Plant., 22:470-472.

Felker, P. and Clark, P.R. 1980. Nitrogen fixation (acetylene reduction) and cross inoculation in 12 *Prosopis* (mesquite) species. Plant Soil, 57: 177-186.

Graham, P.H and Parker, C.A. 1964. Diagnostic features in the characterization of the root nodule bacteria of legumes. Plant Soil, 20: 383-396.

Haq, S.M.I. and Larher, F. 1983. Effect of sodium chloride salinity on the growth and nitrogen status of nodulated cowpea (*Vigna sinensis* L.) and mungbean (*Phaseolus aureus* L.). Z. Pflanzenphysiol., 112: 79-87.

Jarrell, W.M. and Virginia, R.A. 1984. Salt tolerance of mesquite. California Agric., 38 (10): 28.

Keating, B.A., Strickland, R.W. and Fisher, N.J. 1986. Salt tolerance of some tropical pasture legume with potential to adaptation to cracking clay soil. Aust. J. Exp. Agric., 26 : 181-186.

Munns, D.N., Fox, R.L. and Koch, B.L. 1977. Influence of lime on nitrogen fixation by tropical and temperate legumes. Plant Soil, 46 : 591-601.

Nutman, P.S. 1958. The Physiology of nodule formation. In: Nutrition on legumes (Hallsworth, E.G., Ed.), pp. 87-107, Butterworth's Scientific Publications, London.

Pillai, R.N. and Sen, A. 1966. Salt tolerance of *Rhizobium trifolii*. Indian J. Agric. Sci., 36: 80-84.

Rai, R. 1983. The salt tolerance of rhizobium strains and lentil genotypes and the effect of salinity on aspects of symbiotic nitrogen fixation. J. Agric. Sci., 100 : 81-86.

Rhodes, D. and Felker, P. 1988. Mass screening of *Prosopis* (Mesquite) seedlings for growth at seawater salinity concentrations. Forest Ecol. Manag., 24(3) : 169-176.

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Somasegaran, P. and Hoben, H. J. 1985. Methods in legume -*Rhizobium* Technology. NIFTAL Project, Univ. of Hawaii.

Subba Rao, N.S. 1977. Soil microorganisms and plant growth. Oxford and IBH Publishing Co., New Delhi, 289 pp.

Subba Rao, N.S., Lakshmi-Kumari, M., Singh, C.S. and Magu, S.P. 1972. Nodulation of lucerne (*Medicago sativa* L.) under the influence of sodium chloride. Indian J. Agric. Sci., 42: 384-386.

Steinborn, J. and Roughley, R. J. 1975. Toxicity of sodium and chloride ions to *Rhizobium* spp. in broth and peat culture. J. Appl. Bacteriol., 39: 133-138.

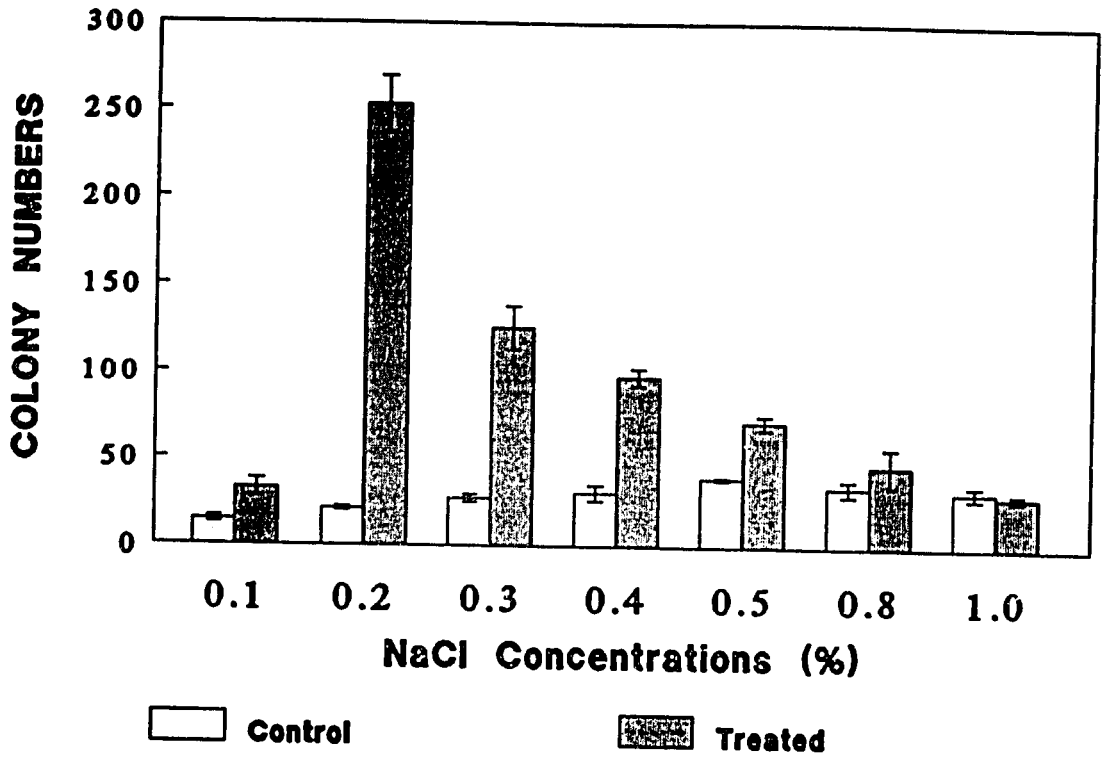
Tu, J.C. 1981. Effect of salinity on *Rhizobium* - root hair interaction, nodulation and growth of soybean. Can. J. Plant Sci., 61 : 231-239.

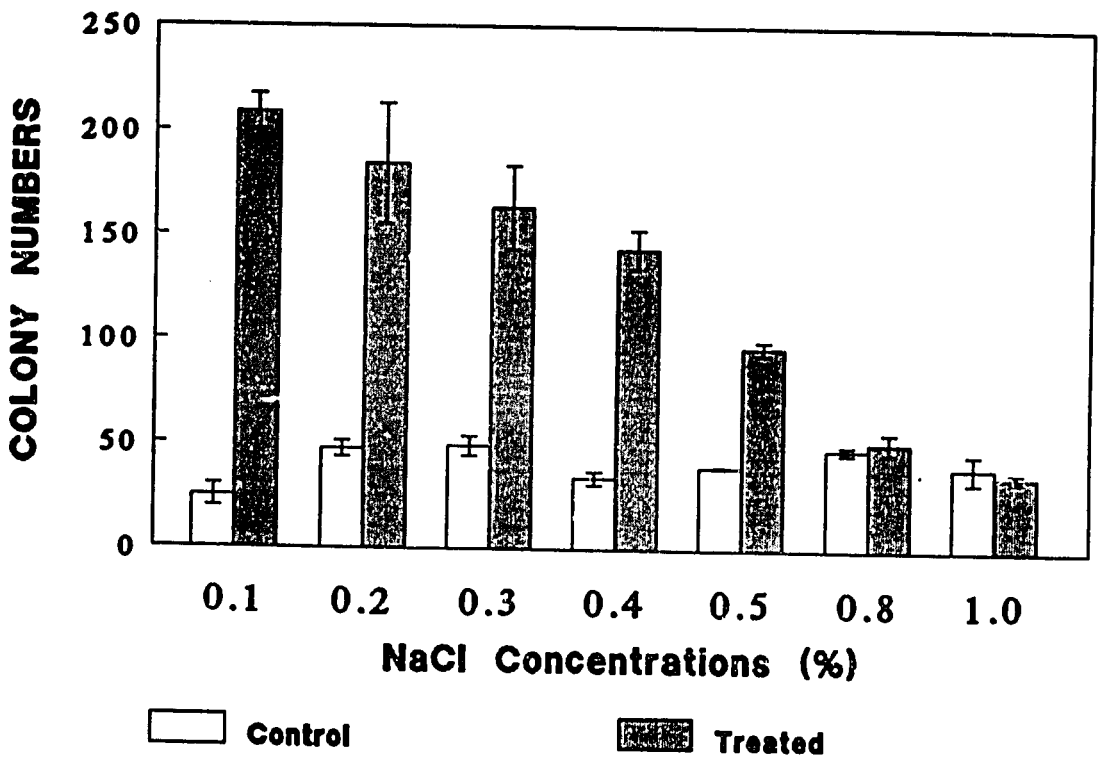
Wilson. J.R. and Norris, D.O. 1970. Some effects of salinity on *Glycine javanica* and its Rhizobium symbiosis. Proc. Int. Grassl. Congr., 11 : 455-458.

LEGENDS

Figure 1. Effects of different salinity regimes on multiplication of *Rhizobium* isolated from *P. glandulosa* (a) and *P. juliflora* (b) on yeast mannitol broth with varying levels of NaCl.

Figure 2. Effects of different levels of NaCl salinity in soil on seed germination of *P. juliflora* inoculated with rhizobia.


COLONY NUMBERS
300
250
200
150
100
50
0
0.1
0.2
0.3
0.4
0.5
0.8
1.0
NaCl Concentrations (%)
Control
Treated


COLONY NUMBERS
250
200
150
100
50
0
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0.2
0.3
0.4
0.5
0.8
1.0
NaCl Concentrations (%)
Control
Treated

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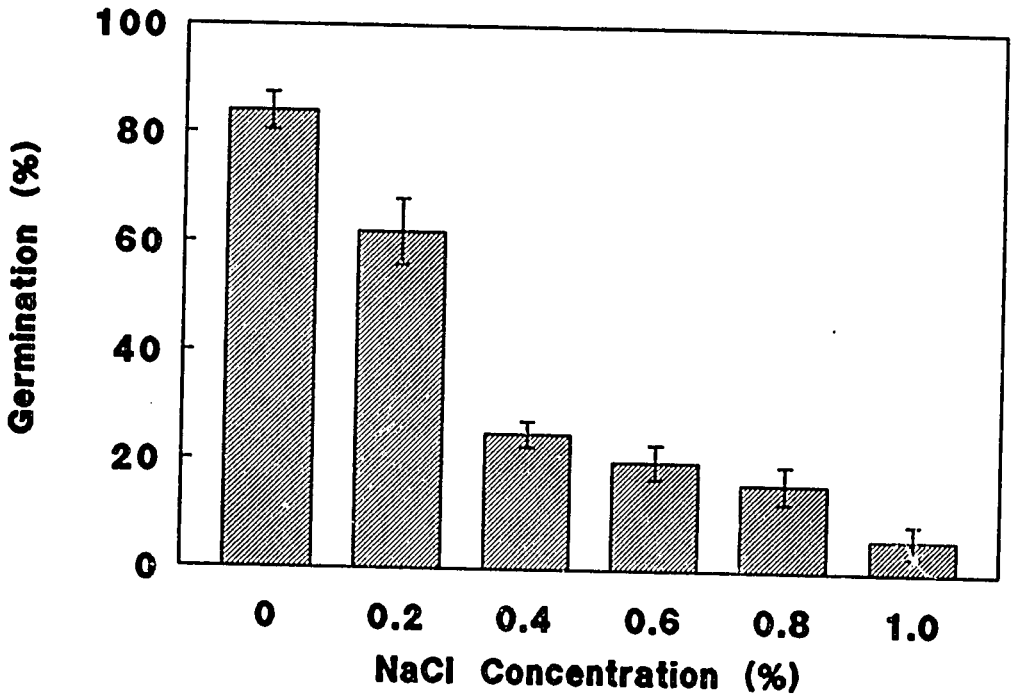
100
80
60
40
20
0
Germination (%)
0
0.2
0.4
0.6
0.8
1.0
NaCl Concentration (%)

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Table 1. Effects of seed inoculation of P. juliflora with Rhizobium sp. at different levels of NaCl salinity in soil on plant height, size and number of nodules.

NaCl Concentration	Number of Nodules Per Plant	Average Diameter of Nodules (mm)	Plant Height (cm)
Control EC: 1.7 $dS.m^{-1}$	5.800 ±1.593	4.400 ±0.599	19.800 ± 1.562
0.2% EC: 3.3 $dS.m^{-1}$	7.600 ±1.435	3.000 ±0.707	14.400 ± 0.678
0.4% EC: 6.6 $dS.m^{-1}$	3.000 ±0.547	2.600 ±0.244	12.400 ± 0.400
0.6% EC: 9.8 $dS.m^{-1}$	5.600 ±1.029	2.600 ±0.244	11.000 ± 0.316
0.8% EC: 12.9 $dS.m^{-1}$	5.200 ±0.374	3.200 ±0.199	11.200 ± 0.583
1.0% EC: 16.0 $dS.m^{-1}$	2.600 ±1.600	2.200 ±0.374	9.800 ± 0.374
F-Value	2.4323	3.0620	21.751
$LSD_{0.05}$	3.5067	1.2834	2.2735

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Table 2. Effects of soil drench with Rhizobium sp. on P. juliflora at different levels of NaCl salinity in soil on plant height, size and number of nodules.

NaCl Concentration	Number of Nodules Per Plant	Average Diameter of Nodules (mm)	Plant Height (cm)
Control EC: 1.7 $dS.m^{-1}$	14.000 ± 1.788	5.000 ±0.707	24.200 ± 2.244
0.2% EC: 3.3 $dS.m^{-1}$	10.200 ± 1.356	3.800 ±0.489	16.200 ± 2.395
0.4% EC: 6.6 $dS.m^{-1}$	9.600 ±2.315	2.600 ±0.927	14.400 ± 0.399
0.6% EC: 9.8 $dS.m^{-1}$	6.600 ±1.029	3.800 ±0.969	13.800 ± 0.374
0.8% EC: 12.9 $dS.m^{-1}$	4.200 ±0.860	2.200 ±0.734	12.200 ± 0.734
1.0% EC: 16.0 $dS.m^{-1}$	3.800 ±0.374	2.800 ±0.663	13.400 ± 0.673
F-Value	7.5280	1.8113	9.4708
$LSD_{0.05}$	4.1860	2.2357	4.1417

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Table 3. Effects of seed inoculation and soil drench with Rhizobium sp. on P. juliflora at different levels of NaCl salinity in soil on plant height, size and number of nodules.

NaCl Concentration	Number of Nodules Per Plant	Average Diameter of Nodules (mm)	Plant Height (cm)
Control EC: 1.7 $dS.m^{-1}$	4.400 ±0.748	3.600 ±0.399	19.000 ± 0.547
0.2% EC: 3.3 $dS.m^{-1}$	9.200 ±3.455	5.400 ±0.244	19.200 ± 1.019
0.4% EC: 6.6 $dS.m^{-1}$	2.200 ±0.734	1.800 ±0.583	14.000 ± 0.707
0.6% EC: 9.8 $dS.m^{-1}$	1.800 ±0.374	1.900 ±0.600	12.000 ± 1.113
0.8% EC: 12.9 $dS.m^{-1}$	3.200 ±0.860	1.320 ±0.287	13.000 ± 1.000
1.0% EC: 16.0 $dS.m^{-1}$	1.200 ±0.374	2.800 ±0.734	10.800 ± 0.734
F-Value	3.6779	8.8695	16.5144
$LSD_{0.05}$	4.4682	1.4799	2.5613

*SCREENING OF MESQUITE (*Prosopis *Spp.) FOR BIOMASS PRODUCTION AT BARREN SANDY AREAS USING HIGHLY SALINE WATER FOR IRRIGATION*

RAFIQ AHMAD, SHOAIB ISMAIL, M. MOINUDDIN AND TARANA SHAHEEN

Biosaline Research Laboratories,
Department of Botany,
University of Karachi,
Karachi, Pakistan.

ABSTRACT

The objective of this study was to screen and identify those mesquite (*Prosopis*) species/accessions/provenances, including both local and exotic genome lines, capable of growing with saline water and producing maximum biomass and fuelwood in semi-arid coastal regions of Pakistan (around Karachi), where good quality water is not available.

Seeds of three indigenous and one exotic *Prosopis* species/provenances were germinated on filter paper saturated with NaCl solutions in petri plates. Electrical conductivity (EC) values of the NaCl solutions ranged from 0 to 30 $dS.m^{-1}$. Seeds of *P. juliflora* and *P. glandulosa* showed higher germination percentages than seeds of *P. cineraria* and the exotic species in controls, as well as under different levels of salinity stress. There was a decrease in germination of all species with increasing salinity levels. The emergence of the three indigenous *Prosopis* species was observed in sand irrigated with NaCl dilutions of the same EC as above. Seedling emergence was completely inhibited above EC 10 $dS.m^{-1}$ in *P. juliflora* and *P. cineraria* and above EC 15 $dS.m^{-1}$ in *P. glandulosa*.

In an another experiment, the growth of three indigenous and two exotic *Prosopis* species/provenances was monitored in pots containing sandy soil and irrigated with dilutions of sea water adjusted at EC_{iw}: 10, 20, 30, and 40 $dS.m^{-1}$. *P. glandulosa* did not survive beyond EC_{iw}: 20 $dS.m^{-1}$. Among the surviving plants, *P. juliflora* obtained from Brazil grew in the highest salt concentration and showed minimum biomass reduction in comparision with other species.

In a field experiment, seedlings of 12 indigenous and 8 exotic species/accessions/provenances of *Prosopis* were transplanted under field conditions and irrigated with saline water (EC: 14-16 $dS.m^{-1}$) obtained from a well. Among the indigenous species/provenances, *P. juliflora* (D.I. Khan) and *P. glandulosa* (Sujawal), and among the exotic species/accessions, *P. alba* (Acc.# 0166) and two South American species, showed better growth in terms of plant height and stem diameter under prevailing halo-xeric conditions.

Shoot stocks of different exotic *Prosopis* species of commercial importance grafted onto fast-growing salt-tolerant indigenous species proved to be successful.

The results of the experiments suggest the importance of mesquite cultivation for providing fodder and fuelwood, supplying timber, and stopping the movement of sand dunes to control desertification.

INTRODUCTION

Meeting present and future global food demand through conventional agriculture is challenging if not impossible. Population growth is exceeding increases in food production in spite of the incorporation of modern scientific techniques in agriculture. Furthermore, management problems such as waterlogging and increasing soil salinity are reducing yields from once-fertile lands. Hence, there is an urgent need to expand vegetation on barren, semi-arid, and arid wastelands in order to meet future food, fodder, and fuel demands.

The objective of this study was to screen and identify those mesquite (*Prosopis*) species/accessions/provenances, including both local and exotic genome lines, capable of growing with saline water and producing maximum biomass and fuelwood in semi-arid coastal regions of Pakistan (around Karachi), where good quality water is not available.

Mesquite, a woody legume, grows naturally in hot semi-arid and arid regions of the world. Its fruits provide an alternative source of food for humans and feed for ruminants. Leaves of *P. cineraria* are grazed by goats and sheep. In addition, mesquite pods provide

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fodder for small ruminants since they contain 9.6-12.5% crude protein, 1-3.4% reducing sugar, and 8.3-12.8% total sugar (Gupta et al., 1984). This genus has provided a dietary base in the United States and Mexican border areas for several thousand years (Nabhan, 1984). Almost all the *Prosopis* species produce good-quality fuelwood and charcoal. Some are capable of growing into trees that can produce wood for making wooden flooring, doors, and furniture. Some multistem species have proved to be excellent in controlling sand dunes, controlling soil erosion and stopping desertification. Enhanced honey production is noted in hives placed in the vicinity of mesquite forests. Overall, mesquite is a good commercial plant species for semi-arid and arid regions. Apart from being able to tolerate water stress, *Prosopis* spp. can tolerate salt stress, up to a high salinity level (Rhodes and Felker, 1988; Ahmad et al., 1986; Jarrell and Virginia, 1984; Felker et al., 1981).

Man-made forests of *P. juliflora* have been established on the barren coastal sandy belt of Pasni, Pakistan, through irrigation with highly saline underground water (Khan et al., 1986). Experiments conducted to determine the effects of irrigation frequency on biomass production have showed greater productivity in selected *Prosopis* species as compared to other tree species under high-temperature/drought conditions (Felker et al., 1983a). Among trees of *P. chilensis, P. alba*, and *P. velutina* grown for 22 years, the latter is reported to produce 7.2 kg dry biomass/plant as compared to other 29 species of *Prosopis* (Felker et al., 1983b). Trees of dry irrigated regimes in these experiments also showed the highest pod production.

There are about 44 species of this genus distributed all over the semi-arid and arid regions of the world (Galera et al., 1992). Proceedings of the *Prosopis* Symposium, held by the Centre for Overseas Research and Development (Dutton, 1992) provide up-to-date information on various aspects of growth and utilization of these plant species. Some of these species are well adapted and grow well under harsh climatic and edaphic conditions. The species that are found naturally in Pakistan include *P. juliflora*, *P. cineraria*, and *P. glandulosa*, which differ morphologically as well as ecologically. *P. juliflora*, a deep-rooted plant varying from shrub to tree habitat was naturalized in Pakistan in the early 1950s to stabilize dunes and provide fuelwood (Mohammad, 1992). Furthermore, *Prosopis* species

are distributed widely from subtropical arid regions to tropical arid regions of Pakistan, mainly in the Baluchistan region and the Thal, Cholistan and Tharparkar areas. Advantage could be taken of this fact to achieve the benefits discussed above. Moreover, *Prosopis* could also be introduced in barren areas elsewhere under similar halo-xeric conditions.

MATERIALS AND METHODS

Germination

Twenty seeds of nine different provenances of three local *Prosopis* species (*P. juliflora*, *P. glandulosa*, and *P. cineraria*) and one exotic species (*P. pallida*) were treated with concentrated sulfuric acid for 20-25 minutes, washed with running tap water and placed in petri dishes on filter paper. The petri dishes were kept in an incubator at 28 ^{o}C for germination. The filter paper was then saturated with non-saline or saline water. Solutions of various salinities were made by dissolving the Analar grade of sodium chloride in distilled water to give electrical conductivity (EC) values of 5, 10, 15, 20, 25, and 30 $dS.m^{-1}$, respectively. Each salinity was replicated three times in a completely randomized design. Germination counts were recorded daily for 8 days. Seeds were considered to have germinated when the hypocotyl emerged from the seed coat.

Emergence

The emergence of the different provenances of *Prosopis* species was also studied, as often the results obtained for germination in petri dishes do not correspond with the emergence of seedlings in soil. The seeds of each of the species used in the germination study were sown in plastic containers filled with sand and irrigated with different dilutions of NaCl, with an EC_{iw} range of 5-30 $dS.m^{-1}$. Three replicates were kept for each treatment in a completely randomized design and observed for 8 consecutive days.

Salt Tolerance in *Prosopis* Species/Provenances

Seedlings of two exotic species -- *P. juliflora* obtained from Brazil and *P. pallida* (supplied by Winrock) -- and two indigenous species *P. glandulosa* (two provenances) and *P. juliflora* (one provenance) were raised from seeds in earthen pots containing 12 kg sand. Once established, the seedlings about 1 cm in height, were irrigated with various dilutions of seawater to give EC values of 10, 20, 30, and 40 $dS.m^{-1}$. Irrigation with non-saline water was used as a control for comparison. Each treatment was replicated five times in a completely randomized design. Data on height, stem diameter and number of branches were recorded every 4 months. Plants were harvested at 1 year of age. At harvest, the whole plant was separated into leaf, stem, and roots, respectively. Samples of plant parts were then placed in an oven at 70 ^{o}C for 48 hours for dry weight measurements. Soil samples from the pots were collected and oven dried. Saturated extract of soil was obtained from these soil samples for measurement of electrical conductivity.

Screening of Local and Exotic Genome of *Prosopis* Under Field Conditions Using Saline Water for Irrigation

Seeds of 21 exotic and 13 local species/accessions were sown after acid treatment in plastic bags filled with pure sand. On the basis of better germination percentage and availability of good-sized seedlings, 20 accessions/provenances of different *Prosopis* species (8 exotic and 12 indigenous) were transplanted in different blocks in a completely randomized design. The whole experimental area was divided into 20 blocks, each randomized for a different species/accession/provenance. There were three rows in each block with three plants/row, for a total of nine replicates of each species/accession/provenance. Plant-to-plant and row-to-row distance was kept at 3 m. Thus 20 blocks covered an area of 1620 m^2 (45 x 36 m^2). Detail of the species randomized in blocks and list of the species/accessions/provenances is presented in Figure 1.

Plants were irrigated by filling each pit with 20 L underground saline water (EC: 14-16 $dS.m^{-1}$), pumped from a 12 m deep well dug at the experiment site. The plants were

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irrigated twice a week during summer and once a week in winter. Extra care was taken to ensure that plants in each pit received the same amount of water at each irrigation. Data regarding survival, plant height and number and length of branches were recorded periodically to determine the growth of the plants. Some plants were destroyed by field rabbits during the first 2 months and were subsequently replanted from the stock. Data pertaining to quantum yield, stomatal conductance, and transpiration rate were recorded using a LICOR (LI-1600) porometer. Chlorophyll and sugar contents of the plants were analyzed as described by Maclachlam and Zalik (1953) and Yemm and Willis (1956), respectively.

Grafting *Prosopis* spp.

Budwood of exotic species (supplied by Dr. Peter Felker, A & I University, Kingsville, Texas) was grafted on 2 year old plants of *P. glandulosa* and *P. juliflora* growing at Karachi University campus. Branches of local *Prosopis* species (about 0.5 mm in diameter) were cut about 50 cm below the tip. The lower end of the budwood about 10 cm long was sliced from both sides into a wedge shape, and inserted into a vertical slit made at the terminal cut end of the fast-growing local genome. Both twigs were kept wrapped tightly with grafting tape at the point of insertion for five weeks when the tape was removed at which time the successful grafts were found to be producing new shoots.

OBSERVATIONS AND RESULTS:

Germination and Emergence Studies

The effects of different dilutions of NaCl on the germination of *Prosopis* seeds is shown in Figure 2. Seeds of *P. juliflora* and *P. glandulosa* from Sujawal showed the best germination percentage under non-saline (control) conditions as compared with the *Prosopis* representing the other sources. Germination was reduced by 5-10% in all the species at EC: 5-15 dS.n pared with the respective controls.*Prosopis* from Sujawal also showed tolerance to salt stress, with a 60% germination even at a saline solution of EC: 20 $dS.m^{-1}$. The overall *Prosopis* germination for the seed source at EC: 20 $dS.m^{-1}$ was as follows:-

P. juliflora (Sujawal) > *P. glandulosa* (Sujawal) > *P. cineraria* (Peshawar) > *P. cineraria* (Karachi) > *P. juliflora* (Bhawani) > *P. cineraria* (Tandojam) > *P. pallida.*

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The emergence of *Prosopis* seedlings from soil irrigated with different salt concentrations is presented in Figure 3. These data do not follow the germination data. The percentage of emergence for the non-saline control was different in the soil than in the petri dishes. Germination development and growth of the seedlings from the three *Prosopis* species were as follows:

P. glandulosa (Sujawal), 80%; *P. juliflora* (Bhawani), 70%; *P. cineraria* (TandoJam), 40%

The seedlings did not develop beyond EC: 10 $dS.m^{-1}$ in the case of *P. juliflora* and *P. cineraria*, and beyond EC: 15 $dS.m^{-1}$ for *P. glandulosa*. Emergence at EC: 10 $dS.m^{-1}$ in *P. juliflora* and *P. cineraria* was about 10%, and that of *P. glandulosa* at EC: 15 $dS.^{-1}$ was only about 5%.

Salt Tolerance in Two Exotic and Three Indigenous *Prosopis* Species/Provenances

The seedlings of all the evaluated species/provenances of *Prosopis* exhibited various degrees of salt tolerance when irrigated with different concentrations of saline water (Table 1). *P. glandulosa* failed to grow beyond an EC_{iw} level of 20 $dS.m^{-1}$, thus exhibiting low tolerance limit to salt stress. These plants also exhibited a 35 to 40% reduction in height and about a 50% reduction in stem diameter at EC_{iw}: 20 $dS.m^{-1}$ as compared with the plants grown in non-saline soil. However, all other *Prosopis* species exhibited a marked reduction in growth only at the highest salinity level (EC_{iw}: 40 $dS.m^{-1}$). *P. juliflora* (Bhawani) appeared quite tolerant at an EC_{iw} of 20 $dS.m^{-1}$, with no effect on plant height, followed by *P. pallida* (Winrock) (8% reduction) and *P. juliflora* (Brazil) (13% reduction). At this salinity level, stem diameter was least affected in *P. juliflora* (Brazil), followed by *P. pallida* (Winrock) and *P. juliflora* (Bhawani). The comparative performance in terms of growth is summarized in Table 2.

Biomass production of *Prosopis* leaf, stem and roots irrigated with water ranging from 0 to 40 $dS.m^{-1}$ is presented in Table 3. The cumulative biomass of shoot and root is shown in Figures 4 and 5. Among the five species/provenances studied, *P. juliflora* (Bhawani) produced the highest amount of both fresh and dry biomass in all the plant parts

under non-saline (control) conditions. In general, root biomass was greater in all the plants followed by stem and leaf.

There was some accumulation of salts in the soil even at low levels (0-10 $dS.m^{-1}$) of salinity in the irrigation water as a result of incomplete leaching during watering, whereas at a higher salinity level (40 $dS.m^{-1}$), the EC_e was less than the EC of the irrigation water. At the 20 $dS.m^{-1}$ level, *P.juliflora* (Brazil) exhibited less reduction in biomass which was still about 50% in both leaf and stem. This compared with reductions in leaf and stemw, respectively, of 53 and 61% in *P. pallida* (Winrock), 40 and 62% in *P. glandulosa* (Sujawal), 70 and 85%; in *P. glandulosa* (Karachi), 85 and 73% in *P. juliflora* (Bhawani). *P. glandulosa* did not tolerate high salinities and failed to grow when given irrigation water with an EC of 20 $dS.m^{-1}$. At the highest salinity level (EC: 40 $dS.m^{-1}$), biomass reduction reached about 85 to 92% in the surviving three species/provenances.

Root biomass was less affected than shoots at all salinity levels. The root biomass of *P. juliflora* (Bhawani) was greatest among the species/provenances studied. Moisture content in the shoots of control plants varied from about 36-50%. The percentage of moisture in some of the plants irrigated with saline water increased up to 67% at the highest salinity level of irrigation water.

Shoot biomass under control conditions was ranked as follows:

P. juliflora (Bhawani) > *P. pallida* (Winrock) > *P. glandulosa* (Karachi) > *P. glandulosa* (Sujawal) > *P. juliflora* (Brazil)

Using irrigation water of 20 $dS.m^{-1}$, shoot biomass was ranked as follows:

P. juliflora (Brazil) > *P. pallida* (Winrock) > *P. juliflora* (Bhawani) > *P. glandulosa* (Sujawal) > *P. glandulosa* (Karachi)

Screening of Local and Exotic Genomes of *Prosopis* Under Field Conditions Using Saline Water for Irrigation

The various plant species exhibited different growth responses depending on their

habitat and growth vigour. The height of *P. juliflora* (D. I. Khan), *P. glandulosa* (Sujawal), and *P. julifora* (Sujawal-2) ranged from 61.1 to 92.4 cm. The first two species exhibited better performance with regard to the number and cumulative length of the branches. The height of *P. alba* (Acc. #0166), *Prosopis* sp. (Peru), and *P. juliflora* (Brazil) ranged from 75.9 to 96.4 cm. With respect to the number of branches, *Prosopis* sp. (Peru), *P. chilensis* (Acc. #0009) and *P. articulata* (Acc. #0598), ranked first, second and third, respectively. With reference to the cumulative length of branches, *P. articulata* (Acc. #0598), *P. alba* (Acc. #0166) and *Prosopis* species (Peru) were the three best exotic species/accesssions. The differences among these six species/accessions, irrespective of origin, for various parameters were significant ($P < 0./05$) as compared with the rest of the species/accessions/provenances. *P. alba* proved to be the best with respect to the height, number, and cummulative length of the branches. *P. alba* also appeared to have a greater tendency to become monostemic as compared with *Prosopis* from local sources.

P. cineraria was the only palatable species among the various species/accessions/provenances used in this experiment. It is generally monostemic and makes a shady crown. *P. cineraria* (Tando Jam), though not as high as *P. juliflora* (D.I. Khan) or *P. alba* (Acc. #0116), is still a good choice because of its grazing value.

There appeared to be salt accumulation in the soil due to saline water irrigation over a period of time and insufficient leaching of the saline water through the root zone (Table 4). This could be attributed to differences in soil texture in different areas of the experimental field, though the area selected was quite homogenous (sandy loam). The salt accumulation in the root zone varied from 5 to 14 $dS.m^{-1}$, and could be one of the factors affecting rooting and growth pattern.

Data on some physiological parameters for the plants grown with saline water are presented in Table 5. *P. articulata* (Acc. #0598), an exotic species, showed the highest quantum yield, followed by *P. glandulosa* (Sujawal), an indigenous species. However, these differences were not significant ($P < 0.05$). The lowest quantum yield was observed for *P. juliflora* (D.I. Khan), which had a significantly lower yield than many of the other species.

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Differences among the remaining species/accessions/provenances in quantum yield were non-significant ($P < 0.05$). With regard to stomatal conductance, seven species/accessions/provenances exhibited significantly higher values as compared with the rest of the species/accessions/ provenances. Within this group, there were no statistically significant differences. *P. alba* (Acc. #0591), an exotic species, ranked highest and *P. juliflora* (Brazil), lowest. The transpiration rate corresponded more or less with the stomatal conductance of the individual species/accessions/provenances. *P. alba* (Acc. #0591) exhibited both a high stomatal conductance value and maximum values for transpiration rate. These species/ accessions/provenances ranking high with regard to transpiration rate were found to be statistically different ($P < 0.05$) with the species at the bottom (*P. juliflora*, Bhawani).

Chlorophyll content in the exotic species/accessions/provenances was higher than in the indigenous ones. In general, the concentration of chlorophyll 'a' was lower than that of chlorophyll 'b,' except in *P. juliflora* (Karachi), and *P. cineraria* (Karachi) where it was nearly equal (Figure 6). However, in *P. cineraria* (Tandojam), the concentration of chlorophyll 'a' was higher than that of chlorophyll 'b'. The amount of total chlorophyll in *P. alba* (Acc. #0166) and *P. nigra* (Acc. #1117) was statistically different ($P < 0.05$) from that in the rest of the species, whereas, it did not differ among the others. The indigenous plants, *P. glandulosa* (Sujawal), *P. glandulosa* (Sujawal, thornless), and *P. juliflora* (D.I. Khan), contained more sugar (Figure 7) than the three species/accessions of the exotic group (i.e. *P. alba*, Acc. #0166; *P. pallida*, Winrock, and *P. juliflora*, Brazil). It is interesting to note that these exotic species occupied the same ranks with respect to total chlorophyll content as well. Among the indigenous species, *P. glandulosa* contained the highest amounts of chlorophyll and sugar. *P. juliflora* (Sujawal) exhibited the highest concentration of proline (Figure 6). This concentration was even higher than that of *P. alba* (Acc. #0166), which showed the maximum concentration of proline among the exotic species.

A low percentage of successful grafts was observed in the grafting experiment. Some of the plants started new sprouts, but within a month the grafts failed (Table 6). The growth of four different species grafted on local *P. glandulosa* was successful, whereas sprouts of Haitian *Prosopis* trees grafted on local *P. juliflora* were stunted after growing slowly for 1 year.

DISCUSSION:

Prosopis Germination and Emergence

With regard to germination, *Prosopis* seeds exhibited differences in salt tolerance across the seed sources evaluated. This may have been due to better seed production and greater adaptation under prevailing saline conditions among the more tolerant species. Reduction in the percentage of germination with increasing salinity levels has been reported previously (Khan et al., 1987). Local *Prosopis* species growing naturally in Sujawal appeared to produce seeds that could tolerate salts during germination. These seeds exhibited a minimum reduction in percentage of germination at low salinity levels (EC: 5-10 $dS.m^{-1}$) and were able to germinate up to EC: 25 $dS.m^{-1}$. Sujawal is situated by the side of Indus River. Though the soil is saline, good-quality water is available which reduces the salt toxicity and allows *Prosopis* species to grow luxuriantly in that area. Hence, seeds produced on these plants have greater vigor. This could be a reason for their better salt tolerance during germination.

The *Prosopis* radical emerges first during germination and initially comes in contact with the soil to form roots; later, the two cotyledons, along with the plumule, elongate to form the shoot. These newly formed cells come in direct contact with the saline soil, and face osmotic and ionic shocks. Some of the seedlings in the experiment were unable to survive, and only those juvenile seedlings (*P. glandulosa*, Sujawal) which were salt tolerant emerged out of the saline soil. Apart from this, the emergence rate of the control plants was also quite low.

Pot Experiments on Growth of Local and Exotic Species Under Different Salinity Regimes.

Plants of different species within the same genus may differ in growth because of genetic differences. Variations among the accessions/provenances of the same species may be due to the habitat where they grow and where the seeds have been collected. When grown under the same environmental conditions, differences in their genetic variability

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were evident. *P. juliflora* appeared to be a more adapted species in this experiment. The taxonomy of *P. pallida* is disputed and is considered very close to *P. juliflora*. *P. pallida* produced the third most biomass compared to the control plants which were not able to survive at salinity levels above EC_{iw}: 20 $dS.m^{-1}$. Only *P. juliflora* (Bhawani), *P. juliflora* (Brazil), and *P. pallida* (Winrock) survived at the highest salinities levels (EC_{iw}: 40 $dS.m^{-1}$). The root systems of these species were comparatively well developed. It may be noted that *P. juliflora* (Bhawani) plants had a comparatively better adapted root system (in terms of biomass) among the *Prosopis* species evaluated, even at the highest salinity level. However, at EC_{iw}: 20 $dS.m^{-1}$, more root biomass was produced by *P. glandulosa* (Karachi) than by *P. juliflora* (Bhawani). Though the shoot biomass of *P. juliflora* (Brazil) grown in the control was less than that of the other four *Prosopis* species, it occupied the top position in salt tolerance at all salinity levels.

Screening of *Prosopis* Genomes Under Field Conditions Using Saline Water for Irrigation

The well water used to irrigate the field plots ranged from 14 to 16 $dS.m^{-1}$. *P. glandulosa*, which did not grow under higher salinity in the pot culture, showed reasonably good growth. *Prosopis juliflora* and *P. glandulosa* were introduced to Pakistan within the last 50 years and and have become as well established as indigenous species of Pakistan. *P. juliflora* grows luxuriantly along the coastline near Karachi and Sonmiami, and is threatening to become weed on fertile land. It is generally multistemic in nature, but pruning of branches gives it a monostemic character. It is very hardy and grows under halo-xeric conditions. The root system persists even if the top is removed, as evidenced by new shoot regrowth. Its biomass has been reported to be 52.3 tons/ha under optimum growth conditions and even in alkali soil after 6 years of growth (Singh and Singh, 1993). *P. glandulosa* is comparatively less salt-tolerant and requires more water than *P. juliflora*. It is also found growing luxuriantly on saline land along the canal sides and rivers. Its biomass production varies greatly depending on the accession (Felker et al., 1983b). *Prosopis* raised from the seeds collected from D.I. Khan and Sujawal was superior to that collected from other places in Pakistan. Although the soil is saline, water remains available and plants exhibit good growth. Hence their seeds are fully developed, and the plants appear to have the vigour for stress toler-

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ance. Among the exotic species seeds obtained from South America (*P. alba, P. articulata, P. nigra* and *P. pallida*) exhibited better growth under the prevailing halo-xeric conditions. Experiments conducted by Felker et al., (1983b) showed that some accessions of these species produce better biomass than others. *P. alba* (Acc. #0166) is reported to be among the highest biomass-producing species in the genus, with an oven-dry mean biomass value reaching 36.8 kg/plant after 2 years of growth (Felker et al., 1983a).

Differences in the utilization of solar energy and in the chlorophyll and sugar content of the leaves of the plants of various species/accessions/provenances are not statistically significant ($P > 0.05$). However, species like *P. alba* (0166), *P. nigra* (1117), *P. juliflora* (D.I. Khan), and *P. glandulosa* (Sujawal), which showed better performance in growth, also ranked highest on these parameters.

The high correlation of chlorophyll and sugar with high quantum yield suggests better photosynthetic efficiency of these species even under saline conditions. Greater amounts of photosynthetic apparatus per leaf have been linked to increased concentration of chlorophyll content (Terry and Waldron, 1984). A relationship has been suggested between accumulation of sugar in plants under salt stress and respiration rate and allocation of carbon for synthesis of organic solutes, which serve as cell osmoticum (Shannon, 1984; Jefferies et al., 1979). The presence of other organic compounds is required to balance the osmotic gradient between internal and external cell potential. Proline, a water soluble amino acid, is considered to have an osmo-regulatory function (Treichel, 1975).

The availability of soil moisture and the associated osmotic potential of the soil solution influences water uptake and related processes. Transpiration rate is known to be influenced by external factors such as ambient air humidity and temperature (Eshel and Waisel, 1984) and internal factors such as anatomical features of the plant. In the experiments, plants with high transpiration demand also exhibited higher stomatal conductance. Thus *P. alba* showed maximum values of both stomatal conductance and transpiration. Among the local genomes, all provenances of *P. glandulosa* exhibited maximum stomatal conductance and transpiration values. This can be attributed to the shape of the leaflets in

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this species, which are broader and have less cuticle as compared with other species. Lower values of stomatal conductance in other species/accessions/provenances may not be due to stress, but to partial closure of stomates or reduced size and density of stomates per unit area (Warne et al., 1990).

Grafting Experiment

Prosopis glandulosa appears to be a good host for grafting shoot stock of other *Prosopis* species. Plants in the genus *Prosopis* seem to be divided physiologically into two groups, one comparatively more salt-tolerant, less water-demanding than the other. Grafting within plants of the same group appeared to be successful, whereas grafting across the groups was difficult. Hence, a graft of a comparatively more salt-tolerant *P. chilensis* shoot stock to a comparatively less salt-tolerant root stock of *P. glandulosa* was not successful. However, more data are needed to prove or reject this hypothesis.

CONCLUSIONS:

The results of the experiments suggest the importance of mesquite cultivation for providing fodder and fuelwood, supplying timber, and stopping the movement of sand dunes to control desertification, specially in saline soils.

The research reported in this paper is only a step toward selection of *Prosopis* species/accessions/provenances capable of producing useful quantities of biomass in saline soils. The plants in the experiments have not grown old enough to show appreciable diameter and height of stems so that predictions about wood production can be made. Results of studies on pruning of multistemic species and determination of the growth rates of remaining single stems should provide this information in the future.

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REFERENCES

Ahmad, R., Ismail, S. and Khan, D. 1986. Saline Agriculture at Coastal Sandy Belt. Coordinated Res. Prog. on 'Saline Agriculture', Karachi University and PARC, Islamabad.

Dutton, R.W. 1992. *Prosopis* species : Aspects of their Value, Research and Development. Proc. of *Prosopis* Symp. Durham, U.K., 320 pp.

Eshel, A. and Waisel, Y. 1984. Effect of salt and soil water status on transpiration of *Salsola kali* L. Plant Cell and Environ., 7 : 133-137.

Felker, P., Cannel, G.H., Clark, P.R., Osborn, J.F. and Nash, P. 1983a. Biomass production of *Prosopis* species (Mesquite), *Leucaena* and other leguminous trees grown under heat/drought stress. Forestry Sci., 29(3) : 592-606.

Felker, P., Cannel, G.H., Osborn, J.F., Clark, P.R. and Nash, P. 1983 b. Effects of irrigation on biomass production of 32 *Prosopis* species (Mesquite) accessions. Expl. Agric., 19 : 187-198.

Felker, P., Clark, P.R., Laag, A.E. and Pratt, P.F. 1981. Salinity tolerance of tree legumes : Mesquite (*Prosopis glandulosa* var. *torreyana*, *P. velutina* and *P. articulata*), Algarrobo (*P. chilensis*), Kiawe (*P. pallida*) and tamarugo (*P. tamarugo*) grown in sand culture on nitrogen-free media. Plant and Soil, 61 : 311-317.

Galera, F.M., Trevission, M. and Bruno, S.A. 1992. *Prosopis* in Argentina : Initial results on cultivation in greenhouses and orchards, and pod quality for food or feed of five native *Prosopis* species of Cordoba province. In: *Prosopis* species : Aspects of their Value, Research and Development (Dutton, R.W., Ed.), pp. 145-156, Proc. of *Prosopis* Symp., Durham, U.K.

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Gupta, A.K., Solanki, K.R. and Kackar, N.L. 1984. Variation for quality of pod in *Prosopis cineraria* Mac Bride. Ann. Arid Zone, 23(3) : 255-258.

Jarrell, W.M. and Virginia, R.A. 1984. Salt tolerance of mesquite. California Agric., 38(10) : 28.

Jefferies, R.L., Rudmik, T. and Dillon, E.M. 1979. Response of halophytes to high salinity and low water potentials. Plant Physiol., 64 : 989-994.

Khan, D., Ahmad, R. and Ismail, S. 1978. Case history of *Prosopis juliflora* plantation at Makran coast raised through saline water irrigation. In: Prospects for Biosaline Research (Ahmad, R. and San Pietro, A., Eds.), pp. 559-585. Department of Botany, University of Karachi, Karachi, Pakistan.

Khan, D., Ahmad, R. and Ismail, S. 1987. Germination, growth and ion regulation in *Prosopis juliflora* (Swartz) DC. under saline conditions. Pak. J. Bot., 19(2) : 131-138.

Maclachlam, S. and Zalik, S. 1963. Plastid structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of barley. Can. J. Bot., 41 : 1053-1062.

Mohammed, N. 1992. Developmental potential of *Prosopis* species in Pakistan. In: *Prosopis* species : Aspects of their Value, Research and Development (Dutton, R.W., Ed.), pp. 71-82, Proc. of *Prosopis* Symp., Durham, U.K.

Nabhan, G. 1984. Mesquite : Another great American legume. Organic Gardening, 31(4) : 114-115.

Rhodes, D. and Felker, P. 1988. Mass screening of *Prosopis* (Mesquite) seedlings for growth at seawater salinity concentration. Forest Ecol. Manag., 24 : 169-176.

Shannon, M.C. 1984. Breeding, selection and the genteics of salt tolerance. In: Salinity tolerance in crop plants : Strategies for crop improvement (Staples, R.C. and Toenniessen, G.H., Ed.), pp. 231-254, John Wiley & Sons.

Singh, G. and Singh, N.T. 1993. Mesquites for the revegetation of saltlands. Central Soil Salinity Research Institute, Bulletin No. 18, 25 pp.

Terry, N. and Waldron, L.J. 1984. Salinity, photosynthesis and leaf growth. California Agric., 38(10) : 38-39.

Treichel, S. 1975. The effect of NaCl on the concentration of proline in different halophytes. Z. Pflanzenphysiol., 76 : 56-68.

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Warne, P., Guy, R.D., Rallins, L. and Reid, D.M. 1990. The effect of sodium sulphate and sodium chloride on growth, morphology, photosynthesis and water use efficiency of *Chenopodium rubrum*. Can.J.Bot., 68 : 999-1006.

Yemm, E.W. and Willis, A.J. 1956. The estimation of carbohydrate in plant extract by anthrone. Biochem. Jour., 57 : 508.

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Figure 1. Field lay-out of the experiment for screening different species/provenances/ accessions of *Prosopis* under saline conditions.

Figure 2. Effect of various salt levels on the germination of different *Prosopis* species/provenances.

Figure 3. Effect of various salt levels on the emergence of different *Prosopis* species/provenances.

Figure 4. Effect of saline water irrigation on shoot biomass productivity of different species/provenances of *Prosopis*

Key to the Species:-
Sp.1 - *P. juliflora* (Brazil) Sp.2 - *P. juliflora* (Bhawani) Sp.3 - *P. pallida* (Winrock)
Sp.4 - *P. glandulosa* (Sujawal) Sp.5 - *P. glandulosa* (Karachi)

Figure 5. Effect of saline water irrigation on root biomass productivity of different species/provenances of *Prosopis*

Key to the Species:-
Sp.1 - *P. juliflora* (Brazil) Sp.2 - *P. juliflora* (Bhawani) Sp.3 - *P. pallida* (Winrock)
Sp.4 - *P. glandulosa* (Sujawal) Sp.5 - *P. glandulosa* (Karachi)

Figure 6. Concentration of chlorophyll content in local and exotic species/accessions/ provenances of *Prosopis* under saline water irrigation.

Key to the Species:-

1. *P. juliflora (Karachi)*
2. *P. juliflora* (Sujawal-1)
3. *P. juliflora* (Sujawal-2)
4. *P. juliflora* (D.I.Khan)
5. *P. juliflora* (Faisalabad)
6. *P. juliflora* (Bhawani)
7. *P. glandulosa* (Sujawal)
8. *P. glandulosa* (Sujawal, Thornless)
9. *P. glandulosa* (N.W.F.P.)
10. *P. cineraria* (Tando Jam)
11. *P. cineraria* (Karachi)
12. *P. nigra* (Acc.#1117)
13. *P. articulata* (Acc.#0598)
14. *P. chilensis* (Acc.#0009)
15. *P. alba* (Acc.#0591)
16. *P. alba* (Acc.#0166)
17. *Prospis* sp. (Peru)
18. *P. pallida* (Winrock)
19. *P. juliflora* (Brazil)

Figure 7. Concentration of sugar and proline content in local and exotic species/ accessions/provenances of *Prosopis* under saline water irrigation.

Key to the Species:-

1. *P. juliflora (Karachi)*
2. *P. juliflora* (Sujawal-1)
3. *P. juliflora* (Sujawal-2)
4. *P. juliflora* (D.I.Khan)
5. *P. juliflora* (Faisalabad)
6. *P. juliflora* (Bhawani)
7. *P. glandulosa* (Sujawal)
8. *P. glandulosa* (Sujawal, Thornless)
9. *P. glandulosa* (N.W.F.P.)
10. *P. cineraria* (Tando Jam)
11. *P. cineraria* (Karachi)
12. *P. nigra* (Acc.#1117)
13. *P. articulata* (Acc.#0598)
14. *P. chilensis* (Acc.#0009)
15. *P. alba* (Acc.#0591)
16. *P. alba* (Acc.#0166)
17. *Prospis* sp. (Peru)
18. *P. pallida* (Winrock)
19. *P. juliflora* (Brazil)

15	9	12	13	20
19	5	6	2	18
16	1	7	3	8
17	4	1	14	10

1. *P. juliflora* (Karachi)
2. *P. juliflora* (Sujawal-1)
3. *P. juliflora* (D.I.Khan)
4. *P. juliflora* (Faisalabad)
5. *P. glandulosa* (Sujawal)
6. *P. glandulosa* (Sujawal) [Thornless]
7. *P. glandulosa* (N.W.F.P)
8. *P. cineraria* (Tandojam)
9. *P. cineraria* (Karachi)
10. *P. cineraria* (N.W.F.P.)
11. *P. juliflora* (Bhawani)
12. *P. juliflora* (Sujawal-2)
13. *P. nigra* (Acc.# 1117)
14. *P. articulata* (Acc.# 0598)
15. *P. chilensis* (Acc.# 0009)
16. *P. alba* (Acc. # 0591)
17. *P. alba* (Acc. # 0166)
18. *Prosopis* spp. (Peru)
19. *P. pallida* (Winrock)
20. *P. juliflora* (Brazil)

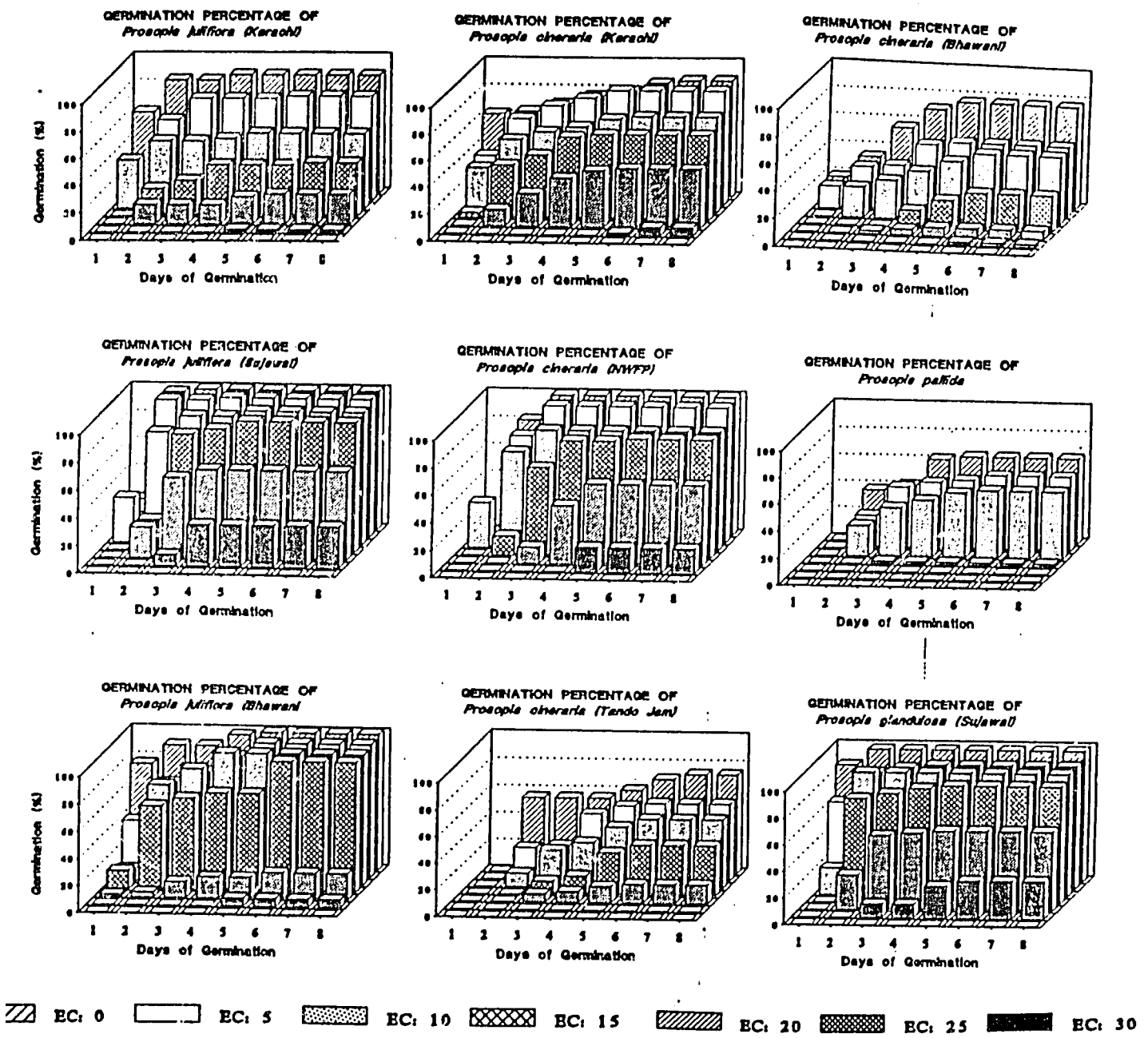
GERMINATION PERCENTAGE OF
Prosopis juliflora (Karachi)
GERMINATION PERCENTAGE OF
Prosopis cineraria (Karachi)
GERMINATION PERCENTAGE OF
Prosopis cineraria (Bhawani)
GERMINATION PERCENTAGE OF
Prosopis juliflora (Sujawal)
GERMINATION PERCENTAGE OF
Prosopis cineraria (NWFP)
GERMINATION PERCENTAGE OF
Prosopis pallida
GERMINATION PERCENTAGE OF
Prosopis juliflora (Bhawani
GERMINATION PERCENTAGE OF
Prosopis cineraria (Tando Jam)
GERMINATION PERCENTAGE OF
Prosopis glandulosa (Sujawal)
Germination (%)
Days of Germination
EC: 0
EC: 5
EC: 10
EC: 15
EC: 20
EC: 25
EC: 30

Fig 2

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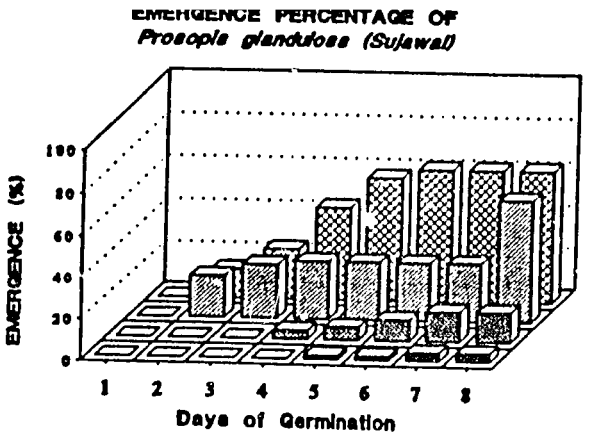
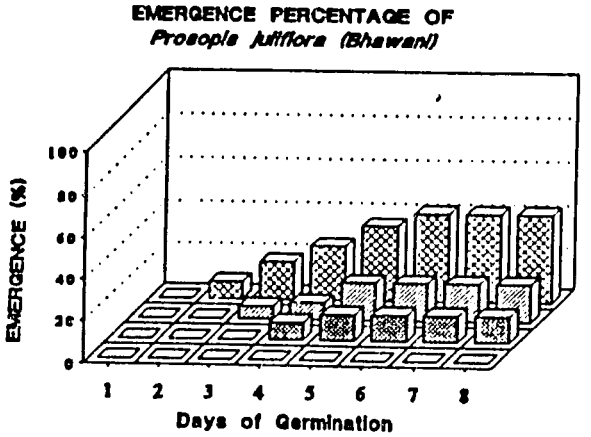
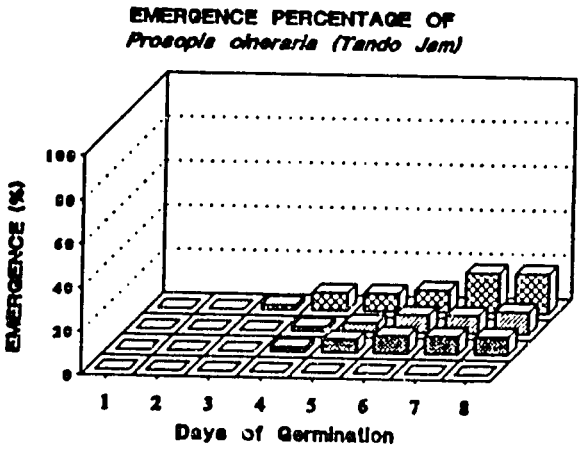
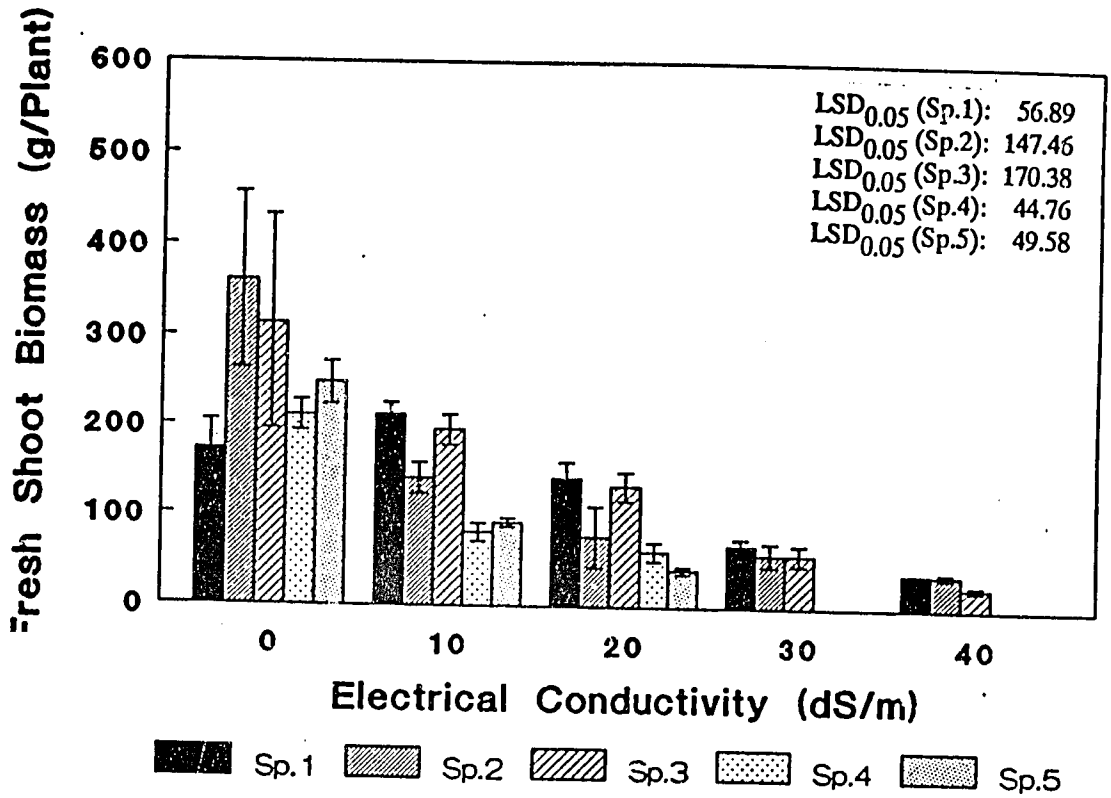
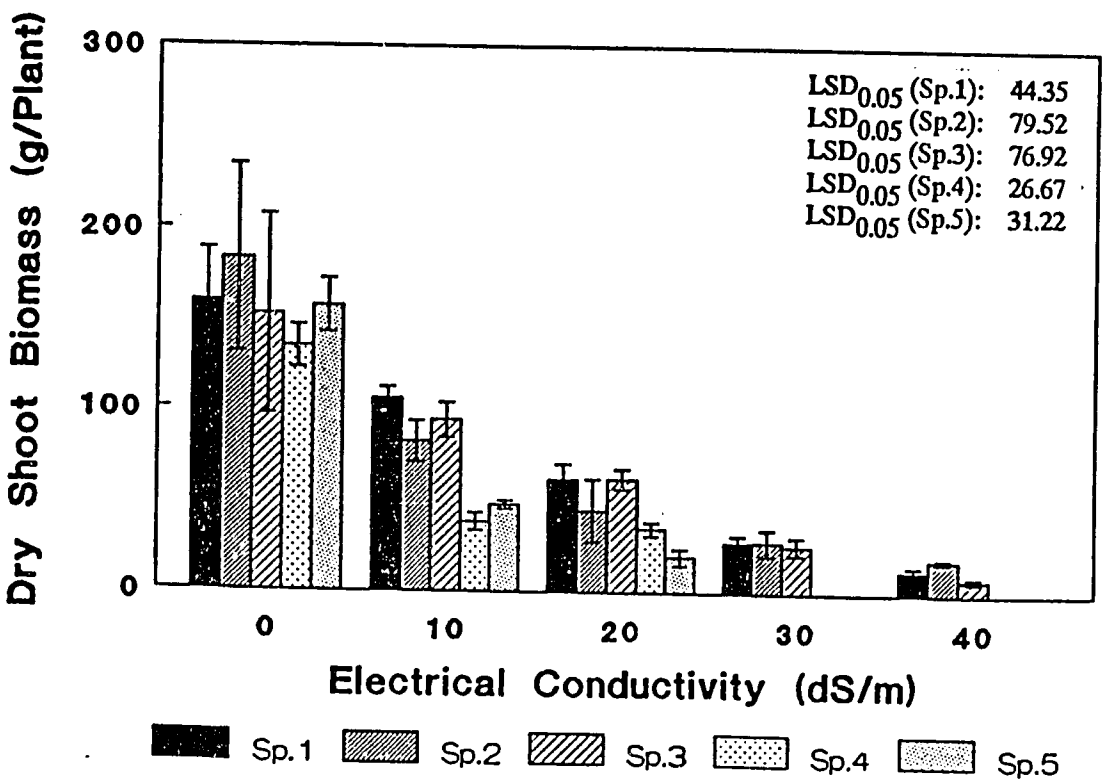


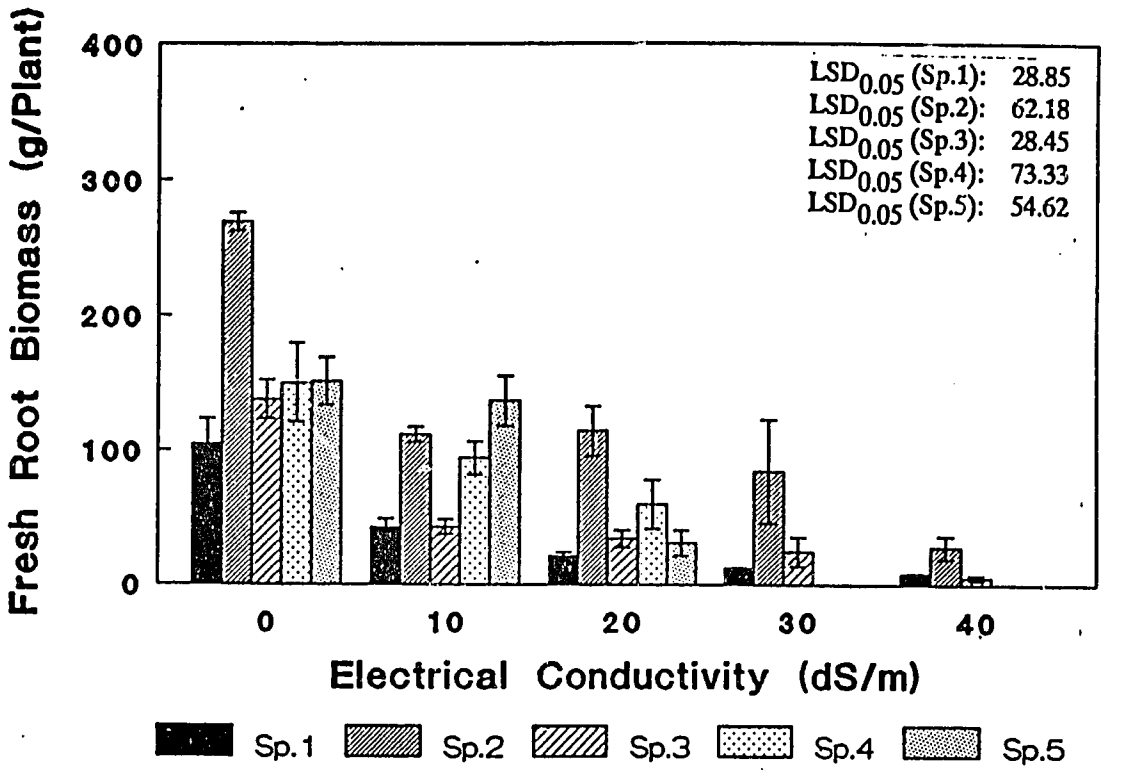


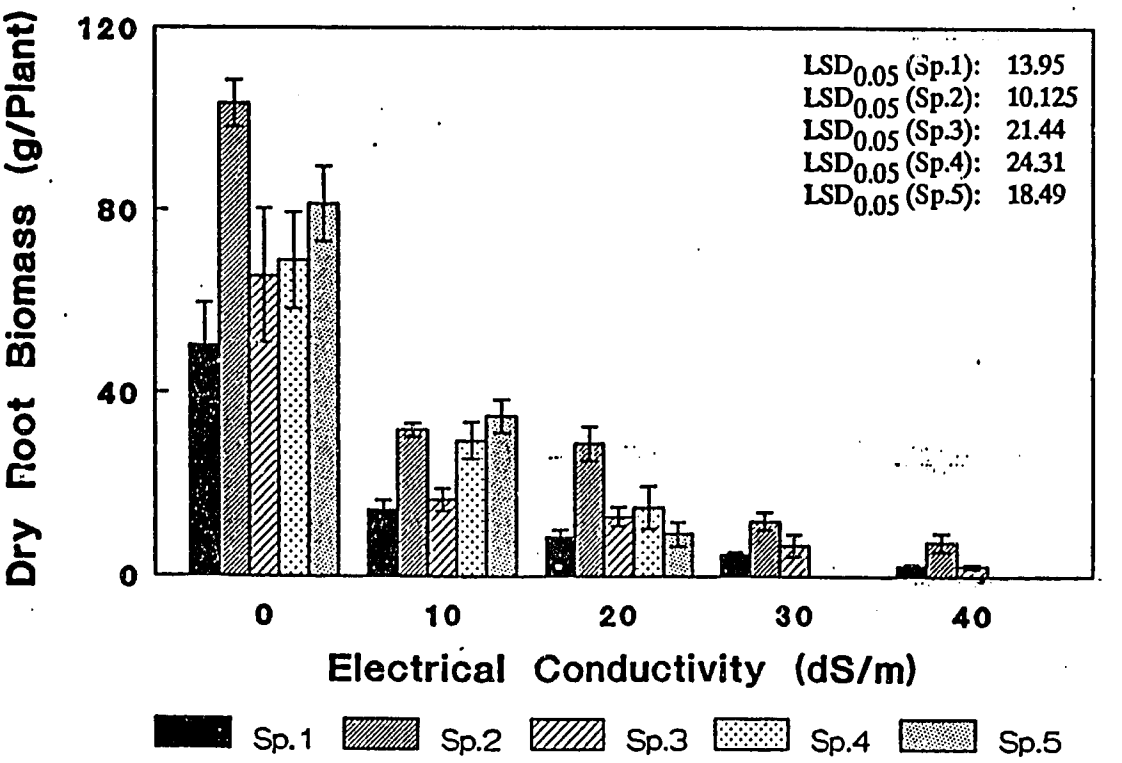


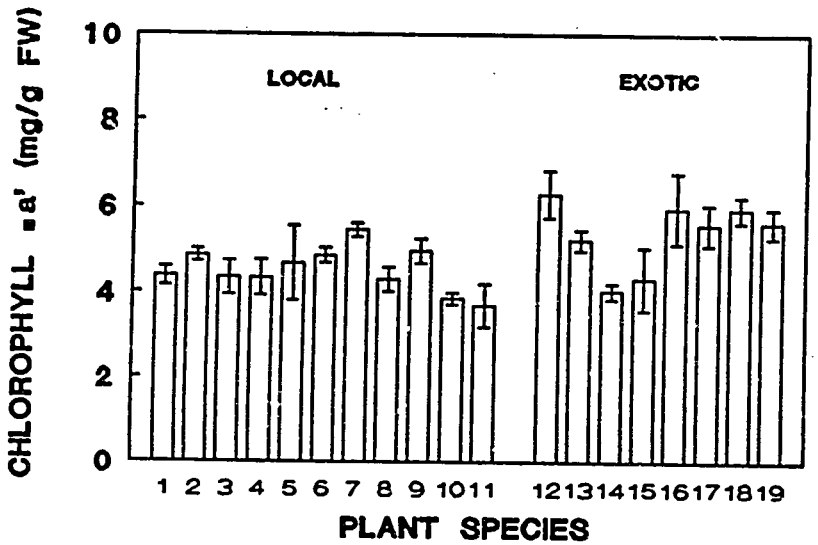
EC: 0 EC: 5 EC: 10 EC: 15

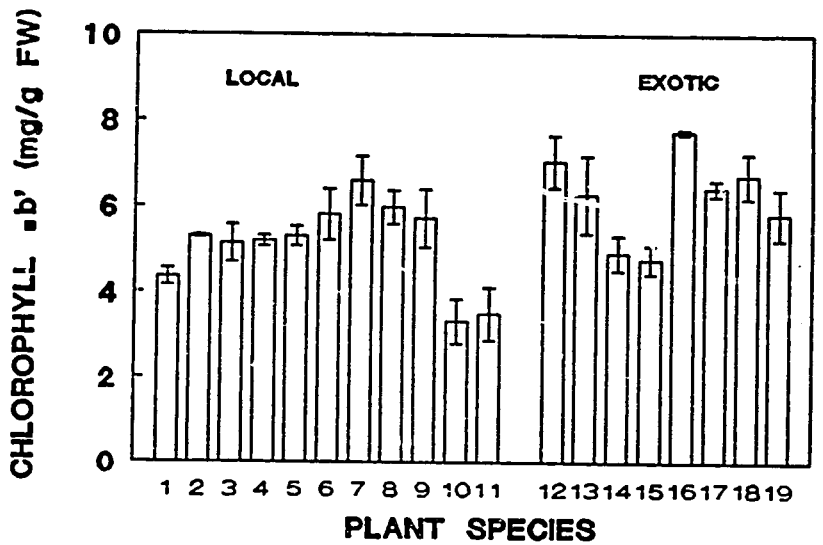

Fresh Shoot Biomass (g/Plant)
600
500
400
300
200
100
0
LSD0.05 (Sp.1): 56.89
LSD0.05 (Sp.2): 147.46
LSD0.05 (Sp.3): 170.38
LSD0.05 (Sp.4): 44.76
LSD0.05 (Sp.5): 49.58
0
10
20
30
40
Electrical Conductivity (dS/m)
Sp.1
Sp.2
Sp.3
Sp.4
Sp.5

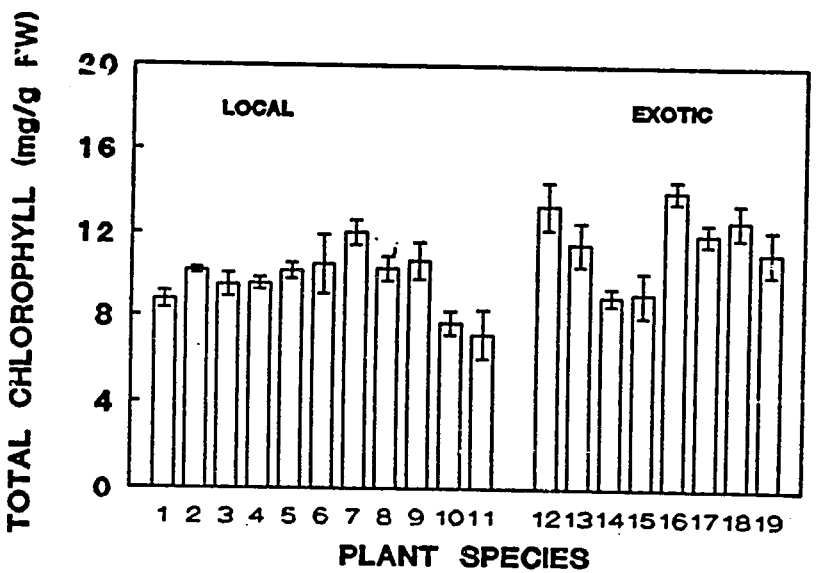

Dry Shoot Biomass (g/Plant)
300
200
100
0
LSD0.05 (Sp.1): 44.35
LSD0.05 (Sp.2): 79.52
LSD0.05 (Sp.3): 76.92
LSD0.05 (Sp.4): 26.67
LSD0.05 (Sp.5): 31.22
0
10
20
30
40
Electrical Conductivity (dS/m)
Sp.1
Sp.2
Sp.3
Sp.4
Sp.5

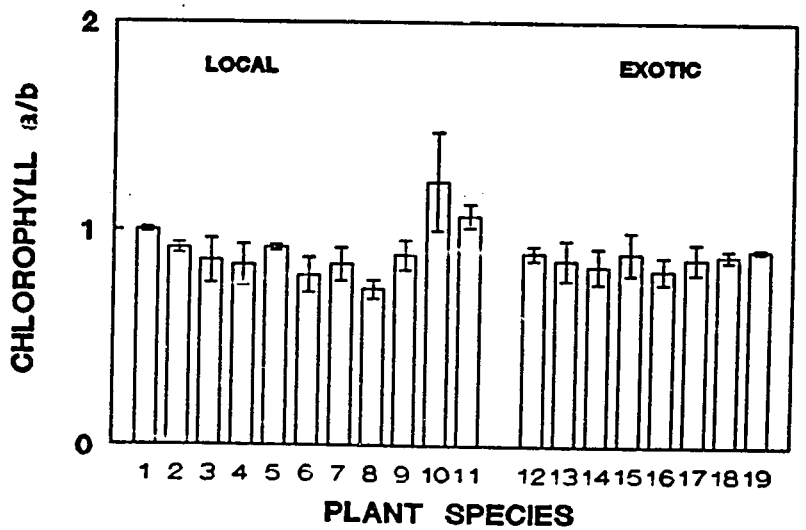

Fresh Root Biomass (g/Plant)
400
300
200
100
0
LSD0.05 (Sp.1): 28.85
LSD0.05 (Sp.2): 62.18
LSD0.05 (Sp.3): 28.45
LSD0.05 (Sp.4): 73.33
LSD0.05 (Sp.5): 54.62
0
10
20
30
40
Electrical Conductivity (dS/m)
Sp.1
Sp.2
Sp.3
Sp.4
Sp.5

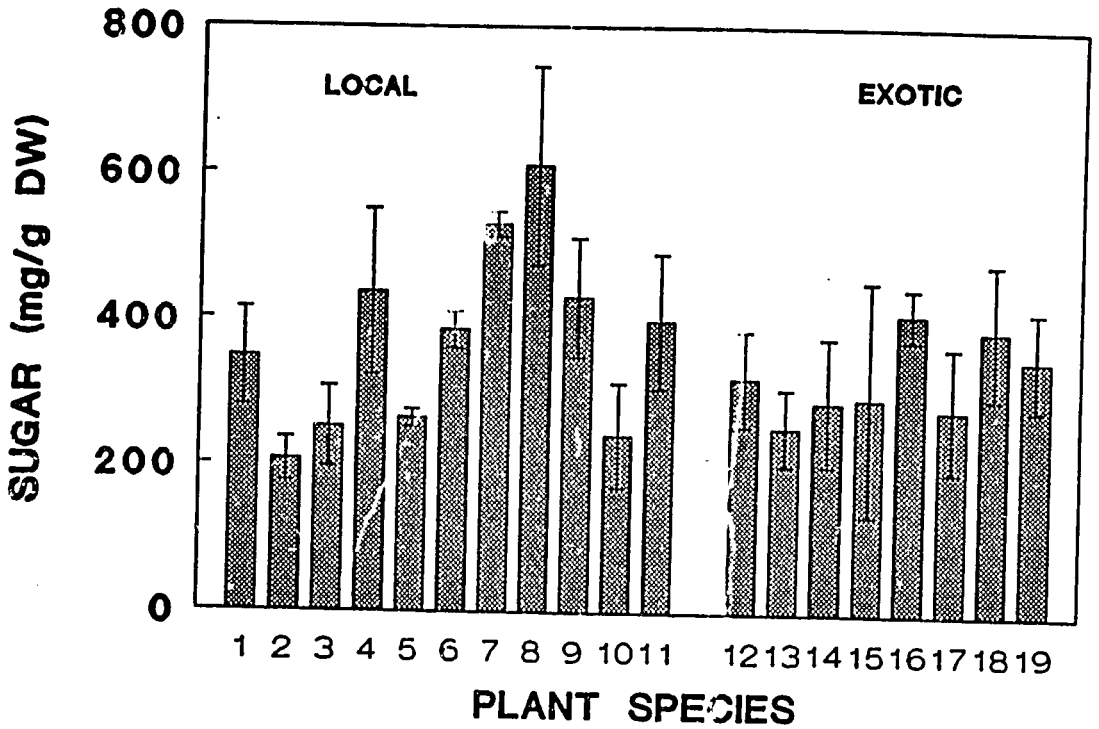

Dry Root Biomass (g/Plant)
120
80
40
0
LSD0.05 (Sp.1): 13.95
LSD0.05 (Sp.2): 10.125
LSD0.05 (Sp.3): 21.44
LSD0.05 (Sp.4): 24.31
LSD0.05 (Sp.5): 18.49
0
10
20
30
40
Electrical Conductivity (dS/m)
Sp.1
Sp.2
Sp.3
Sp.4
Sp.5


CHLOROPHYLL 'a' (mg/g FW)
LOCAL
EXOTIC
PLANT SPECIES


CHLOROPHYLL 'b' (mg/g FW)
LOCAL
EXOTIC
PLANT SPECIES


TOTAL CHLOROPHYLL (mg/g FW)
LOCAL
EXOTIC
PLANT SPECIES


CHLOROPHYLL a/b
LOCAL
EXOTIC
PLANT SPECIES


800
600
400
200
0
SUGAR (mg/g DW)
LOCAL
EXOTIC
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
PLANT SPECIES

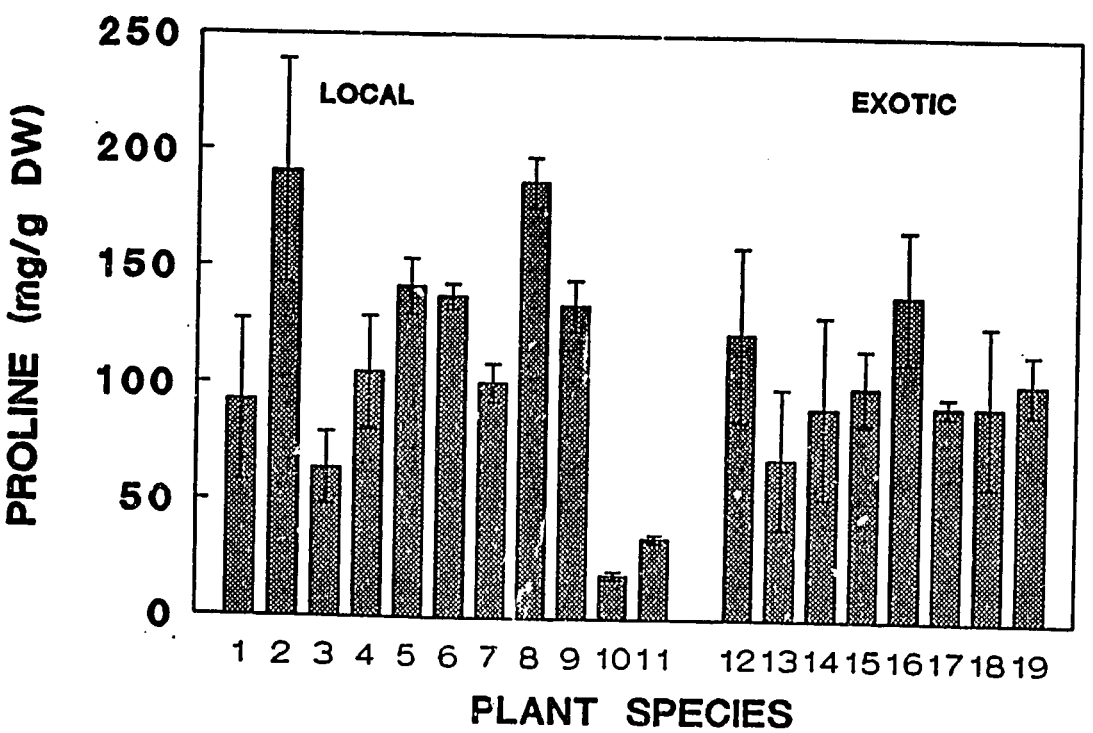

250
200
150
100
50
0
PROLINE (mg/g DW)
LOCAL
EXOTIC
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
PLANT SPECIES

Table 1. Growth responses of *Prosopis* species/provenances under saline water irrigation

Plant Species/ Accesssions	Salinity Levels ($dS.m^{-1}$)	Growth Period (Months) 4		8		12	
		Plant Height	Stem Diam.	Plant Height	Stem Diam.	Plant Height	Stem Diam.
		(cms)					
P. juliflora (Bhawani)	0	102.00 ±11.25	0.78 ±0.04	133.00 ±23.38	1.30 ±0.11	139.33 ±25.82	2.30 ±0.17
	10	61.00 ±4.04	0.74 ±0.02	110.00 ±14.60	1.12 ±0.01	136.60 ±9.20	1.50 ±0.09
	20	44.33 ±5.54	0.70 ±0.05	86.66 ±14.80	0.93 ±0.09	138.33 ±1.66	1.36 ±0.12
	30	48.00 ±6.80	0.65 ±0.02	64.00 ±18.14	0.65 ±0.03	72.00 ±3.78	1.26 ±0.16
	40	40.33 ±2.40	0.55 ±0.08	49.00 ±5.13	0.66 ±0.09	68.33 ±4.60	1.13 ±0.16
P.juliflora (Brazil)	0	102.66 ±5.89	0.83 ±0.07	106.33 ±7.31	1.26 ±0.08	113.33 ±49.69	1.81 ±6.00
	10	69.50 ±4.76	0.62 ±0.01	110.66 ±16.89	1.16 ±0.03	140.00 ±22.90	1.63 ±0.66
	20	58.33 ±7.83	0.60 ±0.00	79.00 ±12.01	0.85 ±0.10	98.66 ±23.90	1.36 ±0.14
	30	56.00 ±3.78	0.55 ±0.03	74.33 ±5.36	0.56 ±0.0	97.33 ±2.66	1.06 ±0.06
	40	47.00 ±2.88	0.43 ±0.03	54.66 ±5.36	0.46 ±0.03	60.66 ±20.46	0.86 ±0.10
P. pallida (Winrock)	0	85.00 ±11.70	0.76 ±0.04	144.00 ±11.59	1.46 ±0.18	136.60 ±23.33	2.33 ±0.17
	10	60.00 ±4.04	0.54 ±0.02	118.66 ±7.69	1.03 ±0.03	143.33 ±11.66	1.66 ±0.14
	20	45.00 ±3.60	0.55 ±0.02	99.33 ±5.66	0.83 ±0.06	126.33 ±13.50	1.50 ±0.09
	30	41.00 ±7.55	0.55 ±0.07	66.00 ±19.52	0.66 ±0.03	100.30 ±12.25	1.06 ±0.16
	40	40.33 ±5.69	0.45 ±0.06	54.33 ±9.26	0.46 ±0.03	60.30 ±5.33	1.01 ±0.04

Plant Species/ Accesssions	Salinity Levels ($dS.m^{-1}$)	Growth Period (Months) 4		8		12	
		Plant Height	Stem Diam.	Plant Height	Stem Diam.	Plant Height	Stem Diam.
		(cms)					
P. glandulosa (Sujawal)	0	16.16 ±1.96	0.43 ±0.02	107.66 ±8.95	1.50 ±0.21	109.00 ±16.46	2.26 ±0.17
	10	14.33 ±0.33	0.53 ±0.03	54.33 ±2.85	0.96 ±0.05	66.00 ±3.03	1.70 ±0.05
	20	19.00 ±3.21	0.48 ±0.06	51.66 ±4.33	0.83 ±0.06	71.33 ±5.33	1.23 ±0.08
	30	15.66 ±2.33	0.40 ±0.00	35.00 ±0.00	0.50 ±0.00	--- ---	--- ---
	40	16.66 ±2.60	0.45 ±0.08	--- ---	--- ---	--- ---	
P. glandulosa (Karachi)	0	30.50 ± 5.00	0.38 ±0.02	125.00 ±14.84	1.36 ±0.08	120.00 ±5.70	2.16 ±0.03
	10	11.66 ±2.72	0.44 ±0.04	69.00 ±8.86	0.94 ±0.04	88.33 ±6.00	1.43 ±0.14
	20	11.66 ±2.72	0.43 ±0.03	59.66 ±18.98	0.70 ±0.10	71.33 ±14.43	1.06 ±0.27
	30	11.33 ±3.93	0.40 ±0.0	30.00 ±0.00	0.50 ±0.00	--- ---	--- ---
	40	9.66 ±1.95	0.38 ±0.02	--- ---	--- ---	--- ---	

Dashes indicate the dead plants.

Table 2. Growth of different species/provenances of *Prosopis* as influenced by low- and high-salinity water.

Growth Parameter	Salinity Level ($dS.m^{-1}$)	Growth in Order of Performance
Plant Height	0	*P. juliflora* (Bhawani) > *P. pallida* (Winrock) > *P. glandulosa* (Karachi) > *P. juliflora* (Brazil) > *P. glandulosa* (Sujawal)
	40	*P. juliflora* (Bhawani) > *P. juliflora* (Brazil) > *P. pallida* (Winrock)
Stem Diameter	0	*P. pallida* (Winrock) > *P. juliflora* (Bhawani) > *P. glandulosa* (Karachi) > *P. juliflora* (Brazil)
	40	*P. juliflora* (Bhawani) > *P. pallida* (Winrock) > *P. juliflora* (Brazil)

Table 3. Biomass production of some *Prosopis* species/provenances grown with different concentrations of saline water.

Plant Species	Parameters Studies	Control	EC: 10	EC: 20	EC: 30	EC: 40	F-Value	$LSD_{0.05}$
		dS.m^{-1}						
Prosopis juliflora (Bhawani)								
Leaf	Fresh Weight (g)	150.00 ±67.10	45.83 ±5.67	22.30 ±11.30	18.66 ±2.30	11.66 ±1.20	3.54*	96.30
	Dry Weight (g)	67.16 ±34.29	17.00 ±1.52	6.66 ±0.66	7.33 ±1.45	4.00 ±1.00	2.99	n.s
Stem	Fresh Weight (g)	209.33 ±41.47	95.16 ±13.58	54.50 ±22.00	39.83 ±10.52	26.00 ±4.00	10.92*	70.7
	Dry Weight (g)	116.00 ±25.32	65.33 ±10.49	34.50 ±13.40	20.66 ±5.54	14.33 ±1.45	9.02*	45.5
Root	Fresh Weight (g)	268.60 ±6.64	111.66 ±5.54	114.33 ±18.20	51.00 ±13.70	28.10 ±8.54	65.8*	36.4
	Dry Weight (g)	106.66 ±7.79	32.00 ±1.52	29.00 ±3.78	12.66 ±2.40	7.33 ±2.20	91.79***	12.8
	EC_e (dS.m^{-1})	0.94 ±0.02	18.53 ±1.18	28.80 ±5.87	33.26 ±4.39	35.10 ±0.59		
Prosopis juliflora (Brazil)								
Leaf	Fresh Weight (g)	80.20 ±22.69	66.33 ±2.90	69.66 ±5.36	27.00 ±4.35	13.00 ±2.08	16.69*	20.12
	Dry Weight (G)	25.66 ±10.08	29.00 ±0.57	25.00 ±1.15	9.00 ±1.15	3.66 ±0.33	6.13*	14.42
Stem	Fresh Weight (g)	153.30 ±62.00	135.00 ±17.00	72.16 ±16.88	39.60 ±6.93	22.66 ±0.88	3.70*	94.30
	Dry Weight (g)	126.33 ±23.34	77.33 ±6.69	36.66 ±8.83	26.00 ±5.03	8.66 ±1.85	15.90*	37.18
Root	Fresh Weight (g)	104.66 ±19.06	42.66 ±6.83	21.00 ±2.88	12.33 ±0.33	7.66 ±0.88	18.87*	28.85
	Dry Weight (g)	50.33 ±9.49	16.66 ±0.33	8.66 ±1.45	5.00 ±0.57	2.33 ±0.33	20.77*	1.65
	EC_e (dS.m^{-1})	1.05 ±0.04	25.36 ±2.42	31.8 ±0.05	32.75 ±2.57	34.80 ±0.83		

Plant Species	Parameters Studies	Control	EC: 10	EC: 20	EC: 30	EC: 40	F-Value	$LSD_{0.05}$
		----------	$dS.m^{-1}$	----------				
Prosopis pallida (Winrock)								
Leaf	Fresh Weight (g)	134.33 ±39.10	89.30 ±1.20	63.16 ±3.34	18.33 ±4.09	10.66 ±3.10	8.30*	55.89
	Dry Weight (g)	56.66 ±13.90	29.66 ±0.88	21.66 ±2.02	7.30 ±2.02	3.60 ±0.66	11.06*	20.07
Stem	Fresh Weight (g)	180.00 ±83.30	106.33 ±16.85	70.33 ±72.77	39.66 ±6.93	13.66 ±1.70	2.81	n.s
	Dry Weight (g)	96.33 ±44.40	64.66 ±9.17	40.33 ±3.66	18.66 ±2.60	5.30 ±0.60	3.18	n.s
Leaf	Fresh Weight (g)	138.00 ±14.50	42.30 ±5.48	34.33 ±6.43	24.66 ±10.90	5.00 ±2.00	32.80*	28.42
	Dry Weight (g)	79.66 ±13.80	17.00 ±2.60	13.00 ±2.08	6.66 ±2.40	2.33 ±0.33	24.10*	20.38
	EC_e ($dS.m^{-1}$)	1.70 ±0.23	24.10 ±2.37	32.73 ±3.10	26.23 ±0.75	33.90 ±0.46		
Prosopis glandulosa (Sujawal)								
Leaf	Fresh Weight (g)	63.66 ±7.85	22.83 ±2.40	38.00 ±3.60	-	-	28.20*	15.59
	Dry Weight (g)	31.00 ±4.58	9.33 ±0.30	10.66 ±0.66	-	-	20.52	n.s
Stem	Fresh Weight (g)	114.60 ±35.10	32.5 ±4.80	43.83 ±6.64	-	-	6.57*	59.41
	Dry Weight (g)	104.00 ±7.20	28.66 ±4.80	24.00 ±3.05	-	-	71.06*	18.43
Root	Fresh Weight (g)	117.00 ±30.13	94.33 ±12.25	60.00 ±18.55	-	-	9.07*	55.71
	Dry Weight (g)	62.33 ±14.80	29.66 ±4.09	15.00 ±4.50	-	-	6.79*	32.16
	EC_e ($dS.m^{-1}$)	1.71 ±0.29	18.92 ±0.86	24.90 ±2.77	33.20 ±0.00	34.64 ±0.28		

Plant Species	Parameters Studies	Control	EC: 10	EC: 20	EC: 30	EC: 40	F-Value	$LSD_{0.05}$
		----------	dS.m^{-1}	----------				
Prosopis glandulosa (Karachi)								
Leaf	Fresh Weight (g)	65.66 ±19.00	29.16 ±1.58	20.33 ±0.88	-	-	4.70*	38.33
	Dry Weight (g)	31.00 ±8.30	11.33 ±0.66	7.00 ±0.57	-	-	6.99*	16.72
Stem	Fresh Weight (g)	182.33 ±16.60	63.16 4.34	21.50 ±4.80	-	-	65.17*	35.78
	Dry Weight (g)	126.66 ±13.67	36.33 ±2.66	16.33 ±2.02	-	-	52.26*	28.13
Root	Fresh Weight (g)	151.66 ±17.50	136.66 ±18.65	31.33 ±9.49	-	-	17.26*	54.61
	Dry Weight (g)	81.33 ±8.11	35.36 ±3.75	9.66 ±2.33	-	-	46.30*	18.45
	EC_e (dS.m^{-1})	0.94 ±0.10	19.40 ±1.66	22.85 ±0.39	30.50 ±0.00	35.64 ±0.24		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4. Some morphological characters in different Prosopis species/ accessions/provenances as influenced by saline water irrigation.

Name of Species	Height of Main Stem (cm)	Number of Branches	Length of Branches (cm)	Salinity of Root Zone ($dS.m^{-1}$)
P. juliflora (Karachi)	16.75 ± 0.80 g	1.50 ± 0.09 b	20.5 ± 1.59 h	11.53 ± 1.08
P. juliflora (Sujawal -1)	30.00 ± 2.40 defg	3.25 ± 0.23 b	61.75 ± 7.20 fgh	15.90 ± 9.28
P. juliflora (Sujawal -2)	61.14 ± 2.47 bcd	4.71 ± 0.46 b	213.14 ± 19.4 cd	11.13 ± 1.58
P. juliflora (D.I.Khan)	92.44 ± 3.1 a	8.44 ± 0.25 a	516.88 ± 16.4 a	8.50 ± 1.78
P. juliflora (Faisalabad)	33.66 ± 4.1 defg	4.33 ± 0.29 b	187.00 ± 12.4 de	13.65 ± 0.10
P. juliflora (Bhawani)	21.40 ± 2.60 fg	1.5 ± 0.14 b	34.25 ± 2.6 gh	8.53 ± 1.07
P. glandulosa (Sujawal)	75.11 ± 4.09 bcd	4.87 ± 0.25 b	164.25 ± 18.03 def	7.70 ± 0.00
P. glandulosa (Sujawal)[Thornless]	40.66 ± 2.80 defg	3.40 ± 0.33 b	136.4 ± 13.16 defg	10.80 ± 2.17
P. glandulosa (N.W.F.P.)	45.50 ± 2.70 cdefg	1.37 ± 0.09 b	36.12 ± 3.52 gh	8.36 ± 0.73
P. cineraria (Tando Jam)	45.37 ± 2.61 cdefg	3.25 ± 0.27 b	134.62 ± 13.1 defg	7.60 ± 0.00
P. cineraria (Karachi)	37.00 ± 1.55 defg	2.14 ± 0.15 b	76.00 ± 11.43 fgh	5.70 ± 0.00
P. cineraria (N.W.F.P.)	23.11 ± 1.29 efg	4.00 ± 0.26 b	100.33 ± 7.97 efgh	5.00 ± 0.00
P. nigra (Acc # 1117)	48.33 ± 3.05 cdefg	2.33 ± 0.13 b	72.00 ± 6.18 fgh	8.70 ± 2.57
P. articulata (Acc # 0598)	75.11 ± 4.09 abc	4.87 ± 0.25 b	347.00 ± 19.05 b	12.83 ± 3.16
P. chilensis (Acc # 0009)	35.2 ± 3.11 defg	5.00 ± 0.67 b	81.2 ± 11.4 efgh	11.16 ± 0.94
P. alba (Acc # 0591)	55.75 ± 4.50 bcde	3.33 ± 0.17 b	123.00 ± 8.01 defgh	11.86 ± 2.26
P. alba (Acc # 0166)	96.44 ± 5.94 a	4.660.20 b	325.33 ± 15.79 b	6.36 ± 0.49
Prosopis Spp. (Peru)	83.44 ± 3.70 ab	5.33 ± 0.34 b	295.66 ± 13.4 bc	8.00 ± 0.87
P. pallida (Winrock)	40.8 ± 5.97 defg	1.2 ± 0.12 b	43.25 ± 10.7 gh	13.53 ± 1.76
P. juliflora (Brazil)	75.85 ± 7.39 abc	4.28 ± 0.25 b	92.28 ± 13.9 efgh	7.10 ± 1.61
MS (Species)	4355.18	22.44	130246.800	
df	19	19	19	
F - Value	5.04*	5.67***	13.184**	
$LSD_{0.05}$	27.40	1.85	92.78	

Table 5. Physiological measurements of different *Prosopis* species grown with saline water irrigation.

Name of Species	Stomatal Conductance ($m.mol.m^2 S^{-1}$)	Transpiration Rate ($m.mol.m^2S^{-1}$)	Quantum Yield ($umol.S^{-1}m^2$)
P. juliflora (Karachi)	20.23 ± 2.14 ab	1.09 ± 0.15 abc	1650 ± 195.11 abc
P. juliflora (Sujawal -1)	12.98 ± 1.68 def	0.88 ± 0.14 bcd	1688 ± 67.28 abc
P. juliflora (Sujawal -2)	15.72 ± 1.86 bcd	0.89 ± 0.12 bcd	1760 ± 66.95 abc
P. juliflora (D.I.Khan)	13.30 ± 0.83 def	0.77 ± 0.05 bcd	1415 ± 177.39 c
P. juliflora (Faisalabad)	12.98 ± 1.83 def	1.06 ± 0.15 abc	1575 ± 155.52 abc
P. juliflora (Bhawani)	9.35 ± 1.49 ef	0.49 ± 0.12 d	1700 ± 49.30 abc
P. glandulosa (Sujawal)	16.12 ± 2.93 bcd	0.99 ± 0.16 abc	1582 ± 92.40 abc
P. glandulosa (Sujawal) [Thornless]	14.74 ± 1.71 cde	1.16 ± 0.13 ab	1775 ± 31.91 ab
P. glandulosa (N.W.F.P.)	19.04 ± 1.31 abc	1.20 ± 0.05 ab	1659 ± 75.60 abc
P. cineraria (Tandojam)	11.22 ± 1.03 def	0.65 ± 0.13 cd	1556 ± 120.18 abc
P. cineraria (Karachi)	14.2 ± 1.30 cdef	1.07 ± 0.0 abc	1650 ± 195.11 abc
P. cineraria (N.W.F.P.)	11.73 ± 4.26 def	0.76 ± 0.07 bcd	1587 ± 89.04 abc
P. nigra (Acc # 1117)	8.76 ± 1.95 f	1.02 ± 0.08 abc	1721 ± 45.41 abc
P. articulata (Acc # 0598)	12.60 ± 1.28 def	0.66 ± 0.63 cd	1840 ± 70.23 a
P. chilensis (Acc # 0009)	9.03 ± 1.64 f	0.82 ± 0.87 bcd	1504 ± 113.32 abc
P. alba (Acc # 0591)	23.6 ± 5.30 a	1.42 ± 0.17 a	657 ± 204.84 d
P. alba (Acc # 0166)	9.26 ± 1.34 ef	0.66 ± 0.10 cd	1470 ± 97.88 bc
Prosopis spp. (Peru)	13.53 ± 1.39 def	0.76 ± 0.08 bcd	1587 ± 89.04 abc
P. pallida (Winrock)	13.63 ± 0.96 def	0.89 ± 0.07 bcd	1528 ± 137.18 abc
P. juliflora (Brazil)	8.76 ± 1.95 f	0.80 ± 0.16 bcd	1585 ± 20.44 abc
MS Treatment (Species) (df = 19)	80.80	0.29	219757.00
MS Error (df = 108)	24.24***	0.15*	94561.00**
F - Value	3.33	1.92	2.32
$LSD_{0.05}$	4.60	0.36	287.30

Means in columns followed by same alphabets indicate non-significant differences as determined by Duncan Multiple Range Test at $P < 0.05$.

Table 6. Grafting performance of some *Prosopis* species.

Shoot Stock (Exotic)	Root Stock (Local)	Number of Grafts made	Number of Successful Grafts (sprouted within a month)	Growth Condition After One Year
P. chilensis	*P. glandulosa*	19	2	Failed
P. chilensis	*P.glandulosa*	14	2	Failed
P. juliflora	*P. glandulosa*	17	2	Shoot stock has produced two stems about 90 cm long each.
P. alba $B_2 V_{50}$	*P. glandulosa*	18	4	Shoot stock has produced two stems about 90 cm long each.
P. alba 0088	*P. glandulosa*	13	4	Shoot stock has produced three branches, two about 90 cm long and one about 30 cm long.
Haitian Field Prosopis Trees				
419 BLKI Ln 4 Tree 2	*P. juliflora*	16	2	Shoot stock has produced two branches about 30 cm and 10 cm long, respectively.
419 BLKI Ln 1 Tree 1	*P. juliflora*	17	zero	Failed
419 BLK4 Ln 1 Tree 3	*P. juliflora*	12	2	Shoot stock has produced two branches about 10 cm long each. Further growth retarded.
419 BLK Ln 10 Tree 4	*P. glandulosa*	25	2	Shoot stock has produced two branches, 90 and 45 cm long, respectively.
420 BLK 1 Ln 8 Tree 3	*P. glandulosa*	15	zero	Failed

MANIPULATION OF THATTA LEGHARI RANGELAND TO IMPROVE THE PRODUCTIVITY OF SMALL RUMINANTS IN PAKISTAN

M. F. Khan[1], N. M. Butt[1], M. I. Nutkani[2] and N. A. Chaudhry[3]

ABSTRACT

Thatta Leghari rangeland in the Dera Ghazi Khan district of Pakistan covers an area of 1004 ha. Its topography is undulating, soils are calcareous and low in organic matter and support a native standing crop of mainly unpalatable herbaceous vegetation. Due to uncontrolled grazing the range is in very poor condition. Between 1992 and 1993 the native range(1000 ha) was not grazed. Then in 1992 and 1993, a 600 ha area was reseeded with two improved grasses gorkha (*Lasiurus sindicus*) and buffel grass (*Cenchrus ciliaris*). Forage production on the reseeded and natural range was determined in 1992 and 1993 under weather conditions that were average with respect to temperature and precipitation. The reseeded area produced 10 times (4000 k/ha) more forage than the native range (424 k/ha) in 1992 and more than twice the forage (1250 k/ha vs 534 k/ha) in 1993, a year with 37mm less rainfall. Proximate analysis of the hand harvested plants growing in the area was determined. Buffel grass had a higher crude protein value (7.8%) than did gorkha and chimber (*Eleusine flagellifera*). The shrub, lana (*Salsola foetida*) had a higher energy value 2.7 Mcal/kg than all of the othe forage species. Daily goat and sheep diets consisted of 45% and 39% shrub respectively. Live weight gain in goats and sheep was 34 gm/day 14 gm/day respectively. These preliminary results indicate reseeding may be an important intervention in Thatta Leghari rangelands improvement, for improving nutrition for small ruminants in Pakistan.

KEY WORDS: Pakistan, Rangeland, Forage Species, Reseeding, Sheep and Goat.

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[1] Senior Scientific Officer, Pakistan Agricultural Research Council, Islamabad.

[2] Veterinary Officer, Taunsa

[3] Range Management, Dera Ghazi Khan

INTRODUCTION

Rangeland reseeding has been recommended to improve forage production when coupled with proper management if natural conditions warrants seedling germination and establishment. Rangeland vegetation consisting of more unpalatable plants than palatable ones and low forage production is reliable indicator for the range need of improvements such as reseeding (Mohammad, 1984). Gorkha has been successfully reseeded in Thall, Cholistan and Kohistan ranges in Pakistan. Buffel grass is very drought resistant and has been recommended for reseeding in arid and semi arid rangelands of Pakistan (Mohammad and Naqvi, 1987; Butt et al; 1990). Mixtures of buffel and gorkha have successfully been seeded in row 75 cm apart at the rate of 6 k/ha on denuded silt flats near Rajastan (Mohammad, 1984). The objectives of this study were to improve forage production and livestock gain through reseeding native range with improved forage grasses and controlling livestock grazing.

Study Area

Rangelands of the Dera Ghazi Khan are located between the Sulaiman mountains and the Indus River situated between latitude 28° to 31° south and longitude 69° to 70° in east. The area covers 2638 ha and has twelve rakhs or range sites. One rakh, the Thatta Leghari (Fig. 1) was chosen for this study. It has an area of 1004 ha and is about 20 km north of Taunsa a city of .2 million papulation situated on highway 90 km north of Dera Ghazi Khan. Its topography is undulating, the soil is deep, well drained, calcareous, medium–textured and low in organic matter (Mohammad 1984).

The weather is typical of very arid sub–mountainous sub–tropical continental climates characterized by cold winters and very hot summers. Ambient winter temperatures occasionally drop to 0°C, especially in January and February while June and July are the hottest months with mean maximum ambient temperatures of 42°C. Annual precipitation varies from 75 to 162 mm, with most occurring during the months of July and August, the monsoon season (Alizai and Habib, 1987).

Rangelands in D. G. Khan are in poor condition because of overgrazing by nomadic and local livestock and illicit cutting of shrubs for fuel and fodder purposes during last several centuries. More than 70% of the area is bare and is vulnerable to water and wind erosion. Many

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of the palatable forage species have been replaced by unpalatable ones. Farming and livestock rearing are the important income sources in D. G. Khan. Women play a major role in livestock production. They do major part of crop harvesting, herding and feeding of animals.

MATERIAL AND METHODS

The study was initiated in August 1992 on 1000 ha of Dera Ghazi Khan rangeland in collaboration with the Punjab Forest Department. Following this reseeding was done on about 300 ha during July of 1992 and an additional 300 ha during July of 1993. The 300 ha seeded in 1992 was disc plowed three times between March and April 1992 to remove unwanted shruby and herbaceous vegetation and to prepare the seed. Seeds of buffel grass and gorkha grass were mixed in a ratio of 9:1, based on seed availability, and broadcast manually followed by dragging large brush behind a tractor to cover the seed. Domestic grazing was excluded for one year to allow seedling establishment. The same land preparation, seeding and grazing exclusion procedures were repeated during 1993. However, buffel and gorkha seed grass were mixed in a ratio of 1:9 ratio again based on seed availability. Forage production and species composition data on both native and reseeded range were recorded using line transect sampling at the end of the 1992 and 1993. Close to the watering point a point was selected randomly and an imaginary line was drawn in one of the four cardinal directions on both reseeded and natural range sites. Ten randomly plots each located separated by 100m were sampled on each of the imaginary lines. A 1.0 m^2 square quadrate was thrown by the technician to his right side of each of the 10 selected points. All plants within the 1.0m^2 quadrate were recorded by species and then harvested at 15cm stubble height during October of 1992 and 1993. Air dried forage production and chemical composition of intact plants were determined by the standard methods (AOAC, 1985). Regression equations (Ahmed and Muller 1986) were used to predict energy values of the different plants sampled. Meteorological data for 1991, 1992 and 1993 were recorded in a rain gauge installed by the Punjab Forest Department on an adjacent range about 5 km from the sampled plots. Twenty five adult ewes of Damani breed and 25 goat of the Nachi breed were grazed on the seeded and native range sites to study the weight gain and grazing behavior. Individual live weight were obtained initially and every 30 days following on overnight fast off both feed and water. Behavior was observed and recorded every 5 min on 10 sheep and 10 goats for a period of 6 consective days between 0700 and 1200 hours and again between 1500 and 1800 hours. No sheep and goats were lost or died during the study period.

RESULTS AND DISCUSSION

Vegetation species present in the rakh were recorded and presented in table 1. Based on two years of data the native range had only unpalatable species. Chimber (*Eleusine flagellifera*) was the dominant grass and lana (*Salsola foetida*) was the dominant shrub. Together these two species comprised over 80% of the vegetation.The remaining 20% of the plants were sehnwar (*Rhazya stricta*), bui (*Aerva javanica*), phog (*Calligonum polygonoides*), kikri (*Acacia jacquemontii*), karir (*Capparis decidua*), khip (*Leptadenia spartium*), jand (*Prosopis cineraria*), mallah (*Zizyphus nummulria*) and ak (*Calotropis procera*) (Table 1).

In October 1992, mean forage production from the reseeded area was 3936 k/ha compared to 424 k/ha from the native range plants (Fig 3). Species frequency and forage production during 1993 is given in table 1. Total plant production during 1993 from reseeded and natural range was 1250 k/ha and 534 k/ha, respectively (Fig–3). Higher forage production during 1992 compared to that of 1993 was probably due to higher precipitation during the growing season. Precipitation data for 1991, 1992 and 1993 are given in Fig 2. During 1993, there was 17 mm less rainfall, which has the profound effect on the range forage production in that year.

Chemical Composition of Forage

The chemical composition of forage species sampled are presented in table 2. The native grass (chimber) was lower in crude protein (5.9%) and energy than buffel and gorkha. Buffel had a highest crude protein (7.8%) followed by gorghka (6.2%) and then chimber (5.9%). The shrub lana had the highest crude protein (11.5%) of all the forage species sampled. The energy value was higher for lana and phog as compared to grasses found on the rangeland (Table 3). The higher energy values for these shrubs were due to higher carbohydrate content.

Sheep and Goat Liveweight Changes

Live weights of sheep and goats were recorded monthly between February and December, 1993 (Fig. 4). Goats and sheep gained a mean of 34 g and for 14 g/day respectively. Sheep gained weight from February to August and then lost weight from September to December. Goats gained weight continuously throughout the study period. Weight gains were greater during

the early growing season for the both kind of animal species probably due to the higher nutrient contents of the forage, this pattern has also been reported by Holecheck (1984). During the dry period of the year sheep lost weight while goat continued to gain weight probably as a resort of changing their diet to include leaves and flowers/fruits of shrubs. Similar observations were made in Kenya by Shwartz and Said (1981) where flowers and fruits of Acacia spp represented the major portion of goats diets during the dry season. Malecheh and Provenza (1983) reported that because of goat adaptability to harsh conditions they are ideal candidates for marginal rangeland.

Sheep and Goat Grazing Behaviors

The botanical composition of sheep and goats diets were determined by observing each species.The results presented in Fig.5 suggested that sheep prefer grass (49%),While goats prefer shrubs (45%). These results are similar with Bryant et al (1979) and Malechek and Provenza (1983) that among grass, forbs and shrubs, goat usually prefer shrub diets whereas sheep select high forb diets. Due to higher nutritive value of shrubs (table 3) goats gained weight throughout the study. These results are similar with those of Rafique at al. (1992) who found shrubs and forbs effectively serve to improve diet quality in small ruminant diet fed low quality grass hay. The higher weight gains of goat in this study may be due to higher intake of shrubs as compared to sheep. Shrubs improve the efficiency of N and energy utilization in rumen, which ultimately resulted in higher weight gain in the goat. These results suggested that for the efficient utilization of rangelands, mixed species of livestock should be introduced in stead of single livestock species.

Conclusion

This study can serve as a model for the inhabitants of the Thatta Leghari area.It is also a contribution to the knowledge of sustainable systems in the desertifying rangelands of semiarid lands elsewhere in the world where similar environmental and social conditions exist. Increased demands on these rangelands in the very near future will dictate the reestablishment of plant species, both native and introduced.

These preliminary results indicated that controlled grazing and reseeding of rangeland with palatable species improve forage production and increases livestock productivity expressed

as live weight gain. The results, moreover, suggest that for the efficient utilization of rangelands, mixed species of livestock should be employed, rather than a single species. The goats' adaptability to harsh conditions makes it an ideal livestock for such marginal rangeland areas in Pakistan.

To both scientists and land users, the extremely dry environments of such regions present great challenges. These challenges can be met only by conducting continued research and by applying its results with care and constraint.

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REFERENCES

Ahmad, S. T. and Z.O. Muller. 1986. Tables of chemical composition of feedstuffs in Pakistan converted into various energy value. PAK /80/019 Islamabad, Pakistan.

Alizai, M. Y. and G. Habib 1987. Livestock resources and potentialities of Dera Ismial Khan, District Sarhad. J. Agri. 3:23–49.

AOAC (Association of Official Analytical Chemists) 1985. Official Method of Analysis (15th ed). Washington D.C.

Bryant, F.C; M.M. Kothmann, and L.B Merrill. 1979. Diets of sheep, Anogra goats, Spanish goats and white tailed deer under excellent range conditions. J. Range Manage 32:412–417.

Butt, N.M.,. M Sartaj and M.I. Sultani. 1990. Performance of tropicai grasses under subhumid subtropical conditions of Pakistan. Pak. J. Forestry. 318–320.

Holochek, J.L. 1984. Comparative contribution of grasses, frobs, and shrubs to the nutrition of range ungulates. Rangelands 6:261–263.

Malechek, J.C. and F.D. Provenza 1983. Feeding behaviour and nutrition of goats on rangeland. Wold Anim.Rev. 47:38–48.

Mohammad, N. 1984. Improvement potential of D.G. Khan rangelands. Progressive Farming. 4: 31–35.

Mohammad, N. and A. H. Naqvi 1987. Dry matter yield of promising grasses in tropical rangelands of Sind, Pakistan. Tropical Agri. (Trinidad). 64: 70–71.

Rafique, S; J.D. Wallance, J.L Holechek, M.L. Galyean and D.P. Arthun. 1992. Influence of forbs and shrubs on nutrient digestion and balance in sheep fed grass hay. Small Ruminant Res 7:113–122.

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Schwartz, H.J. and A.N. Said 1981. Dietary preferences of goats and nutritive value of forage on semi-arid pasture in Northern Jenya. In Nutrition and Systems of Goat Feeding, Proc. In Symp., Tours, France, Paris, INRA. ITOVIC.

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Table 1: **Mean dry matter yield (kg/ha) and percent composition of vegetation growing on native and reseeded Thatta Leghari range during Oct, 1993.**

Species	Reseeded		Native	
	DM (kg/ha)	% Frequency	DM (kg/ha)	% Frequency
Cenchrus ciliaris (Buffel)	290	23	–	–
Lasiurus sindicus (Gorkha)	225	18	–	–
Eleusine flagellifera (Chimber)	198.0	18	280	54
Salsola Foetida (Lana)	538.0	41	235	30
Calligonum polygonoides (Phog)	–	–	10	8
Aerva javanica (Bui)	–	–	9	8
Total	1251	100	534	100

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Table 2 **Chemical composition of Important forage species found on Thatta Leghari range land during July to September.**

	Buffel	Gorkha	Chimber	Lana	Phog
Dry matter (%)	91.3	93.6	93.6	89.3	93.3
Crude Protein (%)	7.8	6.2	5.9	11.5	8.4
Ether Extract (%)	1.5	1.8	2.4	2.4	1.8
Crude Fibre (%)	24.7	38.6	29.1	33.5	37.8
TDN (%)	58.4	61.3	63.9	71.2	61.2
Digestible energy (Mcal/kg)	2.6	2.7	2.4	3.1	2.7
Metabolizable energy (Mcal/kg)	2.2	2.3	1.5	2.7	2.3

Crude Protein = N% x 6.25

TDN = Total digestible nutrients

Fig. 1 Map of Thatta Leghari Rangeland, Dera Ghazi Khan District

Scale 1 inch = 1 mile

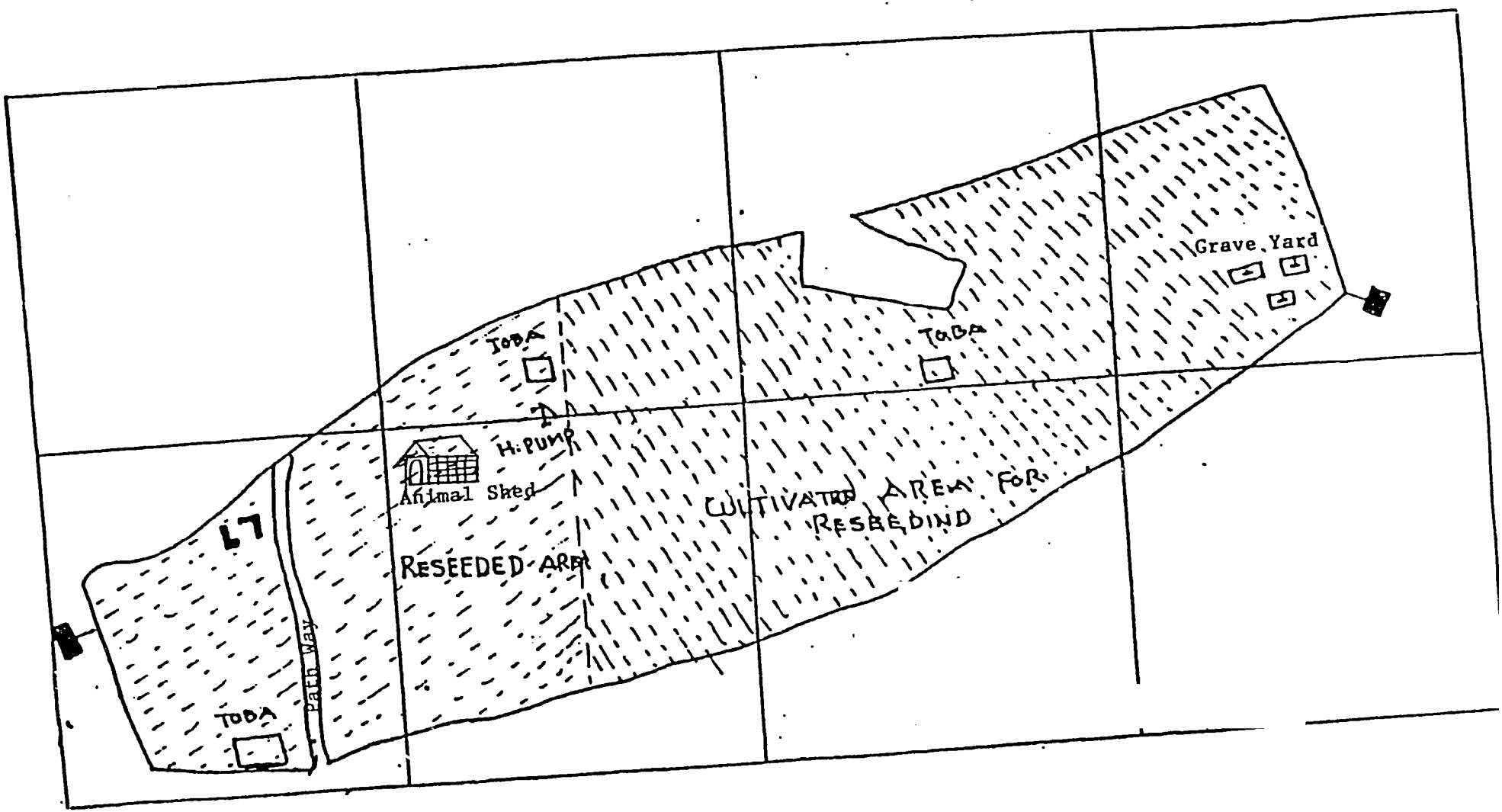


Fig: 2 Mean Precipitation for Dera Ghazi Khan

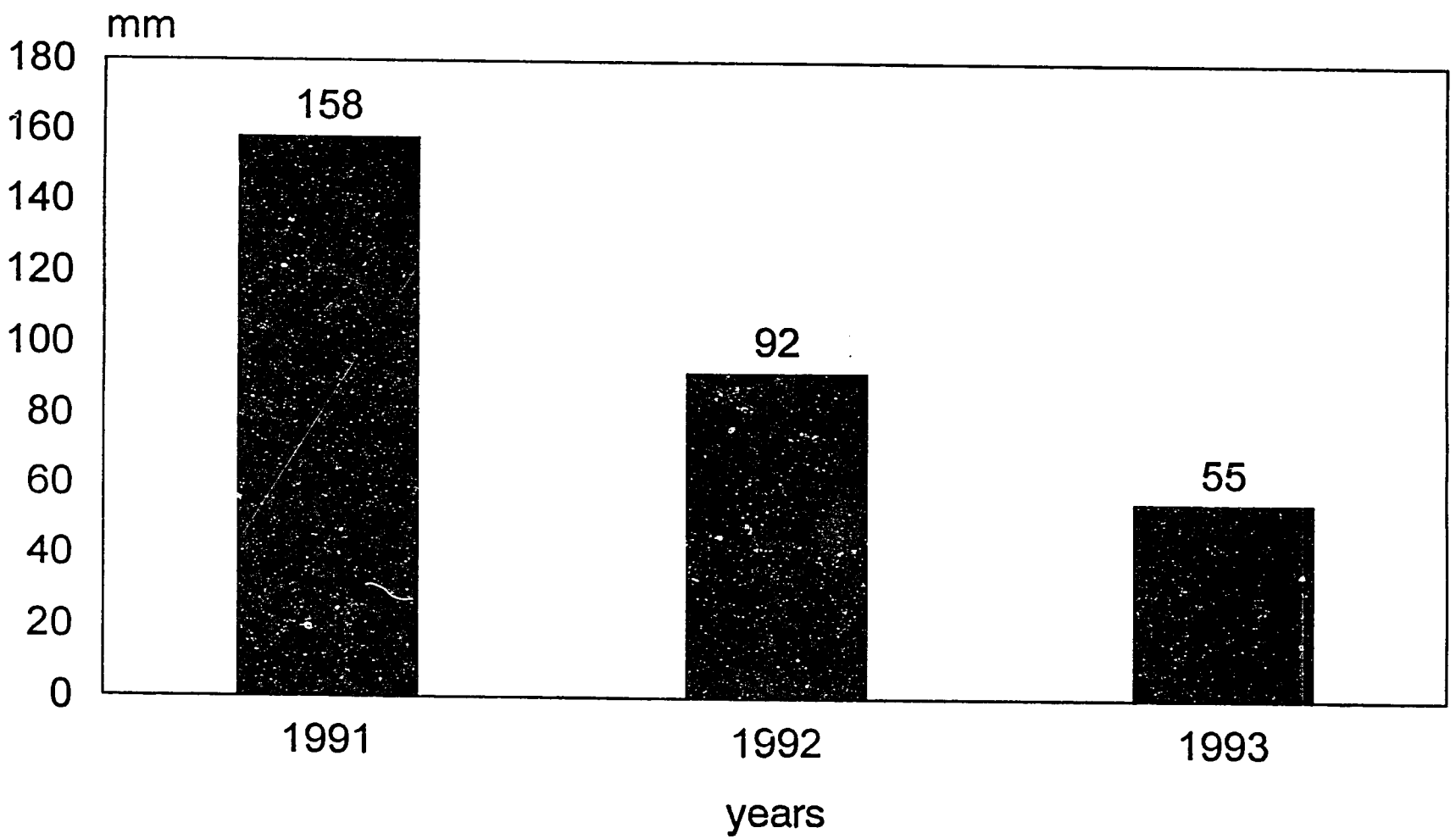


Fig: 3 Mean Production of grasses, forbs and shrubs on Thatta Leghari rangeland, Pakistan

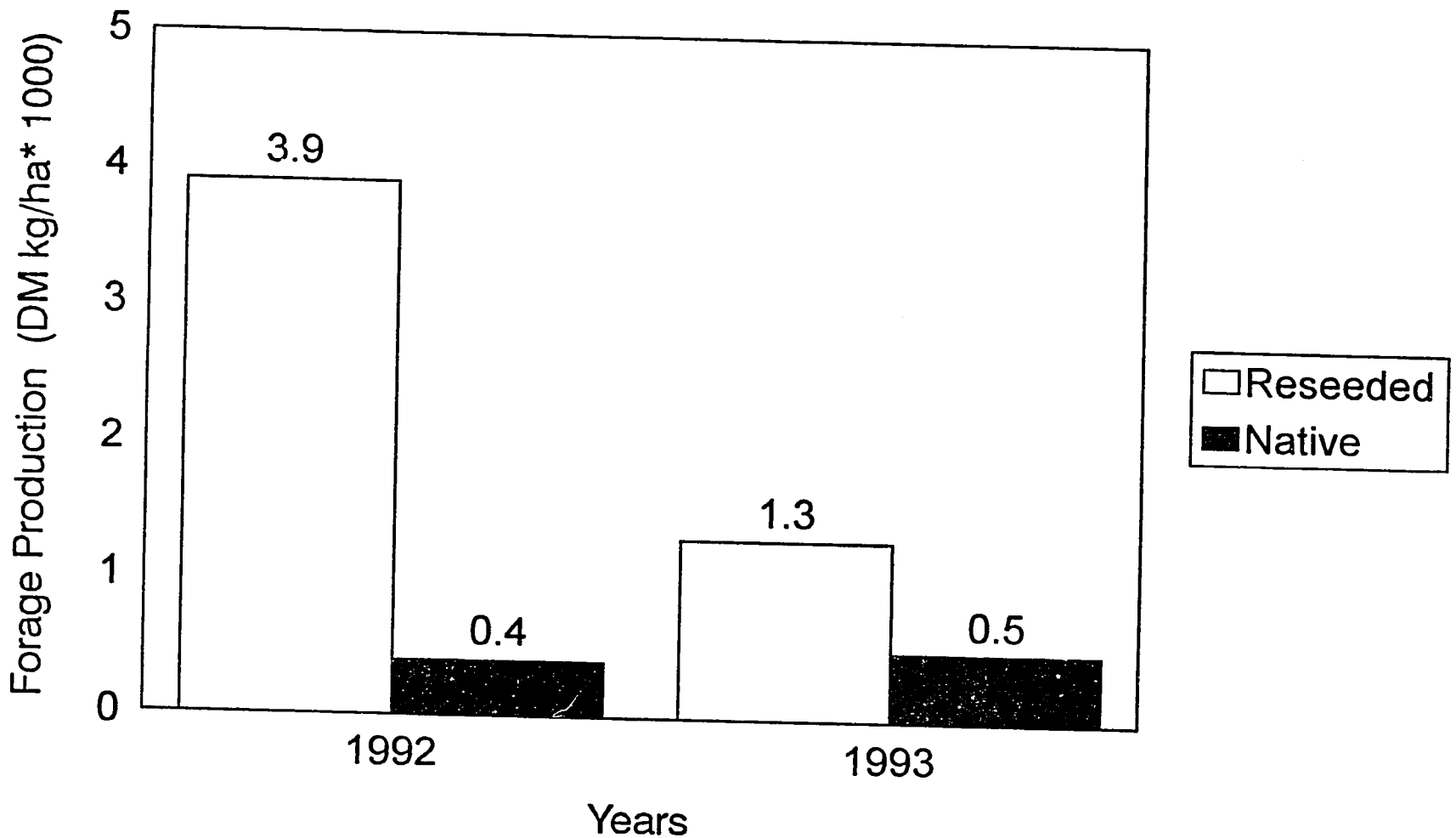


Fig: 4 Mean live weight gain of sheep and goat during 1993 grazing Thatta Leghari rangeland in Pakistan.

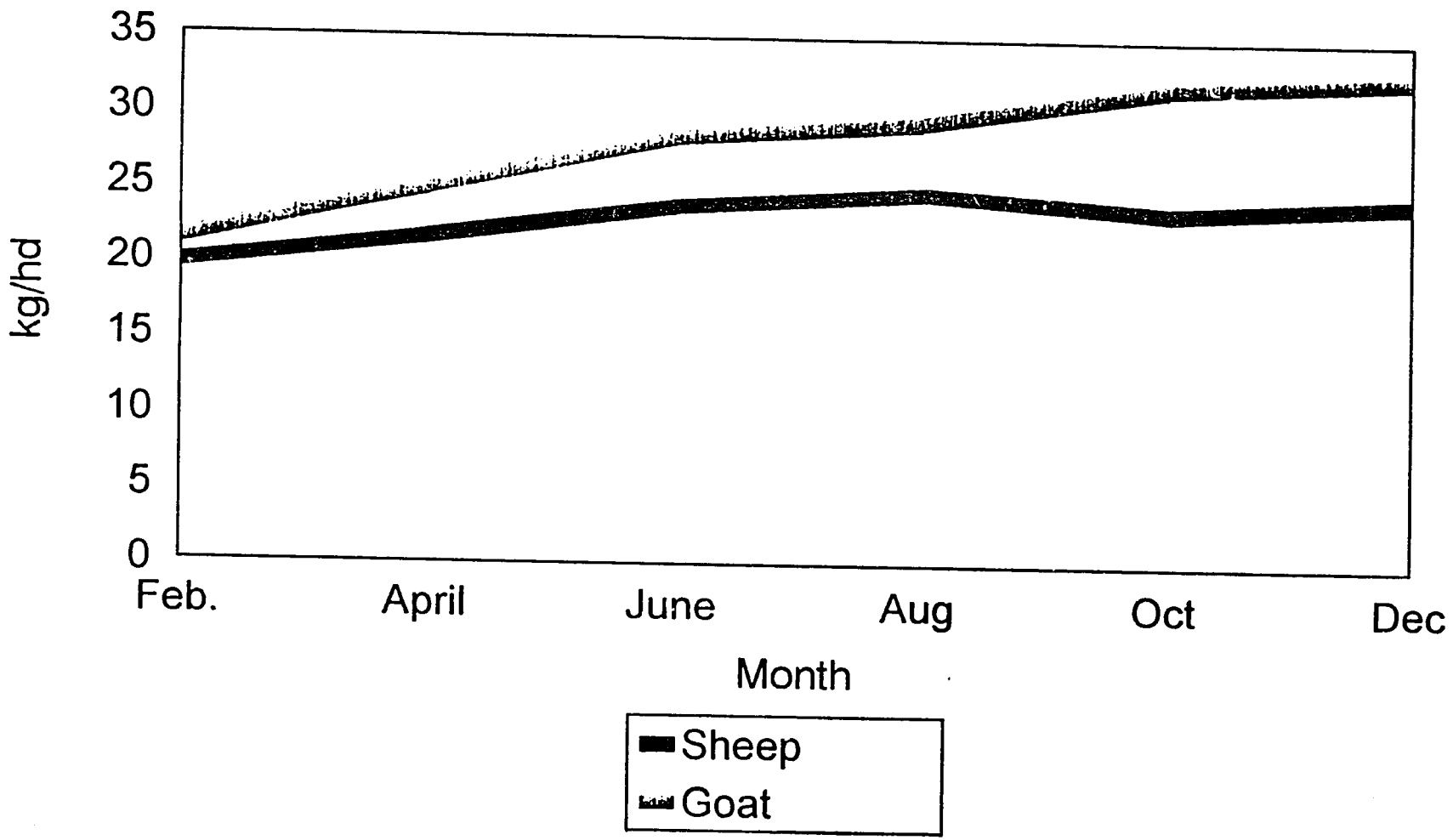
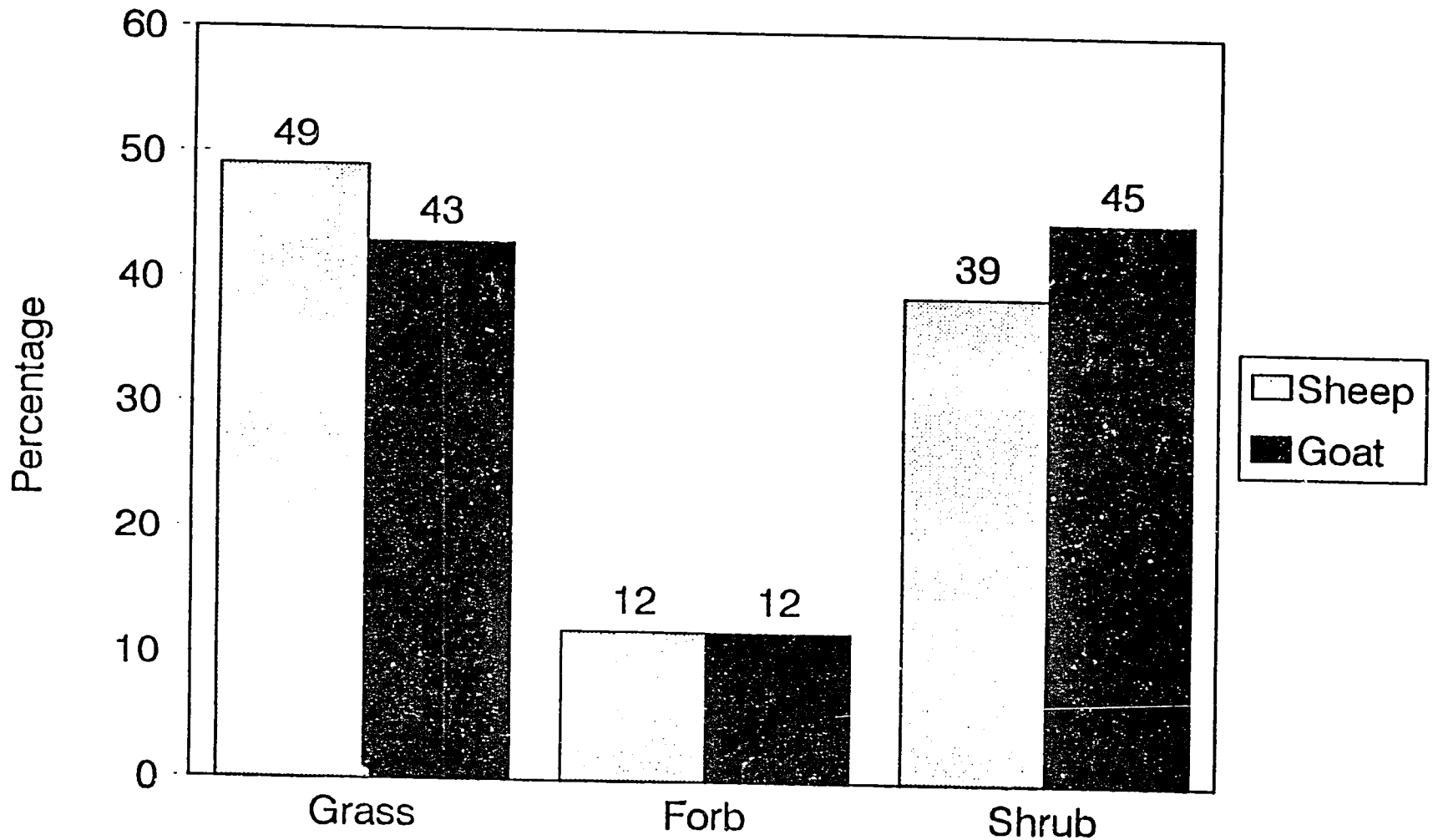


Fig: 5 Grazing behavior study of sheep and goat on Thatta Leghari rangeland, during Sep,1993



MOLECULAR BIOLOGY

GROUP ADVISOR:

Dr. Peter Day
Center for Agricultural Molecular Biology
Rutgers University Cook College
New Brunswick, New Jersey

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Immunosuppressive Potential and Pathogenicity of an Avian Adenovirus Isolate Involved in Hydropericardium Syndrome in Broilers. K. Naeem, T. Niazi, S.A. Malik, and A.H. Cheema.

A Study on Lateral Spread of Aetiologic Agent(s) of Hydropericardium Syndrome in Broiler Chickens. S. Akhtar.

IMMUNOSUPPRESSIVE POTENTIAL AND PATHOGENICITY OF AN AVIAN ADENOVIRUS ISOLATE INVOLVED IN HYDROPERICARDIUM SYNDROME IN BROILERS

Naeem, K., T. Niazi, S. A. Malik, A. H. Cheema
Animal Health Laboratories, Animal Sciences Institute,
National Agricualtural Research Centre, Park Road,
Islamabad, Pakistan.

ABSTRACT

The role of avian adenovirus iosolate PARC-1 as an immunosuppressive agent was investigated in this study using a Newcastle disease virus (NDV) vaccine immune response procedure. The immunosuppressive effect on the humoral immune response was investigated up to 21 days after inoculation with adenovirus. Infected birds showed a reduced serologic response to NDV as compared with the control. To further investigate the effect of the virus on major lymphoid organs, the pattern of virus dissemination in various organs was studied at various time intervals after inoculation. Spleen, thymus, bursa of Fabricius, and caecal tonsils of broilers were examined using a dot-immunobinding assay (DIA). The virus was found to have a predilection for lymphoid organs; virus from lymphoid organs was capable of producing disease when inoculated into healthy birds. The relationship of virus predilection to its immunosuppressive effect was also studied.

INTRODUCTION

Hydropericardium Syndrome (HPS) has been prevalent in poultry in Pakistan since its first appearence in 1987. To date, no disease with similar symptoms and such a high rate of mortality has been

reported from any other country. HPS is seen primarily in broilers, 3-5 weeks old, although outbreaks have occasionally been reported in layers and breeders. All broiler strains commonly found in Pakistan have been affected. The course of the disease is usually 10-15 days, during which a mortality rate of 12-75% has been reported.

Postmortem lesions observed include hydropericarditis, pulmonary oedema, hepatitis, and nephritis (Cheema et al., 1989); microscopic lesions in the heart include nectrotic areas in the myocardium of the ventricular walls and accumulation of mononuclear cells in some areas. In the liver, there are small multifocal areas of coagulative necrosis, and many hepatocytes have large round basophilic intranuclear inclusion bodies. In the kidneys, massive areas of necrosis in the epithelium of the renal tubules has been described (Anjum et al., 1989; Cheema et al., 1989).

HPS was earlier believed to be a nutritional disorder (Jaffery, 1988). However, reproduction of the disease by subcutaneous inoculation of liver homogenate from infected birds and the development of a successful killed vaccine have demonstrated that HPS is infectious in nature (Chishti et al., 1989). Several different organisms have been proposed as causal agents of HPS in the past, but a new avian adenovirus isolate has been recently identified as the major etiologic agent (Naeem et al., 1994). The present study was undertaken to assess the extent of tissue tropism of this HPS-associated fowl adenovirus, with special reference to its predilection for lymphoid organs, and to assess its immunosuppressive potential in broilers.

MATERIALS AND METHODS

Inoculation preparation

Ten 15-day old chickens were inoculated with 20% HPS-infected liver homogenate. The homogenate was prepared by blending liver in sterile normal saline and centrifuged at 800 G for 15 minutes. The supernatant was collected and subsequently passed through 0.45 um and 0.22 um filters. Streptomycin (200 ug/ul) and gentamicin (100 ug/ul) were added to the filtrate before 1 ml per bird was inoculated subcutaneously. Livers were collected from birds that died showing hydropericarditis within 24-48 hours after inoculation. The livers were again homogenized, clarified and filtered as above. The filterate was pelleted at 80,000 G for 2 hours using an SW 28 rotor. The pelleted virus was suspended in Tris buffer saline (TBS) (10 mM Tris, 150 mM NaCl,pH 7.6) and further purified by centrifuging through a 35% sucrose cushion at 90,000 G for 90 minutes.

The LD50 for the purified preparation was calculated after inoculating 15-day-old chicks using the Reed and Muench method (Reed and Muenchm 1938). A virus titer of $10^{3.4}$ LD_{50} in 0.1 ml was used for inoculation of chicks throughout this study.

Chickens

The birds used in this study were commercial broiler chickens obtained from a local hatchery, which used eggs from Hubbard breed hens reared locally. The parent stock was never vaccinated with HPS vacccine, but Newcastle Disease virus (NDV) Lasota vaccinations were performed on day 2, 15, and 28 via drinking water and at 20 weeks of age by inoculation with a killed oil emulsion NDV vaccine

(Solvay, U.S.A). All the chicks were tested for the presence of antibodies against Reovirus, infectious bursal disease virus, NDV and adenovirus-127, and adenovirus serotype-1 group antigen. The birds showing negative titres against the above viruses were included in the HPS experiment. The chicks were maintained in an isolation room at the Animal Science Institute. National Agricultrual Research Centre, Islamabad.

NDV Vaccine

To assess the serologic response in adenovirus-infected birds, the chickens were first vaccinated with HPS vaccine, and 4 days later were vaccinated using the eyedrop method with 0.03 ml ($10^{6.2}$ EID_{50}/chick) of the B-1 type lasota strain (Intervet, U.S.A). The birds were examined for serologic response evaluation against NDV vaccine using a haemagglutination inhibition (HI) test.

Agar gel precipitation (AGP) test

AGP tests were conducted on serum samples to detect HPS-associated adenovirus antibodies, following the standard protocol (Crowl, 1973).

Haemagglutination inhibition test

An HI test was used to measure antibodies to NDV using 10 haemagglutinating units according to the Beta procedure as described elsewhere (Giambrone et al., 1976). HI titres were determined in all chickens, and the geometric mean titer (GMT) was calculated for each group.

Dot-immunobinding assay (DIA)

For the detection of viral antigens from the organs of infected birds, DIA was standardized and then employed as follows.

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Antigen preparation

Selected organs were triturated in TBS and clarified by centrifuging at 800 G for 10 minutes.

Antisera preparation

The above-described adenovirus preparation was used to raise hyperimmune serum in chickens. For this purpose, three 4-week old birds were injected intramuscularly with 1 ml of formalin-treated HPS-associated adenovirus isolate PARC-1, emulsified in an equal quantity of Fruend's complete adjuvant. Two weeks later, a similar virus preparation was emulsified with incomplete Fruend's adjuvant, and 1 ml per bird was injected. Three weeks later, serum of these birds was collected and tested by AGP to detect adenovirus antibodies.

Assay procedure

The DIA was conducted using a dot-blot apparatus (Bio Rad, U.S.A.). Nitrocellulose sheets of 0.45 um pore size were used for transferring the antigen according to a standardized procedure (Gorden and Biling, 1988). The antigen-blotted sheets were air-dried for 1 hour at room temperature, and unreacted protein sites were blocked by immersing and soaking the sheet in 2% fish gelatin (Sigma, U.S.A.) for 90 minutes. The sheets were placed in HPS-associated adenovirus hyperimmune serum diluted to 1:50 in TBS, for 1 hour at room temperature on a shaker. After washing three times in TBS for 5 minutes each, the sheets were submerged in anti-chicken HRP-labeled conjugate (Sigma, U.S.A.), diluted 1:1000, for 90 minutes. The sheets were washed three times, developed in 50 mM TBS containing 0.15% diaminobenizidine, 0.04% $NiCl_2$, and 0.02% H_2O_2.

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The reaction was allowed to proceed for 10 minutes. The sheets were washed in tap water and air- dried before visual interpretation of the results. In positive samples, the site of Ag-Ab complex turned slate black.

Experimental Design

Experiment #1

For the immunosuppression experiment 40 day-old broilers were divided into four groups of ten each (Table 1). The birds were housed in separate cages. At 2 weeks of age, all the chickens were bled, and the serum was collected for AGP and HI tests for antibody detection against HPS infection and NDV vaccine, respectively. The birds of groups 1 and 4 were inoculated with HPS-associated adenovirus subcutaneously at 15 days of age. At 20 days of age, groups 2 and 4 were vaccinated with NDV vaccine by eye drop. Group 3 was inoculated with liver from uninfected birds. The birds were bled every third day up to 36 days of age, and HI antibody titres against NDV were determined (Table 1). All the groups were tested for HPS-associated adenovirus antibodies by AGP 21 days after inoculation.

Experiment # 2

For virus dissemination studies, 30 one-day old broilers were divided into six groups of 5 birds each and kept in separate cages. The birds in the first five groups were infected with adenovirus subcutaneously at 15 days of age, birds from the 6th group were uninfected controls. All five birds from each test group and one control were sacrificed at 12, 24, 36, 48 & 60 hr post-inoculation. Liver, spleen, thymus, bursa of Fabricius and caecal tonsils were

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collected from each bird and frozen at -20C to be used for DIA.

Experiment #3

For the reproduction of disease from various organs of HPS-infected birds, 20-day-old broilers were divided into two groups: a test group of 15 and a control group of 5. The lymphoid organs collected from the experiment on virus dissemination were triturated and injected into the test group. Of the 15 birds used for the virus infection, 3 were sacrificed at 12, 24, 36, 48, and 60 hours post-infection. One bird from the uninfected control group was included each time the birds were sacrificed.

RESULTS

Experiment #1

In the immunosuppression experiment (Table 1a), group 4 acted as the test group for studying the immunosuppressive effect of HPS-associated adenovirus. No clinical signs of infection were seen in 8 birds out of group 4. Two of the birds from this group died 36 hours post-inoculation with typical lesions of HPS. Birds in groups 1 and 4 examined for HPS antibodies by AGP test at 2 weeks post-infection were positive, with 1:80 dilution of the serum. Birds in groups 1 and 3 tested for NDV antibodies had GMT HI titre of >10, whereas group 2 had GMT of 160, and group 4 had GMT of 49 between 3 and 20 days post-vaccination (Table 1b). These results indicate lowered immune response in adenovirus-infected birds of group 4.

Experiment #2

Results of the DIA conducted on the organs for the detection of viral antigens are shown in Table 2. Adenovirus antigens were

detected in spleen, thymus, bursa, and liver at varying time intervals post-inoculation. No antigen was detected in specimens of caecal tonsils at any stage of infection. Two of the 5 birds in group 4, which were to be sacrificed at 48 hours post-inoculation, died of HPS, showing typical hydropericarditis. Liver and spleen were the only organs that retained the viral antigens beyond 60 hours post-infection. Clearance of viral antigen was detected in thymus and bursa after 48 hours post-infection (Table 2).

Experiment #3

In the disease reproduction experiment, HPS was reproduced using liver and all the lymphoid organs from the birds sacrificed at varying time intervals post-inoculation (Table 3).

DISCUSSION

Based on various biological properties of the HPS-associated fowl adenovirus and the nautre of lesions produced by this isolate, a close association is indicated between this isolate and other isolates of fowl adenovirus incriminated in inclusion body hepatitis (MacPherson *et al.*,1974). The mechanism of HPS-associated fowl adenovirus has not been thoroughly investigated thus far. Earlier attempts to demonstrate a pathogen in tissues other than liver from birds that died of HPS were unsuccessful (Ahmad *et al.*,1989). Liver has been identified as the major organ showing histopathological changes along with the presence of intranuclear basophilic inclusions in the hepatocytes (Anjum *et al.*, 1989; Cheema *et al.*, 1989; MacPherson *et al.*, 1974). No evidence has been recorded regarding the presence of HPS-associated adenovirus in the lymphoid organs or its involvement as an immunosuppressive

agent.

In this study the lymphoid organ tropism of the virus in experimentally infected chicks was investigated using DIA. The results indicate that systemic infection by the virus is extensive, involving spleen, thymus, and bursa of Fabricius. The role of HPS-associated adenovirus in nonlymphoid organs remains to be elucidated, especially in the heart and kidneys where gross pathological lesions have been reported (MacPherson *et al.*, 1974). The rate of clearance of the viral antigens from the lymphoid organs was variable in this study. Viral antigen detection up to 60 hours post-inoculation in liver and spleen, its depletion from thymus and bursa in 48 hours, and its undetectable titre in caecal tonsils all indicate variation in the predilection sites of the virus. Further investigation regarding histopathological changes in these organs would help in identifying the level of damage caused by the virus.

Variation has also been observed in the detection of viral antigens by DIA and its ability to reproduce the HPS-associated lesions upon reinoculation with various organs. At such a low level of infection as that induced by the inoculum during this study, only a few birds presented typical lesions of HPS. It has earlier been observed in the field that some birds dying during HPS outbreaks have had very few detectable lesions at an early stage of infection (personal communication). No extra pericardial fluid has been seen in such birds. The disease can now be diagnosed in such birds at early stages of infection using DIA. The presence of infectious virus in lymphoid organs indicates that the virus may be

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involved in modulating responses of the immune system against other viral infections. Alternatively, these infected cells could serve as an additional source of virus.

In addition to the antigenic evidence of viral involvement of the lymphoid organs, the results of this study also indicate that birds infected with a pathogenic strain of fowl adenovirus (FAV) have a decreased ability to mount an antibody response to other viral antigens. Immunosuppression was confirmed by the reduced serologic response of infected birds against NDV vaccine. The immunosuppressive effects were seen in the experimental birds surviving infection, suggesting that a low level of virus infection in the field may be immunosuppressive. Furthermore, it is possible that under field conditions, immunosuppression could be exacerbated by untimely HPS vaccination, poor management practices, and the presence of other immunosuppressive agents. Some other strains of FAV have been reported to compromise the immunological capabilities of infected chickens(Saifuddin and Wilks, 1991).

Although viral antigens were not detected in most of the lymphoid organs 60 hours post-infection, the presence of virus in these organs at low undetectable levels may have a role in immunosuppression. This could be investigated by employing more sensitive techniques of viral nucleic acid detection. At present, formalin-treated autogenous vaccine is being used to combat HPS in the field. It remains to be determined whether this killed vaccine has any role in producing an inmmunosuppressive effect in the vaccinated birds. It would also be appropriate to conduct more studies to confirm virus replication in lymphoid organs, as well as

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many extra-hepatic sites.

This study can serve as a basis for more extensive analysis of the kinetics of adenovirus infection of various tissues during the natural course of virus infection. The systemic distribution of the virus in lymphoid organs observed here during the course of experimental infection is critical to understanding the overall pathologic mechanisms of an adenovirus-induced disease.

MANAGEMENTAL IMPLICATIONS

The results obtained in the present study would help in devising a new vaccination schedule for the HPS vaccine in broilers. Given the immunosuppressive potential of adenovirus isolate PARC-1 as identified in this study, it would be more appropriate to vaccinate the birds at an earlier age, i.e., at day 10 rather than the usual day 17. This would help in avoiding vaccine failures due to earlier exposure of birds to adenovirus infection, as the virus exposure before day 15 of age is known to cause permanant immunosuppression in birds.

REFERENCES

1. Ahmed, I., M. Afzal, M.I. Malik, Z. Hussain and W. Hanif. Disease pattern and etiology of Hydropericardum syndrome in broiler chickens in Pakistan. Pak. J. Agri. Res. 10(2):195-199, 1989.

2. Anjum, A.D., M.A. Sabbri and Z. Iqbal. Hydropericardum symdrome in broiler chicken in Pakistan. Vet. Rec. 124:247-248. 1989.

3. Cheema, A.H., M. Afzal, and J. Ahmed. Adenovirus infection of poultry in Pakistan. Rev. Sci. Tec. Off. Intern. Epiz. 8:789-795. 1989.

436

4. Chishti, M.A., M. Afzal and A.H. Cheema. Preliminary studies on the development of vaccine for "Hydropericardium syndrome"of Poultry. Rev. Sci. Tech. Off. Intern. Epiz. 8:797-801. 1989.

5. Crowle, A.J. Immunodiffusion. Academic Press NewYork, 1973.

6. Giambrone, J.J., C.S. Edison, R.K. Page, O.J. Fletcher, B.O. Barger, and S.H. Kleven. Effects of infectious bursal disease virus in established lymphoid cell lines and chickens to New Castle disease and Marek's disease vaccination. Avian Dis. 20:534-544, 1976.

7. Gordon, J., P. Biling. Dot immunobinding: General principles and procedures. In: CRC handbok of immunoblotting of proteins. Vol. 1., O.J. Bjerrum, N.H.H. Heegaard, eds. CRC Press, Inc., Boca Raton, Fl. pp. 27-30. 1988.

8. Jaffery, M.S. A treatise on Angara Disease in chicken. Pak. Vet. Med. Assoc. Karachi. pp. 1-33. 1988.

9. MacPherson, I., J. S. McDougall, and A.P. Laursen-Jones. Inclusion body hepatitis in a broiler integration. Vet. Rec., 95:286-289. 1974.

10. Muneer, M.A., M. Ajmal, M. Arshad, M.D. Ahmed, Z.I. Chudhary, and T.M. Khan. Preliminary studies on hydropericardium syndrome in broilers in Pakistan. Zootech. Intern. May, 46-48. 1989.

11. Naeem, K., B. Penigrahy, and M. Hussain. Etiology and biological characterization of the agent of Hydropericardium syndrome in briolers. Am. J. Vet. Res. (submitted).

12. Reed, L.J., and H. Muench. A simple method of estimating fifty percent end points. Am. J. Hyg. 27:493-497. 1938.

437

13. Saifuddin, M., and C.R. Wilks. Effects of fowl adenovirus infection on the immune system of chickens. J. Comp. Path. 107:285-294. 1991.

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Table 1a Experimental design for studying Immunosuppressive effects of Adenovirus isolate PARC-1 in broilers.

Group[a]	Tests performed and prescibed age of the bird			
	Adenovirus infection (15 days)	NDV vaccination (20 days)	NDV HI (24,27,30) 33,36 days)	Adenovirus Ab titre by AGPT (36 days)
1	+	-	-	+
2	-	+	+	-
3[b]	–	–	–	–
4	+	+	+	+

[a] 10 birds per group.
[b] Group-3 is uninfected control

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Table 1b Immune response against hydropericardium syndrome and Newcastle disease virus vaccines in immunosuppression experiment

Group[a]	HPS antibody by AGPT (36d)	NDV HI antibody titres (GMT)[b] 15d	24d	27d	30d	33d	36d
1	+ (80)	20	18	18	16	10	10
2	- (<10)	20	17	35	57	98	160
3	- (<10)	16	12	15	10	10	10
4	+ (80)	20	28	46	35	43	49

[a]10 birds per group.
[b]Reciprocal of the highest dilution of serum that completely neutralized 25 ul of 10 HA of NDV-antigen and expressed as log base 2 titre.

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Table 2 Detection of Adenovirus isolate PARC-1 antigens at varible time intervals in different organs of infected broilers using DIA.

Organs examined	Viral Antigen detected at hours post-infection[a]				
	12h	24h	36h	48h[b]	60h
Liver	+(3/5)	+(4/5)	+(5/5)	+(5/5)	+(5/5)
Spleen	+(2/5)	+(3/5)	+(4/5)	+(5/5)	+(5/5)
Thymus	+(2/5)	+(2/5)	+(3/5)	+(4/5)	-(5/5)
Bursa of Fabricius	+(1/5)	+(3/5)	+(3/5)	+(3/5)	-(5/5)
Caecal tonsils	-(5/5)	-(5/5)	-(5/5)	-(5/5)	-(5/5)

Note: All the organs in negative control birds were negative for adenovirus antigen.

[a]Figures in parentheses indicate number of birds found positive of those tested.

[b]2/5 birds died of HPS between 36-48 hours post-inoculation.

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Table 3. Reproduction of HPS in broilers using various organs from the infected birds.

Organs used	Mortality with HPS from organs collected at different time intervals				
	12 h	25 h	36 h	48 h	60 h
Liver	1/3	2/3	1/3	1/3	0/3
Spleen	1/3	2/3	1/3	2/3	1/3
Thymus	0/3	1/3	2/3	0/3	0/3
Bursa	1/3	1/3	1/3	1/3	1/3
Caecal tonsils	1/3	0/3	1/3	1/3	1/3

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A study on lateral spread of aetiologic agent(s) of hydropericardium syndrome in broiler chickens

Saeed Akhtar
Animal Sciences Institute
National Agricultural Research Centre
Park Road, Islamabad 45500,
PAKISTAN

Note: This article is under consideration for publication in "*Veterinary Record*" an official organ of British Veterinary Association

ABSTRACT

The rate of lateral spread of the agent(s) of hydropericardium syndrome (HPS) was investigated in three controlled experimental trials at stocking densities of 0.83, 1.00, and 0.71 ft^2/bird. Deliberately inoculated birds served as a source of infection in exposed groups of 60, 50, and 70 birds in trials I, II, and III respectively. In each trial, a control group of birds was also reared simultaneously. The nonparametric survival functions (Kaplan-Meier product limit) were fitted to the data and compared by Mental-Cox and Breslow tests.

In trial I, HPS occurred fairly rapidly, with a median latent period of 9.5 days (range 5 to 18 days). In trials II and III, HPS occurrence followed a nearly similar pattern, with a median latent period of 13 days (range 6 to 27 days) and 14.5 days (range 7 to 28 days), respectively.

The median daily mortality rates in trials I, II, and III were 3.4% (range 1.8 to 11.6%), 4.5% (range 2.1 to 10.0%) and 4.5% (range 1.6 to 8.6%) respectively. The percent cumulative mortality was 40, 54, and 55.7% in trials I, II, and III, respectively.

Results on median latent period, median daily mortality, and cumulative mortality from trials I, II, and III did not differ significantly on both Mantel-Cox (statistics = 3.530, p = 0.1712) and Breslow (statistics = 2.647, p = 0.266) tests, indicating nonsignificant differences among the nonparametric survival functions. The implications of the results are discussed.

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INTRODUCTION

Hydropericardium syndrome (HPS) was first observed in the broiler production area near Angara Goth, Karachi, during August 1987 (Anonymous, 1988), and spread rapidly to broiler production units throughout Pakistan within a year (Anjum et al., 1989). The syndrome is typically observed in 3- to 5-week-old growing broiler chickens, and results in up to 60% mortality. It is characterized by the accumulation of clear, straw-colored fluid in the pericardium; a swollen, discolored and friable liver; and pale enlarged kidneys with distended tubules (Anonymous, 1988; Jaffery, 1988; Anjum et al., 1989; Cheema et al., 1989; Afzal and Ahmad, 1990; Ahmad et al., 1989).

The aetiology of the disease is unknown. The disease can be reproduced by the inoculation of susceptible birds with a bacteria-free liver homogenate from an affected bird (Khawaja et al., 1988; Ahmad et al., 1989; Anjum et al., 1989; Cheema et al., 1989; Chishti et al., 1989). An adenovirus has also been detected in liver of infected birds by electron microscopy (Cheema et al., 1989). However, several serotypes of adenovirus have also been isolated from infected livers (Saagy, 1989). Further aetiological studies have indicated that in addition to an adenovirus, some other agent is also involved in causing the disease, but that agent requires co-infection by an adenovirus for the reproduction of typical signs of the syndrome. The nature of this agent remains unknown because no virus or virus-like particle could be seen by electron microscopy (Afzal et al., 1991).

Epidemiological studies suggest that HPS is contagious in nature (Akhtar et al., 1992), and that once it appears in the floc' the syndrome continues to affect susceptible birds (Akhtar and Cheema, 1990). However, information on the rate of lateral spread of the aetiologic agent(s) of HPS is lacking.

The objective of the present study was to investigate the kinetics of lateral spread of the agent(s) of HPS under experimental conditions using a method of survival analysis. This analysis provides a quantitative summary of 'survival', a term applying to individuals that appear to have escaped infection at any time a group is experiencing a spreading infection. Survival analysis also adjusts for those birds that fail to develop the disease by the termination of the experiment.

Information on the rate of lateral spread may be useful for pinpointing the time when the infection might have been introduced into the flock, and may help in tracing the source of infection and formulating a disease prevention programs.

MATERIALS AND METHODS

Chickens

Day-old chickens of the Hubbard strain were procured from a commercial source.[1] The birds were determined to be free of HPS at the start of each experiment. This was done by conducting necropsy of three birds from the lot before the start of the experiment,

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[1]SB Chicks, Rawalpindi

with the absence of gross lesions typical of HPS (Cheema and others, 1989) being considered as freedom from HPS.

Housing and Management

Two pens, with steel walls 4 ft high and open tops, were set up in the poultry rearing house, which had natural ventilation. The floor area of each pen was 50 ft^2 and covered with sawdust. Feed and water were available ad libitum, and light was provided continuously. Brooding and rearing followed standard poultry husbandry procedures. The pens were thoroughly cleaned and disinfected between experiments, following standard methods. Experimental birds were not vaccinated against bacterial or viral diseases.

Inoculum

A 20% suspension of liver from birds that had died of HPS resulting from experimental infection was prepared in phosphate buffer saline in a blender (Cheema et al., 1989; Afzal et al., 1991). The suspension was centrifuged at 2500 g for 30 minutes. The supernatant was filtered with positive pressure through a 0.2 µm filter (Nalgene Company) and used for inoculation of chicks.

Experimental Design

Three trials were carried out over a period of 1 year at three stocking densities and three exposure levels (number of experimentally inoculated birds). In trial I, 5 birds 3 weeks of

age from the exposed group (group A) of 60 birds (stocking density 0.83 ft^2/bird) were inoculated subcutaneously with 1 ml of inoculum. A control group (group B), also of 60 birds, was held in another pen. Trials II and III compared two other stocking densities: in trial II, the exposed and control groups each consisted of 50 birds (stocking density 1.00 ft^2/bird), and in trial III they consisted of 70 birds (stocking density 0.71 ft^2/bird). Exposure to HPS agents was accomplished by subcutaneously inoculating 3 birds in trial II and 7 birds in trial III from the exposed groups. Both exposed and control birds were monitored at regular intervals for HPS development, which was confirmed by necropsy of dead birds. Serological monitoring of experimental birds was not carried out because of the nonavailability of specific antigen and a serological test with high specificity to avoid any misclassification of HPS-negative birds. At the end of the eighth week post-inoculation, all surviving birds were killed and necropsied to determine the presence of lesions of HPS.

Statistical Methods

Birds that survived until the end of the study without hydropericardium and other gross pathological lesions of HPS (Cheema et al., 1989) were assumed to be free from infection with HPS agents. The Kaplan-Meier product limit method (Kaplan and Meier, 1958), a nonparametric method, was used to estimate a survival function S(t), the probability that a bird survives HPS

agent(s) infection longer than time (t). Survival functions from the three trials were compared using the generalized Savage (Mantel-Cox) and generalized Wilcoxon (Breslow) tests (Dixon, 1992).

RESULTS

The results far HPS development and its lateral spread in the three trials, are given in Table 1. In all three trials HPS occurred in inoculated birds and subsequently spread to uninoculated birds in the exposed group. The rate of spread of HPS agent(s) varied slightly, but followed a common pattern. The birds in the control groups of all three trials remained HPS-free.

In trial I, HPS occurred fairly rapidly, with a median latent period of 9.5 days (range 5-18 days). In trials II and III, HPS occurrence followed nearly a similar pattern, with median latent periods of 13 days (range 6-27 days) in trial II and 14.5 days (range 7-28 days), in trial III.

The median daily mortality rates in trial Is, II, and III were 3.4% (range 1.8 to 11.6%), 4.5% (range 2.1 to 10.0%) and 4.5% (range 1.6 to 8.6%), respectively. The percent cumulative mortality was 40.0, 54.0, and 55.7% in trials I, II, and III, respectively.

Median latent period, median daily mortality rates and percent cumulative mortality in trials I, II, and III, conducted at three different stocking densities (0.83, 1.00, and 0.71 ft^2/bird), did not differ significantly according to both Mantel-Cox (statistics = 3.530, p = 0.1712) and Breslow (statistics = 2.647, p = 0.266)

tests, indicating nonsignificant differences among the nonparametric survival functions (Figure 1).

DISCUSSION

In the absence of knowledge about the specific aetiology of HPS, information on the latent period of its infectious agent(s) and its rate of lateral spread may be useful for pinpointing when an infection might have been introduced into a flock. Such information may be used to trace the source of infection and formulate preventive measures.

In this study survival data analysis facilitated comparison of incubation periods, daily mortality rates, and cumulative mortalities from the three trials to investigate the lateral spread of HPS agent(s).

The daily mortality rate did not differ significantly among the three stocking densities and exposure levels investigated. This was probably because of the narrow range of stocking densities encompassed by the three trials. Furthermore, higher rates of lateral spread and HPS-related mortality may require additional stress factors, such as accumulation of ammonia and carbon dioxide, as has been documented previously (Akhtar et al., 1992). There are no published data on the daily spread rate of HPS agent(s) under controlled conditions to compare with the results of the present study. Cumulative mortality ranged from 40.0 to 55.7% in this study. In the field, reported mortality ranged from 60 to 70% (Anjum et al., 1989; Ahmad et al., 1989). Some of the risk factors

operating in the field, such as use of kerosene oil as a source of light/heat (Akhtar et al., 1992), were controlled in the present study, which may have accounted for the lower mortality rates found.

In the three trials, the median incubation period ranged from 9.5 to 14.5 days, suggesting that in the event of an epidemic of HPS within a flock, any management error prior to the epidemic within that period must be investigated to trace the source of the infection.

This paper represents the first report on the kinetics of rate of lateral spread of HPS agent(s). Further studies are needed on a wider range of stocking densities. Studies on the natural routes and dynamics of transmission are needed as well.

Potential Benefits to the Poultry Industry of Pakistan

The specific aetiology of HPS is still uncertain. However, it has been established that the causative agent(s) of HPS are viral and contagious in nature. The present study investigated the median length of incubation and rate of lateral spread of potential viral agent(s) of HPS. In the event of an HPS epidemic, knowledge about the incubation period of causative agent(s) will help in determining the errors in flock management that might have introduced the infection into the flock. Subsequently, this information may be used to formulate disease prevention measures.

REFERENCES

AFZAL, M. and AHMAD, I. (1990). *Veterinary Record* 126, 59.

AFZAL, M., MUNEER, R. and STEIN, G. (1991). *Veterinary Record* 128, 591.

AHMAD, I., AFZAL, M., MALIK, M.I., HUSSAIN, Z and HANIF, W. (1989). *Pakistan Journal of Agricultural Research* 10, 195.

AKHTAR, S. and CHEEMA, A. H. (1990). *Proceedings of 3rd International Veterinary Congress*. November 28-29., 229.

AKHTAR, S., ZAHID, S. and KHAN, M. I. (1992). *Veterinary Record* 131, 481.

ANJUM, A.D., SABRI, M.A. and IQBAL, Z. (1989). *Veterinary Record* 124, 247.

ANONYMOUS, (1988). Synopsis of the proceedings of the 1st National seminar on Hydropericardium-Pulmonary Oedema-Cum-Hepatonephritis Syndrome, Karachi, Pakistan Veterinary Medical Association publication.

CHEEMA, A.H., AHMAD, J. and AFZAL, M. (1989). *Revue Scientifique et Technique del l' Office International des Epizooties* 8, 789.

CHISHTI, M.A., AFZAL, M. AND CHEEMA, A. H. (1989). *Revue Scientifique et Technique del l' Office International des Epizooties* 8, 797.

DIXON, W. J. (1992). *BMDP Statistical Software*. Los Angeles, University of California Press. 795.

JAFFERY, M. S. (1988). A Treatise on Angara disease (Hydropericardium-Pulmonary Oedema-Hepatonephritis Syndrome). Karachi, Pakistan Veterinary Medical Association.

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KAPLAN, E. L. AND MEIER, P. (1958). *Journal of American Statistical Association* 53, 457.

KHAWAJA, D. A., AHMAD, S., RAUF, A. M., ZULFIQAR, M., MAHMOOD, S. M. I. AND HASSAN, M. (1988). *Pakistan Journal of Veterinary Research* 1, 2.

QURESHI, A. A. (1989). *Poultry International* 28, 44.

SAAGY, E. I. (1989). Broiler chicks disease outbreak. Personnel communication.

Table 1. Some descriptive statistics on HPS related mortality in broiler chickens in three controlled experimental trials, 1992-93.

Day[a] of HPS related Mortality	No. Died/ No. Exposed	Mortality Rate/day (%)	Cumulative total HPS related mortality (%)
Trial I[b]			
5[e]	0	0.0	0 (0.0)
3	2 (seeder)	0.0	2 (3.3)
4	3 (seeder)	0.0	5 (8.3)
5	1/55	1.8	6 (10.3)
6	2/54	3.7	8 (13.3)
7	1/52	1.9	9 (15.0)
8	3/51	5.9	12 (20.0)
9	1/48	2.1	13 (21.7)
10	2/47	4.3	15 (25.0)
11	5/45	11.1	20 (33.3)
12	1/40	2.5	21 (35.0)
13	2/39	5.1	23 (38.3)
18	1/37	2.7	24 (40.0)
Trial II[c]			
3[e]	0.0	0.0	0 (0.0)
2	2 (seeder)	0.0	0 (4.0)
3	1 (seeder)	0.0	0 (6.0)
6	1/47	2.1	4 (8.0)
8	2/46	4.3	6 (12.0)
9	4/44	9.1	10 (20.0)
10	4/40	10.0	14 (28.0)
11	2/36	5.6	16 (32.0)
12	1/34	2.9	17 (34.0)
13	2/33	6.1	19 (38.0)
14	3/31	9.7	22 (44.0)
15	1/28	3.6	23 (46.0)
16	1/27	3.7	24 (48.0)
17	1/26	3.8	25 (50.0)
27	2/25	8.0	27 (54.0)
Trial III[d]			
7[e]	0.0	0.0	0 (0.0)
2	2 (seeder)	0.0	0 (2.9)
3	4 (seeder)	0.0	0 (5.7)
5	3 (1 seeder)	3.2	9 (12.9)
7	1/62	1.6	10 (14.3)
8	3/61	4.9	13 (18.6)

9	5/58	8.6	18 (25.7)
10	2/53	3.8	20 (28.6)
11	3/51	5.9	23 (32.9)
13	2/48	4.2	25 (35.7)
14	3/46	6.5	28 (40.0)
15	2/43	4.7	30 (42.9)
16	1/41	2.4	31 (44.3)
17	2/40	5.0	33 (47.1)
18	1/38	2.6	34 (48.6)
20	1/37	2.7	35 (50.0)
23	1/36	2.8	36 (51.4)
27	2/35	5.7	38 (54.3)
28	1/33	3.0	39 (55.7)

[a] Day of hydropericardium syndrome-related mortality.
[b] Stocking density = 0.83 ft^2/bird; [c] Stocking density = 1.00 ft^2/bird; [d] Stocking density = 0.71 ft^2/bird,
[e] Number of birds (seeders) inoculated deliberately with infectious inoculum (1 ml/subcutaneously).

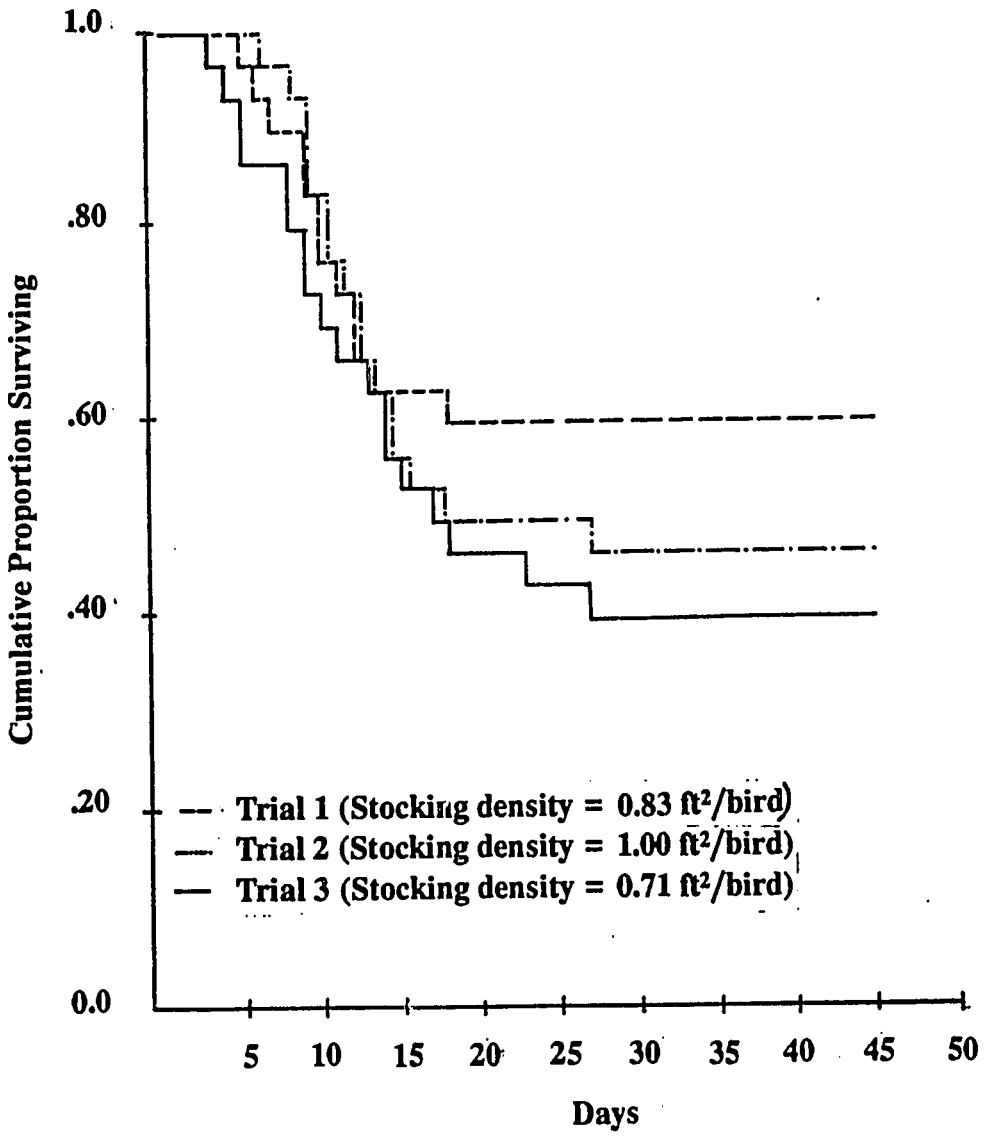

Figure 1. Survival functions (product limit) for chickens exposed to agents of hydropericardium syndrome at three stocking densities.

INTEGRATED PEST MANAGEMENT

GROUP AUTHOR:

Dr. David Pimentel
Department of Entomology
University of Massachusetts
Amherst, Massachusetts 01003

CHAPTER TITLES:

Insect and Pest Management in Corn in North America: 1. Natural Enemies of Insect Pests, A. A. Pimentel and A. P. Kelley

Pest Management in the Soybean Ecosystem: 1. Insect Pests, Richard D. Cooper and Robert Lynn, A. A. Pimentel and A. P. Kelley

Pest Management in Cotton in Mexico and Texas as Practiced Since 1940, Alan Gomez-Cendra and Jorge Vandermeer

Pest Management in the Use of Chemical Pesticides, Richard Wolfenbarger and Paul Bishop, A. A. Pimentel and A. P. Kelley

Economics of Pest Management, A. A. Pimentel and A. P. Kelley

Pest Management in Agroecosystems: Ecological Considerations, Miguel A. Altieri and Peter Gliessman, A. A. Pimentel and A. P. Kelley

INTEGRATED PEST MANAGEMENT IN COTTON IN SINDH, PAKISTAN: I NATURAL ENEMIES OF INSECT PESTS.

By

AHMED ALI BALOCH
and
ALI MOHAMMAD KALROO
Cotton Research Institute, Sakrand,
District Nawabshah, Sindh, Pakistan.

ABSTRACT

The major pests of the cotton crop in Sindh, Pakistan include cotton jassid, whitefly, thrips and several species of bollworm. In the present work, more than 20 predators and 15 parasites that attack these pests were identified and their effects analysed. Among many discoveries were the following. The natural enemies are active against the cotton pests mainly during August and September. They may parasitize up to 34% of the whiteflies, and when the level tops 15% the crop need not be sprayed. When field populations of predators and parasites exceed 24,700/ha the bollworm damage is suppressed. The maximum number of natural enemies -- 116,090/ha -- was recorded during the first week of August. In the off-season, when no cotton plants were available, most of the natural enemies were found residing on wheat, maize, barseem (Trifolium alexandrinum), brassicas (Brassica rapa) and akk (Calotropis procera). The broad-spectrum pesticides now recommended for cotton reduce the population of natural enemies by almost 25%. Thus the use of such pesticides should be avoided, especially when the crop is between 50 and 90 days old.

By utilizing this knowledge, in a well integrated IPM Program, farmers can easily save 2-3 pesticide applications. The cotton crop in the fields studied herein was sprayed only three times, and yet gave maximum return.

KEY WORDS: Pakistan, integrated pest management, cotton, whitefly, bollworm,

INTRODUCTION

Pakistan can truly be called the land of cotton. Cotton fabrics as old as 5000 years have been found in the Indus Valley. Today, the crop is the mainstay of the country's economy. It creates about 65% of the foreign exchange earnings; produces more than 55% of the domestic edible oil; supplies raw material to about 270 textile mills, 1035 ginning factories and 5000 oil expellers; and provides jobs for millions. It s significance to the economy may be gauged by the fact that cotton alone accounts for about 10-12% of the farm area and the recent crop failure, which resulted in a loss of 39% of the expected cotton, caused a loss to the exchequer of between 2 and 4 billion rupees (equivalent to between about 500 million and 1 billion U.S. dollars).

For all the crop's importance, cotton yields in Pakistan are far below those in the advanced cotton-growing countries. Whatever improvements are being achieved, are both modest and out of step with the country's needs.

Pests are a major cause of the low productivity. Cotton is a delicate crop and it is the only one to be attacked by different pests at each of its different stages of growth. Halimie (1980) reported that up to 56% of the potential crop is lost to insect pests. Latif (1984) reported that insect pests in cotton cause Pakistan an annual loss of more than Rs. 400 crores (i.e. 400 million rupees or roughly 160 million dollars).

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Since no single method has proved universally effective against pests in Pakistan's cotton fields, an integrated pest management approach has been recently advocated. This broad, ecologically based approach relies on a variety of compatible techniques for modifying the producing system so as to suppress the major pests to the point where the crop losses never reach disastrous levels. Knowledge of the major pests' lifecycles and relationships forms the basis of the various combinations employed. The objective is to reap the maximum return at the minimum cost, and to do so without unduly disrupting the cotton agro-ecosystem.

Insect pests

In Pakistan, about 150 species of insects and mites attack the cotton crop. Only a few have been found to be notably destructive, but these alone cause losses of between 30 and 40% almost every year (Huque, 1972).

Cotton Jassid	Amrasca devastans (Dist.)
Whitefly	Bemisia tabaci (Genn.)
Thrips	Thrips tabaci Lind. (Schirtothrips dorsalis)
Pink bollworm	Pectinophora gossypiella (Saund)
Spotted bollworm	Earias Vitella (F.)
Spiny bollworm	Earias insulana (Boised)
American bollworm	Helichoverpa (Heliothis) armigera (Hb)
Armyworm	Spodoptera litura (F.)
Cotton leafroller	Sylepta derogata (F.)
Spider mites	Tetranychus sp.
Aphid	Aphis gossypii Glov.
Termite	Microtermes obesi Hlmgr.
Field cricket	Gryllus bimaculatus Deg.

Economic Threshold

The integrated pest management system revolves around the concept of coexisting with pests, just so long as their numbers do not get out of hand and cause unacceptable economic loss. The particular level at which the losses are deemed unacceptable -- the so-called "economic threshold" -- has been developed for the above cotton pests in Pakistan (Baloch et al., 1990). These figures were followed during the present studies and are given below.

Cotton jassid	1-2 nymphs or adults per leaf
Whitefly	4-5 nymphs or adults per leaf
Thrips	8-10 per leaf
Mites	10-15 per square inch (1.5 - 2.3/sq cm.)
Bollworms	Cotton up to 75 days: 15% populated cotton between 75 and 90 days: 10% populated cotton after age 90 days: 5% populated.

Natural Enemies of Pests

The Commonwealth Institute of Biological Control in Rawalpindi has identified 17 species of insect predators and 22 species of insect parasites found in the cotton agro-ecosystem of Pakistan. From the foothills of Islamabad as well as from the Rawalpindi Plains, Iqbal and Mohyuddin (1990) recorded 7 species of parasitoids of the American bollworm (Heliothis armigera).

Attique (1988) reported that in Pakistan about 100 natural enemies are associated with different cotton pests. Of these, the majority were recorded from bollworms. The main predators were Chrysopa sp., Orius sp., Geocoris sp., spiders and coccinellids. The main parasites were Apanteles sp., Elasmas sp. and Bracon sp.

MATERIALS AND METHODS

The field studies were carried out during the 1991/1992 and 1992/1993 cotton-growing seasons at the Cotton Research Institute in Sakrand, Sindh. The pest and predator observations were recorded from 18 locations, each with an area of about 2 ha (5 acres).

Upland cotton (G.hirsutum) is the commercial species of Pakistan, and occupies approximately 96% of the cotton-growing area. A variety named CRIS-9 was employed in all the experiments. The government released this high-yield variety for commercial cultivation in 1992. The plant reached its maximum height of 123 cms during the fourth week of September. The maximum number of fruiting parts (immature and mature) were recorded during that same week as well as during the third week of October: 912,245/ha and 880,975/ha, respectively.

At the research site in Sakrand, the maximum weekly average temperature (22-44 °C) were recorded in May and June and the minimum of 9°C in January. The average relative humidity (measured at 0800 hours), ranged from about 60% in April to 76% in August. The maximum rainfall was 89 mm, during the fourth week of July. The longest day in the season was recorded in the third week of June (14 hours 17 minutes) and the shortest in early January (10 hours 10 minutes).

The population of sucking pests (i.e. jassid, whitefly and thrips) was generally recorded from 20 leaves randomly selected alternately from the top, middle and bottom of the plant. The populations of the various bollworms as well as those of the pests' natural enemies, were determined using a stick (1.3 m long) that was thrown down four times at random.

Stick method sample unit consisted of four sampling points selected at random, each one comprising a segment of a row which is 52.25 inches long (1.32 meters) totalling 209 inches (5.28 meters). For all four points, this is equivalent to 1/1000 of an acre, (1/2470 of hectare). On every sample point, the number of plants, the number of natural enemies, the number of immature and mature fruiting parts, as well as the number of damaged parts were all recorded. The calculations were made on the basis of the sum of four such sampling points.

The parasites were harder to measure. In the case of those affecting whitefly, cotton leaves with large numbers of whitefly immatures were brought to the laboratory and cut into pieces of approximately 1 cm^2. Whitefly nymphs were then counted and placed in glass vials closed with cotton. After 10 to 15 days, the number of emerged whiteflies were counted. The parasitization percentage was calculated from the total number of emerged parasites that were visible on the emerged whiteflies.

RESULTS AND DISCUSSION

The main idea behind the experiments was to provide full opportunity for the natural enemies of cotton pests to develop their populations and maintain a balance in the pest populations. Among many things discovered during the work were the following.

Pest populations

The jassids, whiteflies and thrips started appearing during the fourth week of June, with infestations of 0.27, 0.50 and 0.63 specimens/leaf, respectively (see Figure 1). They reached their maximum populations of 1.86, 1.97 and 8.25 specimens/leaf during the third week of July, the fourth week of August and the second week of September. Similarly, bollworm started to appear from the first week of August with a damage level of 2.5% on immature fruiting parts. They reached a peak during, the second and third weeks of September with damage levels of 12% and 11.3% (on both immature and mature fruiting parts), respectively.

Natural Enemies

The cotton agro-ecosystem in Sindh contains a surprisingly varied and complex predator and parasite fauna. The following natural predators of cotton pests were recorded.

Camytomma sp. nr. Nicolasi (Reuter)
Zanchius breviceps Wagner
Orius albidipennis (Reuter)
Paederus fuscipes Curtis.
Laius melleifer Champion
Anthicus sp.
Brumus suturalis (F.)
Scymnus nubilus Mulsanet
Chrysopa carnia (Steph.)
Polistes sp.
Menochilus Sexmaculata F.
Coccinella Septempunctata L.
Screontindes sp.
Coccinella 11 Panetata L.
Geocoris sp.
Coranus spiniscutis Reuter
Rhynocoris fuscipes (F.)
Geocoris tricolor (F.)
Formicomus caeruleipennis (Laf.)
Catana parcesetosa (Sic.)
Leis dimidiata F.
Spiders (unidentified).

The following indigenous parasites of the cotton pests were recorded.

Apanteles anagaleti Mues
Bracon gelechiae Ashmead
Bracon greeni Ashmead
Camptothlipisis gossypiella Gupta & Bhat
Camptothlipisis dravida Gupta and Bhat
Microchelonus Sp.
Scambus Simulator Gupta and Tikor
Goniozus sp.
Brachymeria tachasdiae Can.
Eretmocerus sp.
Eretmocerus mundus Mercet
Eretmocerus corni Haldeman
Encarsia Lutae (Masi)
Encarsia sp. nr. tricolor Foerster
Prospaltella sp.

The natural enemies started appearing during the fourth week of June, with a population of 2470/ha. They reached their maximum population of 43,645/ha during the third week of September.

The indigenous enemies of the cotton pests started appearing in the very early stage of the crop and continued up to the time of harvest (see Table 2). Their active period began in August and continued until September. This period is especially critical with respect to suppressing bollworm damage.

Parasitization of whitefly

Whiteflies were present in all the cotton fields surveyed, but the level to which they were parasitized varied from locality to locality. The actual results are summarized in Table 1. The highest average seasonal level of parasitization was 27.7%, recorded in Sanghar District. However, the highest actual individual level (34.0%) was recorded in Khairpur District.

Reduced cultural practices

Details of experiments on cultural practices will be given in future papers. However, here a preliminary account of the effects of cultural practices on the natural enemies is in order. The idea behind the experiment was to learn 1) the effect of low inputs on the development of cotton pests and 2) the effects on the pests' natural enemies and 3) ultimate effect on yield. The no-input fields in the test were given no weeding, fertilizer or pesticides.

The results are presented in Table 3. The natural enemies played a dominant role at one site T2, (The population of natural enemies were enhanced with the addition of natural enemies in the treatment area where they completely suppressed the pest population. The maximum cotton yield of 3285 kg/ha was obtained from this site.

At another site T3, (In puts were increased i.e. some fertilizer and pesticidal application) there was evidence that increasing the levels of inputs may actually help the cotton pests to develop damaging population levels.

Overall, there was no significant difference between no inputs and low inputs, a result that is quite encouraging. The difference of yield between the sites T2 and T3 was only of 50 kgs/acre, which is inconsequential.

Availability of predators

The observations were recorded from only one site and the results are presented in Table 4. The most important predators of cotton pests at Sakrand Farm was Orius sp. Spiders were the second most important.

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The maximum number of predators (116,090/ha) were observed during the first week of August.

Alternative Host Plants

During the season following the cotton harvest, a search was made for the cotton pests' natural enemies. The results are presented in Table 5. Many natural enemies of cotton pests were record in barseem (Trifolium alexandrinum), wheat, maize, brassica (Brassica rapa) and akk (Calotropis procera) plants.

CONCLUSION

Any integrated pest management programme requires a thorough study of each pest as well as of its predators and parasites. It calls for a degree of knowledge and biological understanding and operational planning far superior to that required in straight chemical applications. At present, the maximum use of natural enemies should be made by delaying the chemical operations till such time as their use becomes absolutely necessary. Alternatively, selective insecticides could be used. (Those from neem are an example because they affect insects that chew on the sprayed plants but not those that attack the pests.). In the future, there could also be rearing facilities that produce predators and parasities that can be added to the fields so as to augment the populations that grow there naturally.

Looking at the pest population trend and the general fructification pattern of cotton in Sindh, it appears that the most critical period of crop setting and crop protection is approximately 60 days from mid-July to mid-

September. The most profitable minimum number of pesticide treatments would be two: one around 10th July and the other at the end of August or early September. The exact dates will depend on the previous weather that year as well as on other seasonal factors influelncing the pest populations.

The high temperatures in May, June and part of July not only affected the fruiting pattern of the cotton plant but also the insects associated with it. The main cotton pests become important only after the return of moderate temperatures around mid July. The rainfall, in combination with the relative humidity, was found to play an important role on the population dynamics of jassid, whitefly, spotted and pink bollworms. The photoperiod (i.e. length of day) was the most important factor including diapause in pink bollworm.

The predators proved more important pest-suppressors than did the parasites. This was mainly because they are more active in the early part of the cotton season. Parasites multiply by growing on living organisms (notably bollworm larvae) and therefore take time to build up their populations. Most appear quite late in the season, at a time when the crop has already matured. The results indicate that when the population of natural enemies exceeds 24,700/ha the level of bollworm damage starts to be suppressed.

Similarly, the natural enemies play a vital role in controlling the sucking-pest complex, especially whiteflies. During the studies it was observed that when the parasitization level is above 15% the crop need not be sprayed. Thus, the indigenous parasites reached about twice the

levels needed for control. (In one case the parasite infestation was close to 28% and in another it was 34%). These results are quite encouraging and have international implications because whitefly is breaking out in many nations and is becoming a major concern worldwide.

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REFERENCES

Attique, M.R. 1988. Non-toxic Methods of cotton pest control. PAPA Bulletion September, 1988, 29-32.

Baloch, A.A., B.A. Soomro, M.A. Leghari and M.W. Sanjrani, 1990. Studies on economic injury levels of insect pests of cotton. Turk, entomol derg, 14(3); 131-148.

Halimie, M.A. 1980. Determination of need for pest control in cotton. Pakistan Agriculture 2 (8-9): 41-43.

Huque, H. 1972. Cotton Entomology. In: Cotton in Pakistan, Published by PCCC Karachi, PP. 183-238.

Iqbal, N. and A.I. Mohyuddin, 1990. Eco-Biology of Heliothis sp. in Pakistan. Pakistan J. Agric. Res. Vol, 11, No. 4: 257-266.

Latif, A. 1984. Need of Plant Protection. Pakistan Agriculture VI (10): 29-32.

Table 1: Parasitization of whitefly.

	Parasitization levels		
District	Average	Range	Month of max.
Hyderabad	23.0%	17.8 - 32.7%	August
Tharparkar	22.0%	9.9 - 33.3%	September
Sanghar	27.7%	21.6 - 31.2%	June
Nawabshah	18.2%	0.8 - 31.6%	July
Khakpur	16.5%	9.1 - 34.0%	July
Sukkur	12.2%	6.2 - 19.3	September

These figures were measured during the 1991-92 and 1992-93 cotton season (May-November).

BEST AVAILABLE DOCUMENT

Table- 2: Population trend of cotton pests and their natural enemies under natural conditions (without spray).

Date of observations	No. of natural enemies '000'/acre	Average population per leaf of: thrips	jassid	W.fly	Bollworms damage %	Live larvae %
3rd week of June	4	0.93	0	0.17	0	0
4th " " "	3	1.51	0	0.47	0	0
1st " " July	2	3.96	0	1.33	0	0
2nd " " "	3	6.81	0.31	0.49	0.81	0
3rd " " "	4	11.25	0.48	0.23	1.09	0
4th " " "	3	20.56	1.07	0.93	0	0
1st " " Aug.	4	16.02	1.29	0	1.44	0.29
2nd " " Aug.	5	11.84	1.64	0	1.88	0.63
3rd " " Aug.	7	4.11	2.12	0.19	2.01	0.81
4th " " Aug.	10	1.58	3.92	0.33	2.12	0.92
1st " " Sept.	12	1.32	1.12	0.68	7.81	2.83
2nd " " "	22	0.67	0.77	0.96	10.74	3.39
3rd " " "	27	0.31	0.51	0.61	8.01	1.74
4th " " "	13	-	0.33	0.21	5.70	0.62
1st " " Oct.	4	-	0.11	0.09	6.39	0.92
2nd " " "	3	-	-	-	9.26	2.62
3rd " " "	3	-	-	-	7.10	0.78
4th " " "	2	-	-	-	7.18	0.41
Total =	131	80.87	13.67	6.69	71.6	15.96
Average=	7.28	6.22	1.14	0.51	5.11	1.33
Yield kgs/acre=	1258 kgs.					

Table-3: Effect of low inputs on the development of cotton pests and their natural enemies.

Date of observations	POPULATION LEVEL IN T1						T2						T3					
	No. of natural enemies 1000/ac	Per leaf population of Thrip	Jassid	W.fly	Boll-worm damage %age	Earias live larvae %age	No. of natural enemies 1000/ac	Per leaf population of Thrip	Jassid	W.fly	Boll-worm damage %age	Earias live larvae %age	No. of natural enemies 1000/ac	Per leaf population of Thrip	Jassid	W.Fly	Boll-worm damage %age	Earias live larvae %age
7-7-92	3	8.6	-	-	-	-	2+3	7.9	0.22	0.50	-	-	2	7.80	0.30	0.40	-	-
23-7-92	5	11.64	0.36	0.44	-	-	4+6	8.6	0.30	0.40	-	-	6	12.00	0.80	0.90	-	-
9-8-92	15	2.52	2.12	0.08	-	-	17+15	2.0	0.15	0.22	-	-	13	8.90	1.20	1.30	-	-
27-8-92	23	1.44	2.08	0.36	5.79	1.84	34+25	0	0	0	3.91	1.00	23	5.30	0.50	0.90	4.45	0.80
14-9-92	36	0.20	0.64	0.76	9.26	3.20	45+33	0	0	0	5.30	1.40	30	3.90	0	1.20	8.10	1.20
23-9-92	19	-	-	-	8.12	2.62	32+20	0	0	0	6.90	1.80	22	2.20	0.40	1.50	8.75	1.40
27-9-92	8	-	-	-	3.11	0.54	21+15	0	0	0	7.31	2.00	13	1.40	0.90	2.00	7.30	1.00
1-10-92	5	-	0.33	0.60	5.12	0.80	11+10	0	0.22	0.12	7.40	1.90	9	0.85	0.65	1.90	7.90	1.60
6-10-92	5	-	-	-	10.85	2.67	7+8	0	0.10	0	8.00	1.60	7	0.95	0.80	2.50	9.10	2.0
13-10-92	4	-	-	-	9.98	0.87	3+5	0	0	0	8.10	0.95	6	1.20	1.00	2.90	10.30	1.90
Total:	128	24.4	5.53	2.24	52.23	12.54	316	18.5	0.99	1.24	46.92	10.65	131	44.5	6.55	15.5	55.9	9.9
Average:	12.8	4.88	1.11	0.45	7.46	1.79	31.60	6.17	0.20	0.31	6.70	1.52	13.1	4.45	0.72	1.55	7.98	1.41
Yield kgs/acre			1240						1330						1280			

BEST AVAILABLE DOCUMENT

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Table 4 : Availability of natural enemies (predators) of cotton pests on cotton crop.

Month/ Weeks	Population of different natural enemies ('000'/acre)				
	Beetles	Spider	*Orius* sp.	*Chrysopa* sp.	Total
JUNE - 1993					
4th week	0	3	0	0	3
JULY - 1993					
1st week	1	3	1	0	5
2nd "	1	4	4	0	9
3rd "	4	7	8	1	20
4th "	6	9	29	2	46
Average:	3.00	5.75	10.50	0.75	20.00
AUG. 1993					
1st week	8	10	24	5	47
2nd "	6	7	19	6	38
3rd "	5	7	14	7	33
4th "	3	4	11	9	27
Average:	5.50	7.00	17.00	6.75	36.25
SEPT. 1993					
1st week	5	2	18	7	32
2nd "	1	0	12	3	16
3rd "	2	1	12	3	15
4th "	1	1	6	0	8
Average:	2.25	1.00	12.00	2.50	17.75
OCT. 1993					
1st week	2	2	14	0	18
2nd "	5	2	11	0	18
3rd "	5	3	16	0	24
Average:	4.00	2.33	13.67	0	20.00
G.Average:	3.44	4.06	12.44	2.50	22.44

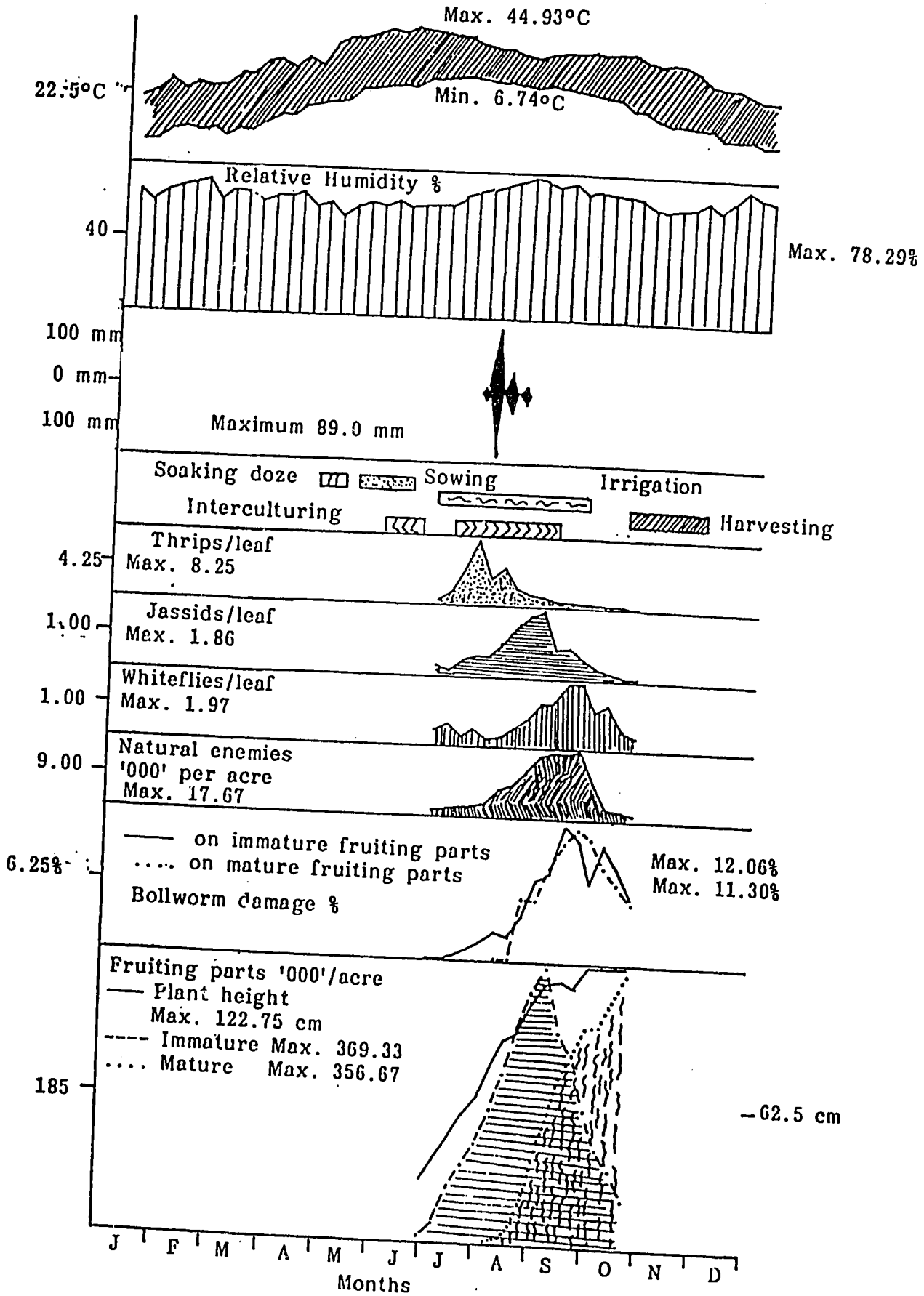

Figure-1: Agro-Eco-System in Sindh, Pakistan.

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DEVELOPMENT OF INTEGRATED PEST MANAGEMENT IN COTTON IN SINDH, PAKISTAN. II- CONTROL BY MANAGEMENT.

AHMED ALI BALOCH AND ALI MOHAMMED KALROO

Cotton Research Institute Sakrand, District Nawabshah Sindh, Pakistan.

ABSTRACT

Studies on Integrated Pest Management in cotton crop with special reference to control by Management in Sindh, Pakistan were carried out. The results are summarised as under:

The cotton agro- eco- system and its components were discussed in details. The results indicated that hairy varieties were resistant to jassid but some what susceptible to Thrips and White fly. Where as nectariless, glabrous and high gossypal content varieties were resistant boll worm complex. varieties CRIS- 9, NAIB- 78 and CIM- 135 could be used in IPM programme. The optimum sowing date for CRIS- 9 in lower to central sindh is from 25th April to 15 May, in upper sindh it may be from 1st of May to 25th of May. The sowing period should be limited upto three weeks. Excessive irrigation and stress during boll formation may be avoided. First irrigation should be delayed as much as possible and the subsequent irrigation should be given with two weeks interval. Excessive fertilizer increase vegetative growth and encourage insect pest to develop. The alternate host plants particularly okra and other Malvaceous plant should be eradicated. The left over bolls should be destroyed by grazing the crop and the ginning waste should also be burned out.

KEYWORDS: Pakistan, integrated pest management, cotton, white fly, thrips, jassid, boll worms.

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INTRODUCTION

Pakistan is basically an agricultural country. The significance of cotton crop is our economy may be gauged from the fact that cotton alone accounts for about 10-12% of cropped area. However the yields are far below than those reported in advanced cotton growing countries. The improvements so far have been limited and remain short of the developing needs of the country's economy.

A Large number of pest species host on cotton and inflict economic losses. It will suffice to recall the recent failure (1993-94) of cotton which resulted in a loss of 39% of our crop and the exchequer suffered a loss of rupees 2 to 4 Billion (0.07 to 0.14 Billion Dollars). Plant protection plays a vital role in the successful production of cotton crop and saves it from the damage of the pests. Since no single method has proved to be effective against all types of pests, an integrated control approach has been advocated.

Knowledge of the pest life cycle and its relationship to plant growth forms a basis for making appropriate changes in the producing system. Thus the objectives of these field studies was to develop an IPM system which aims at an integrated control of major cotton pests under stressed conditions inodrer to get the maximum return at a mimimum cost with mimimal disturbance to the cotton - agro - system.

MATERIALS AND METHODS

The field studies were carried out during the period 1992-93 and 1993-94 cotton season adjacent to cotton. Different experiments were designed to generate the information relevant to the management techniques i.e. the effect of fertilizer, sowing dates, irrigation, plant resistant varieties etc. The details of the experiments are briefly given as under:

In the fertilizer trial, the experiment was designed in randomized complete block design (RCBD) where CRIS- 9 was sown during first fortnight of May. Fertilizers i.e. urea and Amonium Nitrate at the rate of 150, 100, 50 and 0 kgs / ha. were applied individually and also in combination. One third of the doze was applied one day before sowing and remaining 2/3 rd of the doze after about 70 days of planting. Generally the crop was sprayed three times with Systoate 40 Ec.@ 741

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ml / ha., Nuvacron 40 Ec. @ 1235 ml / ha. and Baythroid T.M.525 Ec. @1235 ml / ha. after about 70, 90 and 115 days of planting. The plot size was 22.5 * 12 Feet / treatment.

In the sowing dates trials, the variety CRIS -9 was sown on 10 th April, 25th April and 15th May in RCBD , where the plot size was 22.5 * 12 feet / treatment. Urea at the rate of 123.5 kgs / ha. was given each at the time of first and third irrigation. The crop was sprayed three times with Systoate, Nuvacron and Baythroid T.M. @ 741 and 1235 ml / ha. after 70, 90 and 115 days of planting respectively.

In the irrigation trial three varieties i.e. CRIS- 9, CRIS- 18 and CRIS-21 were used, where the plot size was 100 * 45 Feet / treatment. The experiment was designed in RCBD. The crop was sown during 1st week of May and all the irrigation were given with an interval of two and three weeks. The first observations on pest population was recorded 28 days after sowing and the consequent observations were recorded on weekly interval. The observation were continued upto 3rd week of october.

In host plant resistant varieties experiment some varieties / strains of known genetic markers were tested to see their level of resistance towards different insect pests. All the other required inputs were given as per the official recommendations.

Similarly the population dynamics studies observations were recorded from an area of five acres from each location. In all there were 18 locations.

In all experiments the population of sucking pests i.e. Thrips, Jassid and White fly were recorded using 20 randomly selected leaves alternately from top, middle and bottom foliage of the plant. For boll worm complex, stick method was followed where an stick of 52.25" was used four times at random. on every sample point number of plants, immature and mature fruiting parts and damaged onces were recorded. Statistically the sum of four sampling points i.e. 52.25" becomes 209" which is equal to 1/1000 of an acre, on these basis calculations were made.

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RESULTS AND DISCUSSION

Cotton is very delicate crop and affected by a number of factors but the dominating one is attack of insect pests so to get maximum benefit / return from it, the crop should be seen in its complete eco system. Similarly the practical application of integrated pest control is based upon comprehensive identification of the major factors which comprise the agricultural eco system, forces and counter forces, natural or artificial, physical or biotic, occuring in the environment creat conditions which influence the population dynamics of pests and beneficial organisms. usually those factors are few in number and are called key regulating factors. The management in cotton in sindh, discussed in this article are as under:

COTTON PLANT

In Pakistan, the up land cotton (G. hirsutum) is the commercial cotton and occupies approximately 96% of the area and on the remaining 4% G. arboreum is raised for domestic needs of the farmers. Almost all the plant protection activities are focused on upland cotton.

Cotton Research Institute, Sakrand had developed a high yield potential variety named CRIS- 9, which was released in 1992 by the Government for commercial cultivation. During the course of study in all the experiment CRIS-9 was used. It's development data is given as under:

Growth and Developmental data for upland Cotton Variety CRIS- 9

- The recommended sowing dates are First to 30th May.
- Plant emergence period, average 5days.
- The cede true leaves appear 12 days after emergence.
- The 4th leaf appears 22 days after cede true leaf.
- Time from emergence to square formation average 40 days.

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- Time fromsquare to white bloom average 15 days.

- Time from bloom to open boll average 55 days, which vary for shorter days, low temperatures and other factors.

- Squares formed 35 to 40 days make the flowers 55 to 60 days which conribuate most to yield and quality.

- Boll reach full size in 90 to 95 days after bloom.

- Approximately 65% of blooms make bolls.

INSECT PESTS

In Pakistan, about 150 species of insects and mites have been attacking cotton crop. But the most destructive / major pests are as under:

1. Cotton Jassid	: Amrasca devastans (Dist.)
2. White fly	: Bemicia tabaci (Genn.)
3. Thrips	: Thrips tabaci Lind.
	: Schirto thrips dorsalis
4. Pink boll worm	: Pectinophora gossypiella (saund)
5. Spotted boll worm	: Earias vitella (F.)
6. Spiny boll worm	: Earias insulana (Boised)
7. American boll worm	: Heliocoverpa (Heliothis) armigera (Hb.)
8. Army worm	: Spodoptera litura (F.)
9. Cotton Leaf roller	: Sylepta derogata (F.)
10. Spider mites	: Tetranychus sp.
11. Aphid	: Aphis Gossypii Glov.
12. Termite	: Microtermes obesi Hlmgr.
13. Field Cricket	: Gryllus bimaculatus Deg.

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COTTON AGRO- ECO- SYSTEM

The cotton agro- eco- system (based on two years data) is presented in Fig. 1, which shows the general compositions of the cotton environment. The following are the salient points:

The maximum weekly average temperatures (44.69 to 45.61 c) were recorded in 4th week of March and 2nd week of June, and the minimum of 7.78 c in 2nd week of January. The high temperatures in May, June and part of July not only affected the fruiting pattern of the cotton plant but also the insects associated with it. The main cotton pests only become important after the onset of the moderate temperature by mid July.

The average relative humidity (0800 hours) ranged from about 50.97 in 4th week of April and 80.32 % in 1st week of August . The maximum rainfall was 133.5 mm during 4th week of July. This factor in combination with the relative humidity has been found to play an important role on the population dynamics of Jassid, White fly, Spotted and Pink boll worm.

The longest day in the season was recorded in third week of June (14 hours 17 minutes) and the shortest in early January (10 hours 10 minutes), the photo - period was the most important factor inducing diapause in pink boll worm.

As the activity of key pests are concerned the thrip, white fly and jassid started to appear during 3rd and 2nd week of June and July with a population of 0.93, 0.17 and 0.31 specimens / leaf and reached their maximum i.e. 20.56, 1.33 and 3.92 / leaf during 4th and 1st week of July and 4th week of August respectively. Similarly boll worms started to appear from 2nd week of July with a damage of 0.81 % and reached on the peak with a damage of 10.74 % during 2nd week of September. The natural enemies started to appear during 3rd, week of June with a population of 4000 / acre and reached to their maximum population of 27000 / acre during 3rd week of September.

As the fructification pattern is concerned maximum immature and mature fruiting parts i.e. 3, 69, 638 and 3, 58, 482 / acre were recorded during 4th week of September and 3rd week of October. The plant reached to its maximum height 124.25 cms during 4th week of September.

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Looking at the pest population trend and the general Fructification pattern of cotton as a means to arrive at a strategy, it appeared that ate most critical crop protection is apprxiomately 60 days from 1st week of July to 1st week of September. The most profitable minimum number of pesticide treatments would be two, one around 1st week of July and 2nd by the end of August or early.

INTEGRATED PEST CONTROL

By taking an ecological approach to pest control, consolidating features of different practices and control agents, it is possible to have integrated pest control programme and to manage pest population so as to avoid economic crop damage. Pest management demands truly multi - disciplinary team approaches. Barkin (1988), reported that the integrated pest control programme reduced subsequently pesticide applications by 30 % higher than in fields using traditional chemical approaches to pest control.

We think IPM is an integrated programme in which nature, the farmer and pesticides together combine to control pest population. The key word is integrated i.e. the integration of different control methods particularly Biological, Cultural and Chemical control methods. As the main object of this article is control by management. So the major components of this aspect currently available are discussed as under:

GROWING OF RESISTANT VARIETIES

Growing of resistant varieties is not only a highly effective means of reducing damage to crops but involve minimum production costs, leave no insecticide residues in the plant, do not contaminate the environment, do not harm beneficial insects. Moreover, it is compatible with biological, chemical, cultural and other control methods.

During the course of studies sixteen cotton genetic stocks with known markers were tested against sucking and boll worm complex. The results are summarized in Table - 1, which reveals that maximum population of thrips, jassids and white flies i.e. 4.70, 1.90 and 6.29 / leaf was recorded on CRIS - 7A (Densely hairy), CIM - 91 (Nectariless) and CRIS - 13 (Densely hairy), respectively, ehere as maximum boll worm damage and live larvae i.e. 7.21 and 2.91 % was recorded on MNH - 93 (Nactried variety) and CRIS - 7A respectively. Where as the minimum levels were 3.11 and 0.11 % respectively on HG6 - 1N, i.e. a nectariless

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and high gossypol content variety. As yield component is concerned, maximum and minimum yield i.e. 2810 and 1148 kgs / ha. were obtained from CRIS - 9 and Acala 4 - 42 respectively.

Generally hairy varieties were resistant to jassid, but some what susceptible to thrips and white flies where as nectariless, glabrous and high gossypol content varieties were resistant to boll worm complex. Similar observations were recorded by Sikka (1966) who concluded that the combination of hair length and high density of hairs on the lamina may be the best index of selection to breed for resistance to jassid attack. Similarly Wilson and Wilson (1976) quantified that the nectriless trial gave 40 % reduction in population of pink boll worm while glabrous suppressed the population of 20 %. There are evidences that varieties with increased gossypal content suffer less damage from boll worm attack particularly Heliothis species. (Lukefahr and Houghtaling, 1969). Reduction in egg laying on glabrous and nectriless cotton was also reported by Lukefhar et al (1965). Similarly Frisbi (1983) stated that four plant traits impart resistant to Heliothis sp. are glabrous, nectarliess, increased gossypol content in flower buds and the X factor (antibiosis: heliocides).

In Pakistan characteristics like nectariless, hairy, glabrous, leaf shape and gossypol composition of different plant parts and early maturing character have been widely investigated and utilized to develop resistant varieties against major cotton pests. On the basis of these studies varieties CRIS 9 (Early, smooth to hairy leaved, short to medium stature), NIAB - 78 (hairy) and CIM - 135 (Nectariless variety) which are less susceptible to jassid and boll worm complex particularly pink and spotted boll worm and could be used in IPM programme.

CULTURAL CONTROL

Cultural practices, if performed properly and timely, works two ways, directly improving the growth and general vigour of the crop and indirectly keep the pest population below economic injury levels. Typical examples for the role of different cultural practices in the control of different pests are given below.

Date of planting

The results of the experiment are presented in Table - 2 and 3. Table - 2 revealed that maximum population of thrips, jassids, and white flies i.e. 10.20, 2.24 and 2.83 / leaf was recorded during 2nd week of July, August and September from the plants sown on 15th of May and 10th of April

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respectively.Similarly maximum boll worm and live larvae percentage i.e.5.48 and 1.00 % were respectively recorded during 3rd and 2nd week of October and September from the plots sown on 10th April. Generally pest pressure was low on the crop sown on 25th of April. Maximum number of natural enemies i.e. 34000 / acre were recorded during 4th week of August on the crop sown on 15th of May.

On the basis of seasonal average maximum population of thrips, jassids and white flies i.e. 1.75, 0.47 and 1.43 / leaf was recorded from the plants sown on 25th April respectively. Similarly maximum average boll worm infestation and live larvae i.e. 4.81 and 0.92 % was recorded from the crop sown on 10th April, where as the population of natural enemies was high i.e. 14900 / acre in the plots sown on 25th of April. Maximum yield of 905 kgs / acre (2235.35 kgs / ha.) was obtained from the crop sown on 15th of may (Table - 3).

It is clear from the observations that date of planting has significant effect on the incidence of cotton pests. Early planting attracts the thrips,white flies and boll worm particularly spotted boll worm. So the planting may be regulated in such a way that it can be done during relatively dry season. It is also timed to coincide the optimum temperatures for rapid germination of seed, plant growth and fructification. Late planting may be delay c. maturity and result was a heavy infestation of pink boll worm. So it could be concluded that the optimum sowing dates for CRIS - 9 is from 25th of April to 25th of May. So early crop may be avoided.

Afzal (1991) stated that optimum planting dates be established which could commensurate with increased production and at the same - time offer less / minimised use of chemical control. Similarly Diyuan - bo (1992) suggested that the cotton planting is also limited to coincide with soil temperature and mixture for rapid germination of the seed and growth of seedling. The appropriate seed sowing assists the seedlings escape from soil - born pathogens.

Irrigation intervals

In Sindh provinceof Pakistan on an average cotton crop utilizes about 2570 to 3085 m3 (25 to 30 acre inches) of water in silt loam soils where ground water depth is more than 5 meter. This water is supplied in five to six irrigations during the season. The irrigations also affect crop growth and maturity, and pest activity. To see the effect of irrigation on pest development on different varieties an experiment was designed where the crop was irrigated with different intervals. The results are presented in Table - 4.

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Table - 4 indicates that different pests and varieties behave differently. In two weeks interval maximum population of thrips, jassids and white flies i.e. 6.79, 3.02 and 2.21 / leaf was recorded during 1992-93 season on CRIS - 9 respectively. Maximum boll worm damage and live larvae i.e. 13.52 and 1.98 % was also recorded during 1992-93 on CRIS - 6 and CRIS - 9 respectively. Same trend was also observed in three weeks interval. Generally yields were high in two weeks interval as compared to three weeks interval. Maximum yield of 3582.01 kgs / ha.was obtained from CRIS - 21 in two weeks interval.

From the results it could be concluded that stress of irrigation water do not help in the reduction of pest population but reduced the yield. These findings are in agreement that of Malik et al (1983) who reported that cotton crop should not suffer from water deficit during yield formation phase i.e. 55 - 105 days from date of planting. About 75 - 90 % bolls formed during this period. Similarly Frisbie (1983) reported that an adequate supply of water is essential for a balanced vegetative and fruiting growth of the plant when water is available in excessive quantities,the plants may tend forward vegetative growth and there by be more attractive to leaf feeding Lepidoptera. Too much moisture can destroy seed or seedlings, interfere with proper root development, prevent the plant from reaching maximum boll bearing capacity, contribute rotting of bolls and significantly reduce the yield. Tharp (1960) reported that the cotton plant requires about 562 kgs of water per kg. (dry weight) of total plant materials. Lack of sufficient water can be one of the factors most critically affecting growth of the cotton plant, the most obvious effect is that seeds fail to germinate, seedlings do not survive, mature plants shed fruiting bodies and wilt and die.

From these studies one could conclude that the first irrigation should be delayed as much as possible and the subsequent irrigation should be given with two weeks interval. From 1st October onward watering should be closed. These practices contributed a lot towards higher cotton production of better quality. Irrigation applied after 1st of October does not contribute to yield but it delay the maturity, increase the attack of pink boll worm.

Effect of Fertilizer on pest attack

Fertilizer is one of the most important input required for replenishing soil fertility. Nitrogen, in its various forms, is the fertilizer most commonly used in cotton. In this regard to see its effect on pest development an experiment was conducted and results are summarised in Table - 5 and 6.

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Table - 5 revealed that maximum average population of thrips, jassids and white flies i.e. 16.91, 2.49 and 9.91 / leaf was recorded during 1st, 4th and 2nd week of July, August and September in the plots where 50, 150 and 100 kgs / ha. of urea, urea + Ammonium Nitrate and only Ammonium Nitrate was applied respectively. Similarly maximum boll worm damage and live larvae i.e. 8.61 and 3.19 % respectively was recorded during 2nd week of September in the plots where 50 kgs / ha. of Ammioium Nitrate was applied. Where as on the basis of seasonal average of two year data (Table - 6) maximum population of thrips, Jassids and white flies i.e. 3.05, 1.05 and 3.23 / leaf was recorded in the polts where 50, 150 and 150 kgs / ha. each of urea + Ammonium Nitrate was applied.

But we are of the opinion that the fertilizer application is modified according to varied soil conditions and cropping patterns. The recommendations for the use of fertilizers, are made on the basis of physio - chemical properties of soil, fertilizer and cropping history, quality and quantity of the irrigation water, yield potential of the variety sown and the management practices in vogue.

Eradication of Alternate Host Plants

Almost all cotton pests are polyphagous and can pass the non - cotton period on other plants, so the alternate host plants play an important role in the course of study different host plants of cotton pest were identified and their population on these plants are presented in Tables - 7 and 8.

Table - 7 indicates that during the course of studies 24 host plants were recorded in the vicinity of Cotton Research Institute, Sakrand. Similarly Table - 8 revealed that maximum population of thrips, jassids and white flies and aphids i.e. 1.30, 5.66, 18.66 and 3.93 / leaf was recorded on tomato, brinjal, brinjal and brinjal at Moro, Tando Adam, Shahdadpur and Shahpur Jahania respectively. Maximum Heliothis damage and live larvae i.e. 18.0, and 7.5 % was recorded on tomato at Kazi Ahmed and Sindhri road respectively. It is therefore suggested that the alternate host plants should be eradicated from the field and it will help in reducing the development of cotton pests. Arif and Attique (1990) reported that Earias vitella was comparatively more on okra and Kenaf (Hibiscus cannabinus) where as E. insulana was abundant on Althia rosea (Holly hook) and china rose (Hibiscis rosasinensis and Turk's cap Malvaviscus arboreus.).

Generally okra is planted in the month of February and continues through out the summer months and play an important role in the bridge over of spotted boll worm between the two cotton seasons. Destruction of okra in the cotton area definitely helps to reduce the infestation of this pest.

Destruction of left over bolls
and ginning waste

Left over bolls are considered as major source of carry over of pink boll worm from year to year. In order to confirm the hypothesis studied were conducted on the emergence of pink boll worm moths from the left over bolls, where bolls were kept in the open and at room temperature in two separate lots. To determine the carry over through the ginning waste, samples were collected from the top, middle and base of the heaps lying in 10 ginning factories in Sakrand and Nawabshah at weekly interval. The results are summarised in Table 9 and 10.

Table - 9 revealed that emergence from the bolls kept in laboratory starts from December and continued upto August. Maximum number of emergence i.e. 47.50 was recorded during 2nd week of July. Where as the emergence from the bolls kept in the open also started from December but continued upto April. Maximum number of emergence i.e. 64.0 was recorded during 2nd week of March. This indicated that the population present in the bolls lying under direct sun heat emerged before the next year cotton crop was available.

The data in Table - 10 indicated that larval population was highest at the top and lowest at the bottom of heaps of ginning waste. However, mortality percent was highest at the bottom and lowest at the top. During April percent was zero and no alive was recorded.

From the above studies it could be concluded that the main source of carry - over pink boll worm is from left - over bolls and ginning waste.The destruction of left - over bolls (By goat and sheep grazing) and ginning waste is important to minimize the attack of pink boll worm. Similar observations were also recorded by Ahmed (1991).

In addition to above discussed points some other factors i.e. seed bed preparation, crop rotation, timings of harvesting, removal of weeds, and destruction of stubble after harvest may also play a vital role in minimizing the pest attack. Generally cultural practices are quite inexpensive if they are practice on an area wide basis and the farmers can also easily adopt them.

Acknowledgments

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The species reported were identified by CIBC and Zoological Survey Department Karachi. Voucher specimens are curated at C.R.I. Sakrand.

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Reference cited

Afzal, M. 1991. Integrated pest management in cotton. PAPA Bulletin Jan - June 1919, 23 - 26.

Ahmed, Z. 1991. Cultural control as part of integrated pest management programme in cotton in Pakistan, paper presented in the FAO Regional workshop on IPM in cotton, Feburay 25 - 28, 1991 at PCCC Karachi.

Arif, M.I., and M.R. Attique, 1990. Alternate hosts in carry over of Earias insulana (Boisd) and Earis vitella (F.) in Punjab, Pakistan. Pakistan cottons (in press).

Barkin, D. 1988. Search on alternate solution for a more sustainable Agriculture - The last decade for pesticides?. Pakistan Agriculture X (4):18-22.

Diyuan - bo, 1992. IPM on cotton in Asia and the Pacific region. The Pakistan cottons, 36 (2): 87 - 106.

Frisbie, R.E. 1983. Guide lines for integrated control of cotton pests. FAO plant protection paper No. 58: Xii + 187 p.

Lukefahr, M,J., D.F. Martin and J.R. Meyer. 1965. Plant Resistance to five Lepidoptera attacking cotton. J. Eco. Ent. 58 (3): 516 - 518.

Lukefahr, M.J. and J.E. Houghtaling, 1969. Resistance of cotton strains with high gossypal control of Heliothis sp. J. Eco. Ent. 62; 588 - 591.

Malik, M.N., M.S. Choudhry, and M.T.Javid, 1983. Practices in cotton irrigation, Pakistan Agriculture, V (7- 8): 46.47.

Sikka, S.M. 1966. Studies on the jassid resistance in relation to hairiness of cotton leaves. Euphytica, 15 (13): 383 - 388.

Tharp, W.H. 1960. The cotton plant. How it grows and why its growth rate varies U.S. Dep. Agric. Agric. Hand Book - 178. Govt. Print. Off. Wash., D.C. 17 pp.

Wilson, R.L. and F.D. Wilson, 1976. Ncetoriless and glabrous cotton; effect of pink boll worm in Arizona. J. Eco. Ent. 69: 623 - 624.

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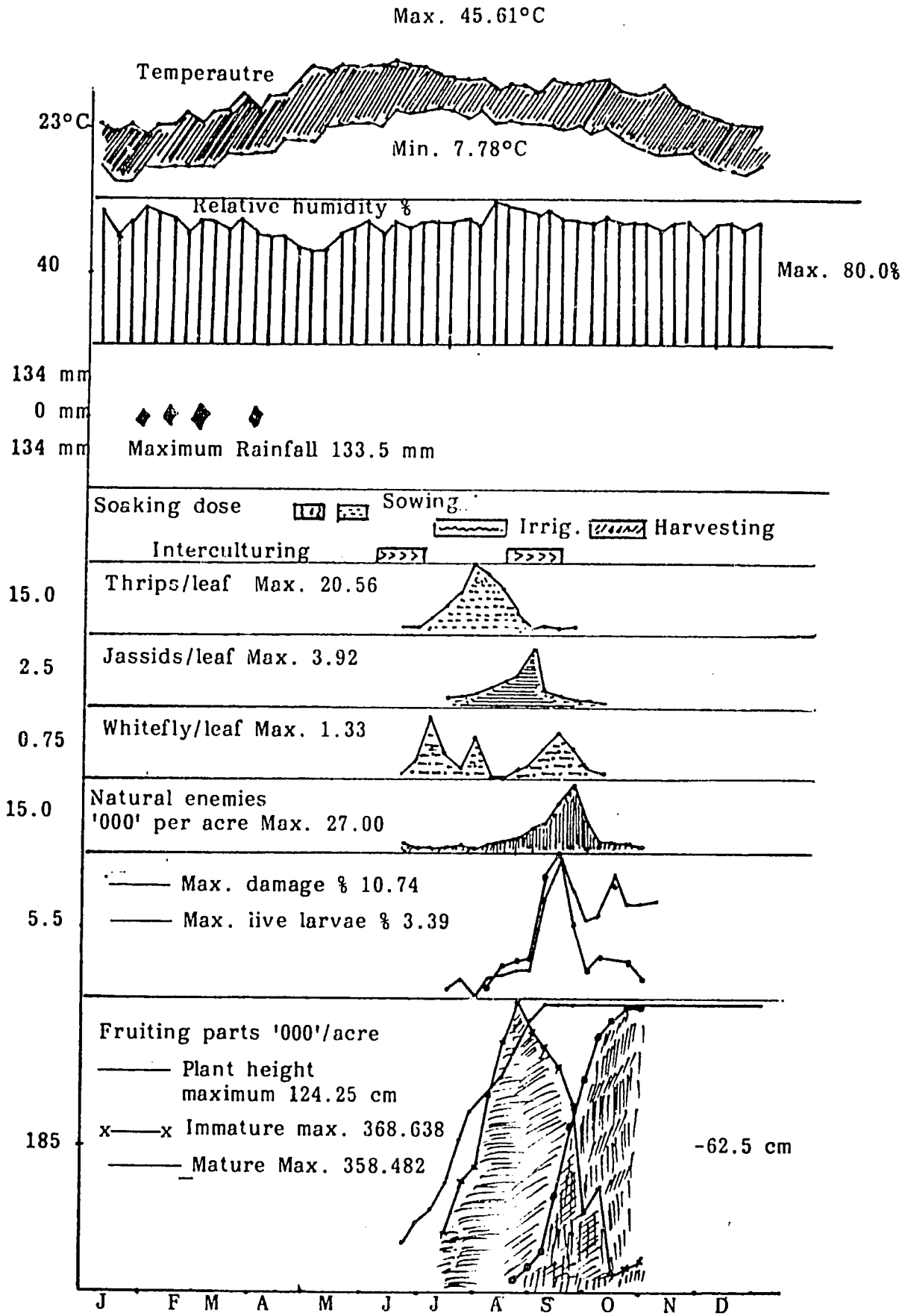

Figure-1: Agro-Eco-System in Sindh, Pakistan.

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Table-1: Average performance of new strains and other varieties tested for their resistance towards major pests of the cotton at CRI - Sakrand.

Sr. No.	Name of Variety	Characteristic features	Per leaf population of sucking pests (Based on two years data)			Level of boll worm infestation (Based on two year data)		Yield kgs/ ha
			thrips	jassid	w.fly	Bollworm damage %	Live larvae %	
1.	CRIS-9	Early, smooth to hairy leaves short to medium statured	0.70	0.07	2.02	5.04	0.92	2810
2.	CRIS-18	Early, smooth leaves, short statured.	1.88	0.97	1.75	6.13	1.49	2678
3.	CRIS-7A	Densely hairy, some what early	4.70	0.51	3.71	7.06	2.91	2776
4.	JBW-76	High gossypol contents	1.2	1.0	0.4	6.1	1.60	2014
5.	CRIS-6	Densely hairy	1.71	0.28	2.39	5.82	1.63	1499
6.	CRIS-17	Densely hairy	3.43	0.26	4.16	6.81	1.34	1648
7.	CRIS-13	Densely hairy	2.69	0.24	6.29	6.92	1.40	1836
8.	CRIS-10	Medium hairy	0.89	0.21	2.45	5.99	1.38	1834
9.	CRIS-15	Medium hairy	1.00	0.40	2.98	6.41	1.37	1932
10.	RA-33-47	Hairy	1.76	0.25	1.64	5.53	1.44	1341
11.	Rajhans	Densely hairy	1.56	0.10	2.18	6.32	1.01	1148
12.	Acala 4-42	Glandless	0.90	1.07	0.49	4.31	0.29	1240
13.	HG6-1N	Nectariless and high gossypol	1.37	1.14	0.62	3.11	0.11	1338
14.	6-1-3	High gossypol	0.93	1.61	1.21	3.12	0.14	1328
15.	CIM-91	Nectariless	1.13	1.90	1.31	4.33	0.29	1438
16.	MNH-93	Nectaried	1.86	2.00	4.02	7.21	2.13	1871

Table-2: Maximum average pest population at different sowing dates (Based on 2 years data)

Name of pest	Sowing dates		
	10/4/1992-94	25/4/1992-94	15/5/1992-94
1. Thrips/leaf	8.20 (2nd week July)	9.31 (4th week Aug.)	10.20 (2nd week July)
2. Jassid/leaf	1.72 (3rd week July)	1.39 (1st week Sept.)	2.24 (2nd week Aug.)
3. W.fly/leaf	2.83 (2nd week Sept.)	1.93 (2nd week Sept.)	2.11 (2nd week Sept.)
4. Bollworm damage %	5.48 (3rd week Oct.)	5.10 (3rd week Oct.)	5.15 (3rd week July)
5. Live Larvae %	1.00 (2nd week Sept.)	0.90 (2nd week Sept.)	1.00 (2nd week Sept.)
6. Natural enemies '000'/ acre	23.0 (1st week Aug.)	26.00 (2nd week Aug.)	34.00 (4th week Aug.)

Table-3: Seasonal average of pest population at different sowing dates.

Name of pest	Pest population in		
	10/4/1992-94	25/4/1992-94	15/5/1992-94
1. Thrips/leaf	1.50	1.75	1.43
2. Jassid/leaf	0.47	0.43	0.46
3. W.fly/leaf	1.43	1.35	1.26
4. Bollworm damage %	4.81	4.60	4.06
5. Live Larvae %	0.92	0.77	0.74
6. Natural enemies '000'/ acre	13.90	14.90	12.90
7. Yield kgs/acre	814.00	851.00	905.00

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Table- 4 : Average pest population on different varieties at different irrigation intervals.

Irrigation Intervals	Name of Pest	Year of the experiment: 1992-93			1993-94		
		Varieties					
		CRIS-6	CRIS-9	CRIS-21	CRIS-6	CRIS-9	CRIS-21
TWO WEEKS	1. Thrips/leaf	6.79	6.38	6.68	3.98	3.83	3.60
	2. Jassid/leaf	3.02	2.26	2.28	0.42	0.48	0.43
	3. W.fly/leaf	1.59	2.21	1.76	0.85	1.02	0.88
	4. Boll worm damage %	13.52	13.44	11.49	2.80	3.05	2.92
	5. Live Larvae %	1.86	1.98	1.19	0.44	0.38	0.39
	6. Yield kgs/ha	2608.31	2428.72	3582.01			
THREE WEEKS	1. Thrips/leaf	6.99	6.66	6.31	3.65	3.71	3.60
	2. Jassid/leaf	2.63	1.99	2.10	0.40	0.52	0.45
	3. W.fly/leaf	1.81	2.09	1.88	0.99	1.04	0.98
	4. Boll worm damage %	13.58	12.58	11.96	2.70	2.97	2.86
	5. Live Larvae %	1.69	1.62	1.54	0.41	0.32	0.34
	6. Yield kgs/ha	2502.33	2257.23	3033.30			

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Table-5: Maximum average pest population levels at different levels of fertilizers applied to cotton crop (Based on Two year data)

Name of Fertilizer and dose kgs/ha	Pest population per leaf			Bollworm	
	thrip	jassid	w.fly	damage %	live larvae %
1. UREA					
150	16.5 1st week July	1.00 2nd week Sept.	8.71 2nd week Sept.	5.42 2nd week Sept.	2.00 2nd Week Sept.
100	16.33 1st week July	1.08 3rd week Sept.	8.33 2nd week Sept.	5.20 2nd week Sept.	1.99 2nd week Sept.
50	16.91 1st week July	0.89 2nd week Sept.	9.41 2nd week Sept.	4.96 2nd week Sept.	1.82 2nd week Sept.
0	15.82 1st week July	0.72 2nd week Sept.	4.84 4th week Sept.	5.00 2nd week Sept.	0.58 4th week Sept.
2. AMONIUM NITRATE					
150	13.61 1st week July	1.11 4th week Aug.	6.82 2nd week Sept.	6.99 2nd week Sept.	2.11 2nd week Sept.
100	12.77 1st week July	1.12 4th week Aug.	9.91 2nd week Sept.	8.13 2nd week Sept.	3.00 2nd week Sept.
50	15.66 1st week July	0.98 4th week Aug.	6.97 2nd week Sept.	8.61 2nd week Sept.	3.19 2nd week Sept.
0	14.13 1st week July	0.81 4th week Aug.	5.99 2nd week Sept.	7.80 2nd week Sept.	0.77 1st week Oct.
3. UREA + AMONIUM NITRATE					
150	15.73 1st week July	2.49 4th week Aug.	9.60 2nd week Sept.	7.31 2nd week Sept.	2.72 4th week Aug.
100	16.33 1st week July	2.48 4th week Aug.	9.81 2nd week Sept.	6.92 2nd week Sept.	2.71 2nd week Sept.
50	15.69 1st week July	2.26 4th week Aug.	8.82 2nd week Sept.	6.73 2nd week Sept.	2.48 2nd week Sept.
0	16.11 1st week July	2.39 4th week Aug.	9.00 2nd week Sept.	6.62 2nd week Sept.	0.48 2nd week Oct.

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Table-6: Population level of different pests of cotton crop as affected by different levels of fertilizers (Based on two years data).

Name of Fertilizer and dose kgs/ha	Pest population/leaf of			Bollworm		Yield kgs/ha
	thrip	jassid	w.fly	damage %	Live larvae %	
1. UREA						
150	2.39	0.47	2.24	6.11	0.94	2218.98
100	2.31	0.49	1.12	3.89	0.77	2147.70
50	2.24	0.44	1.85	3.70	0.70	1911.88
0	2.27	0.42	1.58	3.81	0.62	1781.98
2. AMONIUM NITRATE						
150	1.82	0.50	1.75	3.94	0.88	2105.20
100	1.74	0.46	1.96	4.05	0.98	2229.16
50	2.00	0.42	1.75	4.39	1.05	1945.43
0	1.75	0.38	1.62	3.94	0.53	1731.93
3. UREA + AMONIUM NITRATE						
150 + 150	2.80	1.05	3.23	6.36	1.39	3108.54
100 + 100	2.76	1.00	2.95	6.25	1.40	3117.13
50 + 50	3.05	0.91	2.93	6.18	1.31	2776.69
0 + 0	2.94	0.87	2.73	6.03	0.99	2534.37

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Table-7: Alternate host plants of cotton pests.

Sr. No.	Crop	Pest recorded
1.	Tomato	Heliothis armigera, thrips, jassid, whitefly, aphid
2.	Brinjal	H.armigera, thrips, jassid, whitefly, aphid, mites.
3.	Gourd	Thrips, jassid, whitefly, aphid.
4.	Okra	H.armigera, Earias sp, Pink bollworm, thrips, jassid, whitefly.
5.	Chillies	Jassid, whitefly, mites.
6.	Spinich	Jassid, whitefly.
7.	Cabbage	Whitefly, Aphid
8.	China rose	Spotted boll worm
9.	Kenaf	Spotted boll worm
10.	Holly hock	-do-
11.	Turk's Cap	-do-
12.	Abutilon sp.	-do-
13.	Lantana sp.	Whitefly
14.	Wild mulbery	Whitefly
15.	Cucurbits	Whitefly, thrips
16.	Onion	Whitefly, thrips
17.	Potato	Whitefly
18.	Gurhal	Spotted boll worm
19.	San Kukra	-do-
20.	Cotton Rose	-do-
21.	Sunchal	-do-
22.	Maize	H.armigera, mites.
23.	Sunflower	-do-
24.	Soybean	-do-

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Table- 8: Population trend of some major cotton pests on alternate host plants in different districts of Sindh.

District	Crop	Location	HELIOTHIS Damage %	HELIOTHIS Live larvae %	Per leaf population Thrips	Jassids	W.fly	Aphids
HYDERABAD	Tomato	Saeedabad	5.33	2.33	0	1.06	1.93	0.11
	"	Kameja Shakh	13.90	6.00	0	1.16	2.83	0.30
	"	Hala (New)	10.00	6.00	0	0.47	1.06	0
	"	Tando M.Khan	13.00	6.50	0	0.90	2.10	0
	Brinjal	Matyari	-	-	-	0.19	9.71	2.60
	"	Rahuki	-	-	-	0.20	7.46	0
	Kadoo	Sandhan	-	-	-	0.33	8.2	0
	"	Hala (New)	-	-	-	9.8	9.80	-
	"	Hala (Old)	-	-	-	0.80	1.06	-
	Bhindi	Matyari	1.66	0	0.04	0.96	2.53	0
	Chilies	Barocho Bagh	-	-	-	0.80	1.47	0
	Spinich	Hyderabad	-	-	-	0.63	1.93	0
NAWABSHAH	Tomato	ATI Farm	5.00	2.00	0	0	0.64	0.48
	"	Airport Area	8.00	1.00	0	2.33	0.73	0.33
	"	Kazi Ahmed	18.00	5.00	0	0	0.67	0.40
	Brinjal	Dalel Dero	-	-	-	-	4.43	-
	"	Majeed Keerio	-	-	-	-	2.87	-
	"	Airport Area	-	-	-	0.13	1.67	-
	"	Nawabshah	-	-	-	1.33	1.06	2.06
	Kadoo	ATI Farm	-	-	-	0.22	7.95	2.62
	"	Mehrabpur	-	-	-	0.13	0.60	2.40
NAUSHAHRO FEROZ	Tomato	Moro	8.50	6.00	1.30	0.53	1.89	0
	"	Shahpur Jahania	5.00	1.00	0.40	0.33	1.13	0
	"	Naushahro Feroz	2.00	0	0.20	0	3.47	0.26
	Brinjal	Moro	6.00	0	0.47	0.13	12.33	0
	"	New Jatoi	4.00	0	0.33	2.13	3.73	0
	"	Shahpur Jahania	2.00	0	0	0	1.93	3.93
	"	Naushahro Feroz	-	-	-	1.80	1.13	1.67
	Cabbage	Moro	-	-	-	-	5.63	4.26
SANGHAR	Brinjal	Tando Adam	-	-	-	5.66	12.06	0
	"	Shahdadpur	-	-	-	0.93	18.66	0
	"	Uderolal	-	-	-	0.86	3.87	2.87
	Kadoo	Uderolal	-	-	-	-	10.40	-
	Tomato	Khoja Goth	7.50	4.50	0.16	1.03	2.03	-
	"	Mirpurkhas	10.00	5.00	0.30	0.27	1.06	-
	"	Sindhri Road	12.50	7.50	-	1.06	1.39	0
	"	Umer Kot	9.50	5.00	-	0.23	0.57	-

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Table-9: Emergence of Diapusing pink bollworm from left over bolls (Where size was 1 kg).

Months	Weeks	Av number of moth emerged In Laboratory	Out side Laboratory
December	1	3	1
	2	4	1
	3	6	2
	4	3.5	1
	Total	16.50	5.00
January	1	3.5	0
	2	5.5	4
	3	10.5	5
	4	13.50	2.5
	Total	33.00	11.50
February	1	3	1
	2	5.5	2.5
	3	13.2	4.5
	4	17.5	16.00
	Total	29.00	24.00
March	1	3	1.50
	2	5	64.00
	3	2	12.00
	4	0	6.50
	Total	10.00	84.00
April	1	0	22.0
	2	0	6.50
	3	4.00	31.50
	4	5.00	3.00
	Total	9.00	63.00
May	1	0	0
	2	5	0
	3	0	0
	4	0	0
	Total	11.00	0
June	1	0	0
	2	3	0
	3	17	0
	4	30	0
	Total	50.00	0
July	1	32.50	0
	2	47.50	0
	3	25.00	0
	4	22.50	0
	Total	127.50	0
August	1	4	0
	2	1	0
	3	1	0
	4	1	0
	Total	7.00	0

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Table-10: Pink bollworm larval population in the ginning waste

Months	Weeks	Top			Middle			Bottom		
		Alive	Dead	Total	Alive	Dead	Total	Alive	Dead	Total
December	1	10.00	8.00	18.00	7.00	5.25	12.25	1.75	4.50	6.25
	2	6.90	4.80	11.70	5.40	6.80	12.20	0.00	1.60	1.60
	3	7.30	4.90	12.20	5.70	5.50	11.20	2.40	6.10	8.50
	4	6.60	6.10	12.70	6.6	7.60	14.20	2.00	3.70	5.70
	Total	30.8	23.8	54.60	24.70	25.15	49.85	6.15	15.90	22.05
	% of Total	56.41	43.59		49.55	50.45		27.89	72.11	
January	1	31.60	15.85	34.15	13.65	12.40	26.05	3.80	2.25	6.05
	2	25.10	10.70	25.90	19.65	10.00	29.65	7.45	5.00	12.45
	3	37.90	10.20	48.10	24.30	13.00	37.30	8.70	7.15	15.85
	4	43.45	20.55	64.00	25.05	9.85	23.40	5.85	9.85	15.70
	Total	138.05	57.30	172.15	82.65	45.25	116.40	25.80	24.25	50.05
	% of Total	80.19	33.28		71.01	38.87		57.54	48.45	
February	1	45.20	8.30	53.50	10.87	7.25	18.12	2.35	4.40	6.75
	2	32.25	7.05	39.30	16.65	4.95	21.60	4.40	3.65	8.05
	3	23.85	3.90	27.75	10.75	2.70	13.60	3.25	3.10	6.35
	4	8.10	1.80	7.90	6.80	4.50	11.30	0.70	0.50	1.20
	Total	107.40	21.05	128.45	45.07	19.40	64.62	10.70	11.65	22.30
	% of Total	83.61	16.39		69.75	30.02		47.98	52.24	
March	1	1.15	2.05	5.20	1.80	2.20	1.30	0.30	0.30	0.45
	2	2.29	1.37	3.66	1.55	0.68	2.23	0	1.29	1.29
	3	4.30	2.58	44.73	0.78	4.65	5.43	0	1.16	1.16
	4	1.30	0.75	1.02	0.00	0.76	0.56	0	0.81	0.81
	Total	9.04	6.75	14.61	4.13	8.49	9.52	0.30	3.56	3.71
	% of Total	61.88	46.20		43.38	89.18		0.64	45.96	
April	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	1.70	0	0	1.0	1.00
	Total	0	0	0	0	1.70	0	0	1.0	1.00
	% of Total	0	0		0	100.00	0	0	100.00	

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POPULATION DYNAMICS AND CONTROL OF MELON FRUIT FLIES AT DERA ISMAIL KHAN, PAKISTAN.

By

Said Mir Khan, G.A.Miana and S.Agha Shah Hussain

Department of Entomology, Faculty of Agriculture, Gomal University, D.I.Khan, Pakistan.

ABSTRACT

The population dynamics of Bactocera cucurbitae (Dacus cucurbitae) and B. dorsalis (D. dorsalis) was studied at Agricultural Faculty, Gomal University, D.I. Khan, Pakistan during January to December, 1993 by counts of male flies caught in traps baited with cuelure and methyl eugenol used for the above mentioned pests species respectively. Chemical control trials against these pests were conducted at Agricultural Faculty, Kulachi, Gara Muhabat and Shero Kohna at D.I. Khan Division, Pakistan. The results reveals that B. cucurbitae started building its population in the 4th week of February and reached its peak in the 2nd week of April, then began a gradually decline in the 3rd week of April and completely subsided in the last weeks of December. B. dorsalis remained active through out the year. However, the population of this insect was high in Ist and 2nd week of May and in the 3rd week of September.

In the chemical control trials, all the tested treatments were found effective against the pests compared to control. However, best results were obtained with the application of Dipterex + Molasses followed by Malathion + Molasses, Dipterex and Malathion alone.

INTRODUCTION

Muskmelon (Cucumis melo L) commonly known as "Kharbooza" has its status as an important summer fruit of Pakistan and is thus grown almost throughout Pakistan particularly in D.I.Khan and Tank districts as a cash crop . This fruit is very popular among people due to a number of its nutritional and medicinal characteristics.

Many insect pests such as fruit flies, red pumpkin beetle Aulacophora foveicollis (Lucas), hadda beetle Epilachna demurili (?), jassids and aphids attack this crop and adversely affect its production. Fruit flies Bactrocera cucurbitae, B.dorsalis, B.zonatus, B.ciliatus and Myiopardalis pardalina are the most destructive insects and are become a regular threat to muskmelon growers. B.ciliatus is confined towards the coastal areas and the rest of the four species are

found all over the country. (Marwat and Baloch, 1986).

POPULATION DYNAMICS

Afsar (1987) studied the population dynamics of D.dorsalis at Peshawar, Pakistan with Methyl eugenol baited traps from January to December, 1986. No activity of the fruit fly was observed during January to March. In April this insect started building up of its population, and the average number of flies trapped per week reached from 9 in April to 145.75 in July. The population dropped to 15.75 in November.

Syed et al (1970) studied the seasonal abundance of D.zonatus at Faisalabad,Pakistan and stated that host availability of this insect play a significant role in the building up of its population particularly after winter. Qureshi et al (1974) made effort to study the population fluctuation of D.zonatus with the help of methyl eugenol baited traps and found that lowest population of this insect was recorded in January - February and its peak in March-April, a decline in June-July and again an increase in the population in August which reached its peak in September. Temperature plays a significant role in the building up of fruit fly populations. Syed (1971) recorded a range of 68° F to 78° F as threshold temperature in different localities for D.cucurbitae. In another similar studies Syed et al (1970) stated that the population of D.dorsalis was completely ceased when the temperature dropped to 55° F. Afsar (1987) also reported that at 23.75° C the activity of this fly was ceased.

Marwat(1988) studied the population dynamics of melon fruitfly from March 1986 to February 1989 at D.I.Khan. He recorded melon fruitfly D.zonatus which was active throughout the year. The population started building up in February, during this month only 45 flies were trapped.The population of this insect gradually increased to 92 in March, 186 in April, 698 in May and reached to its peak of 1826 flies in June. The population of this insect again gradually decreased to 1261 flies in July, 355 in August, 167 in September and only 6 flies in January.

CHEMICAL CONTROL

Gupta (1958) obtained best control of melon fruitfly with Malathion 60% EC. at the rate of 0.75 and 0.25 ozs mixed with protein hydrolysate, sugar in 1 gallon of water.

Mannan and Khan (1970) stated that Dipterex was the most effective insecticide followed by Dimecron, Malathion and Anthio for the control of fruitflies.

Harris et al (1971) applied Malathion and nailed

with un-diluted protein hydrolysate against melon fruit fly. They reported that Nailed caused more immediate mortality, while Malathion has longer residual effect against melon fruitfly.

Nawaz (1976) conducted a control trial against melon fruitfly at Dera Ismail Khan and found that three sprays of Dipterex (Trichlorofon) at the rate of 1.6 % (a.i.), first after fruit setting and then subsequently at 10 days interval, significantly reduced the pest infestation.

Hussain et al (1980) conducted a trial on muskmelon against fruitfly at D.I.Khan to test the effectiveness of four insecticides viz; Endosulfan, Malathion, Omethoate and Trichlorofon. The chemicals were used at the rate of 0.025, 0.050 and 0.075 % concentrations. The pest infestation was significantly reduced by all the insecticides compared to the control. In general Trichlorofon was the most effective in suppressing the infestation followed by Malathion, Endosulfon and Omethoate.

Bhatti (1981) used Dipterex, Hostathion and Anthio on apricot against fruitflies at 0.1% concentration in seven different combination. Dipterex was found comparatively better as compared to other insecticides. Suleman (1981) investigated relative efficacy of Dipterex, Malathion, Fenthion and Phosphamidin in three concentrations i.e. low, medium and high against Dacus spp. Chughtai and Baloch (1988) studied the efficacy of various insecticides against Dacus cucurbitae Coq. attacking muskmelon at D.I.Khan. According to them Carbicron (Dicrotophos) at 600 g a.i. and Dipterex (Trichlorofon) at 1920 g a.i. per hectare gave the best results.

MATERIALS AND METHODS

POPULATION DYNAMICS

The population dynamics of fruit flies was studied at the farm of Agricultural Faculty, Gomal University, Dera Ismail Khan, from first week of January to last week of December, 1993 with the help of sex-lure traps.

Two sets (each comprising of eight sex-lure traps) baited with Methyl eugenol and Cuelure mixed with Dipterex for B.dorsalis and B.cucurbitae respectively. These traps were prepared by applying the respective sex lure for each insect species at the rate of 4 ml to a cotton wick measuring 5.5 cm long and 1.5 cm dia.These traps were suspended in the muskmelon fields at a height of 5 feet from ground level. The data was recorded on the basis of number of trapped fruit fly adult males weekly. The cotton wicks of the traps were

replaced after every two month period with a fresh dose of the sex-lure chemicals.

B - CHEMICAL CONTROL

Chemical control trials against fruit flies were conducted at the farm of Agricultural Faculty, Gomal University, D.I.Khan and on farmers fields at Gara Muhabat, Kulachi and Shero-kohna all at D.I.khan, The experiments were laid out in a complete randomized block design, having five treatments and four replications at each locality. Muskmelon was sown with plant to plant and row to row distance 60 cm and 180 cm, respectively. Two seeds per hill were planted and after germination thinning was done to leave only one plant per hill.The treatments included :-

S.No.	Treatment	Concentration
T_1	Malathion	0.5 %
T_2	Malathion + Molasses	0.5 % + 1.0 %
T_3	Dipterex	0.5 %
T_4	Dipterex + Molasses.	0.5 % + 1.0 %
T_5	Control	

The first spray of the crop was done with a knapsack spryer after fruit setting and second spray after a fortnight interval, when the melon fruit reached to two inches size.All agronomic practices were followed equally in all of the treatments.Data were recorded on the basis of 50 muskmelon fruits per replication which were collected randomly in each treatment at the time of maturity. The infestation was determined by dissecting the fruits and locating the larvae of fruit fly in the pulp.The results of treated plots were compared with that of the check (untreated).

RESULTS AND DISCUSSION

POPULATION DYNAMICS

The population data given in table-1, shows that the population of B.cucurbitae started to build up in the 4th week of February and reached to its peak in the 2nd week of April, gradually declined in 3rd week of April and completely subsided in the 4th week of December. B.dorsalis remained active throughout the year.However the population of this insect remained low from 1st week of January to 2nd week of April. Afterward it started building up of its population and reached to its peak in the 1st and 2nd week of May,

then the population started on gradual decline upto 1.18 males/trap in the 2nd week of August. The population of this insect again started gradual increase and reached to its peak in the 3rd week of september, afterward it started gradual decreasing and reached to 5 adult males/ trap in 1st week of December.

It is evident from the table-1, that temperature and relative humidity play an important role in the building up of fruitfly populations. Syed et al (1970) Qureshi et al (1974), Syed (1971), Afsar (1987) and Marwat (1988) also recorded high fruitfly populations at optimum temperature and high relative humidity.

Table-3. POPULATION DYNAMICS OF FRUIT FLIES AT THE FORM OF AGRICULTURAL FACULTY, GOMAL UNIVERSITY, D.I.KHAN DURING THE YEAR 1993.

Weekly average	B. cucurbitae (Cuelure traps)	B.dorsalis (Methyl eugenol traps)	Temperature °C		Percent Relative Humidity
			Max.	Mini.	
January					
1st week	0.00	0.97	22	08	65
2nd week	0.00	1.54	22	06	67
3rd week	0.00	0.54	21	05	55
4th week	0.02	4.84	21	06	62
February					
1st week	0.00	21.60	25	08	64
2nd week	0.03	93.93	23	09	68
3rd week	0.00	33.93	26	07	65
4th week	0.18	1.71	24	10	66
March					
1st week	1.50	7.00	24	11	78
2nd week	0.87	13.36	25	09	75
3rd week	0.78	4.93	26	10	79
4th week	3.97	9.88	25	10	73
April					
1st week	7.38	16.09	28	13	63
2nd week	16.33	19.33	32	17	68
3rd week	4.14	56.95	34	17	80
4th week	1.59	76.71	39	22	59
May					
1st week	2.26	268.83	39	25	66
2nd week	1.53	273.96	39	25	70
3rd week	2.23	159.35	37	24	61
4th week	0.77	51.20	42	26	58

Weekly average	B. cucurbitae (Cuelure traps)	B. dorsalis (Methyl eugenol traps)	Temperature °C		Percent Relative Humidity
			Max.	Mini.	
June					
1st week	0.46	44.36	41	25	79
2nd week	0.07	10.60	43	35	63
3rd week	0.17	11.30	40	36	69
4th week	0.07	4.45	40	28	65
July					
1st week	0.10	5.21	38	29	74
2nd week	0.30	6.38	37	28	76
3rd week	0.02	2.02	35	27	72
4th week	0.20	2.25	36	26	76
August					
1st week	0.66	2.28	37	29	74
2nd week	0.71	1.18	35	27	72
3rd week	0.33	1.52	38	27	71
4th week	0.30	1.85	36	26	6
September					
1st week	0.65	312.25	34	26	63
2nd week	0.76	441.75	34	25	76
3rd week	1.27	480.86	36	25	67
4th week	1.19	322.61	35	24	69
October					
1st week	0.67	58.32	36	24	69
2nd week	0.58	62.45	34	17	62
3rd week	0.71	71.91	33	15	63
4th week	1.44	46.93	30	14	65
November					
1st week	0.80	45.33	29	15	74
2nd week	0.54	26.49	28	13	68
3rd week	0.66	19.95	28	12	69
4th week	0.37	16.46	26	08	64
December					
1st week	0.03	5.89	24	07	66
2nd week	0.00	14.00	23	06	66
3rd week	0.07	23.05	21	04	63
4th week	0.00	16.21	23	05	66

B - CHEMICAL CONTROL

It is evident from the table-2, that all of the treatments significantly reduced the fruitfly infestations in muskmelon, at all of the tested localities at D.I.Khan. Dipterex + molasses proved the best treatment for the suppressing of the infestation of the pest, followed by Malathion + molasses, Dipterex and Malathion compared with control (untreated) plots. Manan and Khan (1970), Nawaz (1976), Hussain et al (1980), Bhatti et al (1981) and Chughtai and Khan (1988) also got effective control of melon fruitfly with the application of Dipterex (Trichlorofon). Gupta (1958) obtained best control of fruitflies with Malathion mixed with protein hydrolysate. Harris et al (1971) applied Nailed and Malathion with undiluted protein hydrolysate for the control of melon fruitfly and stated that Nailed caused more immediate mortality, while Malathion has longer residual effect against the these pest.

Table-2 Percent infestation of muskmelon fruits in the chemical control trials, conducted at farmers fields at various localities of D.I.Khan.

Treatments	Different localities at D.I.Khan				Mean
	Kulachi	Gara Muhabat	Shero kohna	Agric. Faculty	
1. Malathion	25	23	32	24	26.00 B
2. Malathion + Molasses	20	25	24	21	22.50 BC
3. Dipterex	23	25	26	22	24.00 BC
4. Dipterex + Molasses	22	20	24	19	21.25 C
5. Control	88	85	92	93	89.50 A

Means followed by different letters are significantly different from each others at 5% level of significance

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REFERENCES CITED

1. Afsar,K. (1987). Annual population incidence of fruitflies (Dacus spp.) in N.W.F.P. Agric. Univ. fruit orchard. M.Sc(Hons) thesis, submitted to the Department of Entomology, N.W.F.P. Agricultural University, Peshawar, Pakistan.

2. Bhatti,A.M. (1981). Infestation and control of fruitflies of Dacus spp. on apricot fruit at Peshawar University Campus. M.Sc(Hons) thesis, submitted to Department of Entomology, N.W.F.P. Agricultural University, Peshawar, Pakistan.

3. Chughtai,G.H. and U.K.Baloch (1988). Efficacy of various insecticides against the larvae of D.cucurbitae Coq. Pakistan J. Agric. Res. 9(2): 192 - 194.

4. Gupta,R.L. (1958). Preliminary trial of bait spray for the control of fruitflies in India. Indian J.Entomol. 20(4) : 304 - 306.

5. Herris,E.J., D.L.Chamber, L.F.Steiner, D.C.Kamakahi and M.Komura (1971). Mortality of Tephritidae attracted to guava foliage treated with Malathion or Dibrom (Naled®) plus protein hydrolysate bait. J.Econ. Entomol. 64(5):1213 - 1216.

6. Hussain,N.,L.S. Main and N.Khan. (1980). Chemical control of melon fruitfly Dacus cucurbitae Coq. J.Sci. & Tech. 4 (1-3):

7. Manan, K.A. and G.M.Khan (1970). Cultivation of melon (in urdu). Department of Agric. D.I.Khan Division, Pakistan, 21 - 22 pp.

8. Marwat,N.K. (1988). Final report of national fruit fly research laboratory, Agric. Res. Sta. D.I.Khan, Pakistan 129 pp.

9. Marwat,N.K. and U.K.Baloch (1986). A plant having Methyl euginol, a male fruit fly sex-attractant. A research note submitted to PARC News Journal.

10. Nawaz,R.(1976). The infestation and Control of melon fruit fly on muskmelon in D.I.Khan. M.Sc thesis, submitted to University of Peshawar, 32 pp.

11. Qureshi,Z.A., M.Ashraf, A.R.Bughio and Q.H.Siddiqui, (1974). Population fluctuation and dispersal studies of the fruitfly Dacus zonatus (Saund) Sterility principle for insect control. IAEA-SM-186/83: 20 - 207.

12. Suleman,M. (1981). Infestation & Control of fruitflies Dacus dorsalis Hend. on guava at Herripur. M.Sc thesis submitted to University of Peshawar. 41 pp.

13. Syed,A.R., M.A.Ghani and M.Murtaza,(1970). Studies on the Trypetids and their natural enemies in West Pakistan, IV.Further observation on D.dorsalis Hendel. Tech. Bull. No.13. C.I.B.C. : 17 - 30.

14. Syed,R.A. (1971). Studies on Trypetids and their natural enemies in West Pakistan. Tech. Bull. No. 14. C.I.B.C. : 63 - 75.

MODIFIED ARTIFICIAL DIET FOR MASS REARING OF CHICKPEA POD BORER, HELICOVERPA (HELIOTHIS) ARMIGERA (HÜBN.)

Khalique Ahmed, Feeroza Khalique and Bashir Ahmed Malik

Pulses Programme
National Agricultural Research Center (NARC)
P.O. NARC, National Park Road, Islamabad, Pakistan

Abstract

This paper reports on a comparison of a modified diet with other diets and procedures used in mass rearing of Helicoverpa armigera. The results on the biology of H. armigera reared on the modified diet for up to six consecutive generations indicate that the pupal recovery percentage ranged from 71.2±9.59 to 83.7±5.98; the adult emergence percentage varied from 59.6±3.15 to 78.4±3.29. The maximum egg yield/female was recorded to be 273.3±13.6 in the first generation. The average cost to produce one pupa of this insect was calculated to be Rs. 0.60, which is equivalent to half the cost of production per pupa using other diet formulae.

Key words: Modified diet; Helicoverpa (Heliothis) armigera; rearing; oviposition, techniques; larvae.

Introduction

The ever-increasing demand for a large number of laboratory-reared insects has necessitated the development of efficient and economical methods of production. The shifting emphasis in insect control, using biological entities such as natural enemies and insect pathogens (bacteria, viruses and fungi), has increased the demand for constantly reliable sources of such insects. Moreover, the rearing of phytophagous insects on artificial media, rather than on their host plants, is advantageous in a variety of investigations. Laboratory-reared larvae can be used for the study of insect pathogens, plant resistance factors, and effects of insecticides and radiation on fecundity and growth, as well as for the study of insect life cycles.

Beck et al. (1949), reported the first successful aseptic rearing of a phytophagous lepidopteran, the European corn borer, Ostrinia nubilalis, on a meridic diet. Ishii and Urushibara (1954) were able to rear the rice stem borer, Chilo suppersalis, on a modification of the diet reported by Beck et al. (1949). Vanderzant and Reiser (1956) were the first to formulate a meridic diet for the pink bollworm, Pectinophora gossypiella, without a plant adjuvant. Later, Adkinsson et al. (1960), developed a satisfactory artificial medium supplemented with wheat germ for rearing pink bollworm. The wheat germ medium has been used to rear several species of plant-feeding lepidopterans, including the bollworm Helicoverpa (Heliothis) zea (Vanderzant et al., 1962); the cabbage

looper, Trichoplusia ni (Getzin, 1962); the white large butterfly, Pieris brassicae (David, 1965); and the codling moth, Carpocapsa pomonella (Rock et al., 1964).

Since these studies were performed, both diets and rearing techniques have been modified/simplified (Ignoffo, 1965; Shorey and Hale, 1965; Burton and Perkins, 1972; Dulmage et al., 1976, Ahmed, 1983; Ahmed et al., 1985; Kulkarni and Amonkar, 1988; and Novon and Klein, 1990).

The chickpea pod borer, Helicoverpa armigera, is an important, polyphagous insect pest that inflicts economic losses to chickpea, maize, tomato, tobacco, sunflower, cotton, vegetable, and fodder crops (Ahmed et al., 1986, 1989). In northern Pakistan, H. armigera caused 90% pod damage in unprotected fields (Ahmed et al., 1986, 1990; Anonymous, 1987).

To facilitate research on H. armigera, efforts were focused on developing an economical diet formula and rearing techniques. The present study and its results elaborate upon the development of a diet (with all ingredients locally available), tools, and techniques applied successfully for maintenance of healthy insect cultures of H. armigera to conduct bioassays of Bacillus thuringiensis (an insect pathogen currently being used in many biological insecticides for control of lepidopterous pests). The artificial diet formula and procedures reported herein represent further simplification/modification of the artificial diet and rearing techniques reported by the author (Ahmed, 1983).

Materials and methods

At the start, 1050 larvae (different instars) of H. armigera were collected from chickpea fields and reared on artificial diet. The rearing room temperature ranged from 20 to 29 °C, relative humidity varied from 60 to 80%, and a 15:9 light and a dark cycle was maintained.

Ingredients for the preparation of diet

The ingredients and quantities used in preparation of a 4.25 l batch of the diet are as follows:

Ingredients	Quantity
Agar	25.0 g
Cowpea powder (Vigna unguiculata)	600.0 g
Ascorbic acid	7.0 g
Sorbic acid	3.0 g
Vitamin E	0.2 g
Dried active yeast	20.0 g
Methyl-p-hydroxybenzoate	10.0 g
Vitamin mixture	5.0 ml
Formaldehyde (10%)	6.0 ml
Tap water	3500.0 ml

The following vitamin mixture preparation in 200 ml sterile distilled water was used:

Ingredients	Quantity
Calcium pantothanate.....	4.8 g
Nicotine acidamid.....	2.4 g
Riboflavin	1.2 g
folic acid	1.2 g
Thiamine hydrochloride	0.6 g
Pyridoxine hydrochloride	0.6 g
Biotin	0.048 g
Vitamin B_{12}	0.0024 g

The dry ingredients of the diet were weighed carefully and kept in separate containers. The wet ingredients were measured and also kept in separate containers. The entire quantity of agar was suspended in water in a 5 l capacity container and brought to a boil. The total quantity of bean powder (Vigna unguiculata) was added to the boiled agar and mixed, which resulted in cooling of the mixture to nearly 80 °C. Then all the dry and wet ingredients were added to this mixture and the entire mass was mixed. The vitamin mixture, including vitamin E, was added last, and the mass was again mixed thoroughly. The prepared diet was then poured into the desired number of sterilized glass capsule vials (5-6 ml diet/capsule vial) with the help of a squeeze bottle and allowed to cool and harden.

Rearing procedures

Glass capsule vial technique for individual larval development

A standard-sized glass capsule vial (2.5 cm in diameter & 5.5 cm high) was used in this technique. The sterilized capsule vial containing diet was infested with a newly hatched first instar larva with the help of a sterilized camel hair brush. After the vial was infested with first instar larva, its mouth was tightly closed with a sterilized cotton wool plug, which provided an exchange of air and did not allow the diet to dry out before the developing larva pupated. A number of sets, each comprising 25 to 50 vials (one larva/vial) were used for mass larval development.

Petri plate technique for group larval development

This insect develops cannibalism from the late third to fifth instars. We have developed a technique that allows rearing H. armigera in groups of 90 to 100 larvae per petri plate (17.0 x 3.0 cm), with a thin layer of diet at the bottom and in the cover of the plate. After infestation of the petri plate with about 110 first instar larvae, sterilized two ply tissue paper was folded and placed in the petri plate to soak up excessive diet moisture and allow the developing larvae to move into the tissue paper folds, thereby reducing chances of possible larval cannibalism. This

enabled aseptic rearing upto the early third instar larvae for use specifically in second or third instar larval bioassays. This rearing technique significantly reduced the cost of development of third instar larvae as compared with the individual rearing technique (one larva/container).

Adult emergence

Plastic jars (11.5 cm in diameter and 10 cm high) were used for adult emergence. The larvae that pupated in the capsule vials were taken out and placed on a circular piece of blotting paper in the plastic jar (10-15 pupae/jar). The mouth of the jar was closed with muslin cloth held tightly in position by rubber bands. After emergence, the adults were collected individually into capsule vials (one adult/vial). Care was taken not to disturb the adults to reduce the chances of their escape from the emergence jar. The emerged adults were checked and removed daily.

Mating-oviposition cage

A 30 cm cubic box made of acrylic sheet was fabricated into an oviposition cage. Four slits, each 25.0 cm long X 6.0 mm wide, were made at equal spacing on three sides of the cage. The slits were closed with cotton wool strips for oviposition. On two opposite sides of the cage, slits 6.0 cm wide x 30.0 cm long were left open. These wide slits were covered with muslin cloth to allow air to pass through the cage. The adults were also introduced into the cage through these wide slits. Two medium-sized petri plates containing cotton wool pads soaked in 10% sucrose solution were also placed in the cage to provide food for the ovipositing adults.

The desired number of pairs (20-30) of H. armigera were released in the cage through one of the slits. The eggs deposited on cotton wool strips were removed daily, checked, and kept in polyethylene bags for incubation and hatching.

Egg incubation

The eggs were incubated at optimum temperature in the laboratory. Hatched larvae were transferred into capsule vials containing diet. Egg sterilization was not considered necessary as no incidence of bacterial or viral disease was observed. However, a, low level of fungal contamination (air-born contamination) was found after approximately 12 days of larval feeding in the vials.

For evaluation of significance, a range test (DMRT) was done using the MSTAT-C computer programme (version 1.42, developed by Michigan State University, USA).

Results and discussion

Table 1 shows a comparison of the diet formulae developed by Ahmed, 1983; Ahmed et al., 1985; Kulkarni and Amonkar, 1988; and Novon and Klein, 1990 for rearing the chickpea pod borer, Helicoverpa (Heliothis) armigera; Shorey and Hale, 1965 (for rearing nine noctuid species including the bollworm, Helicoverpa (Heliothis) zea, and tobacco budworm, Heliothis virescens); and Shorey, 1963 (for rearing the cabbage looper, Trichoplusia nii). The modified diet developed in the present work, which supported six generations of the insect, is similar in composition to the diet of Ahmed, 1983, except for the addition of vitamin E, an increase in tap water (3500.0 ml), and a reduction of about 30.0 to 45.0% in the quantities of all the ingredients. Vitamin E was incorporated in the diet to prevent a possible reduction in reproductive vigor and egg viability of the insects. The diet reported by Ahmed et al., 1985, supported six successive generations, was similar in composition to the diet developed by Ahmed, 1983 (likewise supported 23 successive generations of H. armigera), except that sorbic acid and vitamins were omitted, and the quantity of ascorbic acid was decreased to 3.0 g for a 2.5 l batch of diet.

The diet formula developed by Shorey and Hale (1965) also contained the same ingredients,but in different quantities and with complete omission of vitamins as compared with the present formula. Shorey (1963), also worked out a simple diet consisting of only six ingredients on which he successfully raised twelve generations of cabbage looper, Trichoplusia ni. The present diet modification contained all the ingredients reported by Shorey (1963). Kulkarni and Amonkar (1988) also used similar diet ingredients, though in different quantities and without sorbic acid and vitamins; they used Bengal gram powder as the basic ingredient instead of bean powder. The diet formula used by Novon and Klein (1990) for rearing H. armigera consisted of 11 ingredients, of which 8 were common to the present modification.

The modified diet developed herein is comparatively inexpensive, and one batch (4.25 l) can be used to rear about 700 larvae (6.0 ml diet/larva). The average cost to produce each pupa, including labor charges, was calculated to be Rs. 0.60, which is equivalent to 1.8 cents (US cent). Twenty eight years ago, Shorey and Hale (1965) reported that approximate cost to produce one H. zea/H. virescens pupa was 0.9 cent (US cent). In Pakistan, analar grade chemicals (many of the diet ingredients) are imported items. Thus their cost is high (e.g., one tin [454.0 g] of Difco Agar-Agar costs Rs. 3300 which is equivalent to $100).

Helicoverpa armigera larval development and pupal recovery data from field to sixth laboratory generations (Table 2) showed no show significant difference among the pupal recovery percentage in all the generations. The maximum pupal recovery (83.7%) was noted in the first generation. Average pupal period ranges were from 20.2 days (minimum in the fifth generation) to 26.3 days (maximum in the

second generation) at temperature ranges of 23-35 °C and 16-26 °C respectively. Similar results were observed by Burton and Perkins (1972), and Dang et al. (1970), who achieved 86.2% pupation of H. zea reared on bean diet and 75.0% pupation of C. zonellus reared on Kabuli gram diet, respectively. Ahmed (1983) and Ahmed et al. (1985) also reported H. armigera pupal recovery ranging from 61.6% (in the second generation) to 97.3 % (in the second generation), respectively.

The data in Table 3 illustrate results on pupal period and adult emergence. The maximum adult emergence (78.4%) noted in first generation, was significantly different from second and third generation. The cause of the high pupal mortality in the third generation is unknown. The pupal period varied from 13.3 to 20.2 days, according to the temperature range. Ahmed (1983), observed the maximum adult emergence of H. armigera (92.8%) in the fouth generation and the minimum (50.2%) in the twenty-third generation. High adult emergence rates of 96.8% for C. zonellus (reared on bean diet) and 90.0% for H. zea (reared on bean diet) were also reported by Dang et al. (1970), and Burton and Perkins (1972), respectively. Ahmed et al. (1985) reported low adult emergence of H. armigera (44.23 to 70.97% in different generations).

The maximum number of eggs laid/female (Table 4) was 273.2, in the second generation. The minimum number of eggs/female (102) was recorded in the third generation. The reason for such significant variation in oviposition per female is unknown. Similar findings (145 to 326 eggs/female of H. armigera) were reported by Ahmed et al. (1985). Burton and Perkins (1972) obtained 1901 eggs/mated female of H. zea reared on WSB diet.

Conclusions

The modified artificial diet reported herein proved to be successful for mass rearing of H. armigera and supported development of this insect for at least six laboratory generations. Moreover, the techniques applied for maintaining healthy cultures of the insects satisfied experimental requirements (bioassays). The present modified diet was also found economical in comparison with other diets. Hence, this diet is recommended for rearing of H. armigera for several generations.

Table 1. Artificial diet formula presently developed by the authors and its comparison with previously published artificial diet formulae used for rearing Helicoverpa (Heliothis) species and Trichoplusia nii

Ingredients[A]	Present formula[1]	Ahmed, 1983[1]	Ahmed et al. 1985[1]	Shorey & & Hale, 1965[2]	Shorey, 1963[3]	Kulkarni &Amonkar, 1988[1]	Novon& Klein, 1990[1]
Agar	25.0 g	50.0 g	50.0 g	128.0 g	4.0 g	19.0 g	74.0 g
Bean powder	600.0 "	400.0 "	400.0 "	2133.0 " *	100.0 "	136.0 " **	1200.0 " ***
Ascorbic acid	7.0 "	11.0 "	3.0 "	32.0 "	1.0 "	2.9 "	16.0 "
Sorbic acid	3.0 "	3.0 "	-----	10.0 "	-----	-----	5.0 "
Vitamin E	0.2 "	-----	-----	-----	-----	-----	-----
Dried active yeast	20.0 "	20.0 "	20.0 "	320.0 "	10.0 "	42.0 "	160.0 "
Methyl-para-hydroxybenzoate	10.0 "	7.0 "	7.0 "	20.0 "	1.0 "	2.7 "	16.0 "
Vitamin mixture	5.0 ml	4.0 ml	-----	-----	-----	-----	-----
Formaldehyde	6.0 " (10.0%)	6.0 " (10.0%)	6.0 ml (10.0%)	20.0 ml (40.0%)	-----	10.8ml (10.0%)	20.0ml (37.0%)
Tap water	3500.0 "	2000.0 "	2000.0 "	6400.0 ml	200.0 ml	825.0ml	3000.0ml
Cholesterol	-----	-----	-----	-----	-----	-----	3.0 g
Chloramphe-nicol	-----	-----	-----	-----	-----	-----	6.0 "
Alfaifa meal	-----	-----	-----	-----	-----	-----	200.0 "
Quantity of diet(approx.)	4250.0 ml	2500.0 ml	2500.0 ml	8500.0 ml	300.0 ml	1000.0ml	4250.0ml
No. of gen-erations raised	6	23	6	-----	12	-----	-----

A All the ingredients were that of analar grade except bean powder, yeast and formaldehyde

1 For rearing Helicoverpa (Heliothis) armigera

2 For rearing Heliothis zea and H. virescens

3 For rearing Trichoplusia nii

* Soaked lima bean

** Bengal gram powder

*** Phaseolus vulgaris presoaked in water for 24 hr

Table 2. Mean±SE of larval development of *H. armigera* from field collected larvae to sixth laboratory generations

Generation	Larvae placed on diet	Average** pupal recovery (%)±SE	Average** larval period (days)±SE	Temperature range (°C)
Field	1050*	71.2± 9.56 a	-----	24-29
first	900	83.7± 5.98 a	22.9±4.04	17-29
Second	500	75.3± 8.85 a	26.3±3.50	16-26
Third	706	76.9±11.45 a	26.2±1.55	18-26
Fourth	723	79.0±11.51 a	26.2±4.72	22-30
Fifth	757	74.5± 5.34 a	20.2±2.54	23-35
Sixth	330	79.4± 4.97 a	23.2±0.81	23-33

A catagory that shares common letters is nonsignificantly different at P<0.05; * Larvae collected from chickpea field were in different instars; ** average of 4 batches.

Table 3. Mean±SE of pupal development and adult emergence of *H. armigera* from field to sixth laboratory generations

Generation	Number of pupae recovered	Adult emergence* (%)±SE	Average* pupal period (days)±SE	Temperature range (°C)
Field	759	73.2± 5.88 ab	13.6±0.26	24-30
first	742	78.4± 7.07 a	17.1±2.34	16-29
Second	372	65.3± 2.05 bc	19.5±0.69	18-26
Third	557	59.6± 3.15 c	20.2±1.82	18-29
Fourth	603	78.4± 3.29 a	15.5±0.45	22-31
Fifth	561	76.0± 7.05 a	13.3±1.38	23-35
Sixth	264	78.2± 5.38 a	14.2±0.53	23-31

A catagory that shares common letters is nonsignificantly different at P<0.05; * Average of 4 batches.

Table 4. Mean±SE of oviposition per female of *H. armigera* from field to sixth laboratory generations

Generation	Number of adults recovered	Number of adult pairs released in cage	Average* no. of eggs/ female±SE		Temperature range (°C)
Field	547	110	202.2±35.4	b	21-28
first	589	130	273.3±13.6	a	16-27
Second	243	115	102.9±13.3	c	18-26
Third	326	144	153.9±17.1	b	18-29
Fourth	476	155	158.9± 8.8	b	22-30
Fifth	424	88	188.3±40.8	b	23-35
Sixth	208	67	166.1±35.3	b	24-32

A catagory that shares common letters is nonsignificantly different at $P<0.05$

* Average of 4 batches

References

Adkisson, P.L., Vanderzant, E.S., Bull, D.L., and Allison, W. E. 1960. A wheat germ medium for rearing the pink bollworm. J. Econ. Ent. 53(5):759-762.

Ahmed, K., Khalique, F. and Malik, B. A. 1989. Studies on Helicoverpa (Heliothis) armigera management in Pakistan. Proc. 1st. Intnal. Conf. Econ. Entomol. 2: 153-161.

Ahmed, K., Khalique, F., Afzal, M. and Malik, B. A. 1986. Pulses Entomology Report, Food Legumes Improvement Programme, National Agric. Res. Center, Pak. Agric. Res. Council. pp 31-39.

Ahmed, K., Lal, S. S., Morris, H., Khalique, F. and Malik, B. A. 1990. Insect pest problems and recent approaches to solving them on chickpea in South Asia. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). Chickpea in the Nineties: proceeding of the Second International Workshop on Chickpea I, Improvement, 4-8 Dec 1989, ICRISAT Center, India. Patancheru A.P. 502 324, India pp.165-168

Ahmed, K. 1983. Development of artificial diets for mass breeding of some lepidopterous insects. M. Phil thesis. Department of Zoology, University of Karachi, Karachi, Pakistan. pp 16-47.

Ahmed, K., Afzal, M., Malik, B. A. and Khalique, F. 1985. A simple low cost artificial diet and some mass rearing techniques of gram pod borer, Heliothis armigera (Hübn.) (Lepidoptera: Noctuidae). Pakistan J. Sci. Indust. Res. 28(4):257-260

Anonymous, 1987. Entomology of chickpea. Annual Technical Report (July 1, 1986 to June 30, 1987) on Food Legumes Improvement Project In Pakistan, National Agricultural Research Center, Islamabad, Pakistan pp 121-134.

Beck, S.D., Lilly, J. H. and Stauffer, J.F. 1949. Nutrition of European corn borer, Pyrausta nubilalis (Hübn.). Development of satisfactory purified diet for larval growth. Ann. Ent. Soc. Am. 42:483-496.

Burton, R. L. and Perkins, W. D. 1972. WSB a new laboratory diet for the corn earworm and the fall armyworm. J. Econ. Ent. 65:385-386

Burton, R. L. 1970. A low cost artificial diet for the corn earworm. J. Econ. Ent 63(6):1967-1970.

Dang, K., Anand, M. and Jotwani, M. G. 1970. Simple improved diet for mass rearing of sorghum stem borer, Chilo zonellus Swinhoe. Indian J. Ent. 32(2):130-133.

David, W. A. L. and Gardiner, B. O. C. 1965. Rearing Peiris brassicae L. larvae on a semi-synthetic diet. Nature 207:882-883

Dulmage, H. T., Martinez, A. J. and Pena, T. 1976. Bioassay of Bacillus thuringiensis (Berliner) δ-endotoxin using the tobacco budworm. Technical Bulletin No. 1528, USDA. ARS pp:1-15.

Getzin, L. W. 1962. Mass rearing of virus free Cabbage loopers on an artificial diet. J. Insect Pathol. 4:486-487.

Ignoffo, C. M. 1965. The nuclear polyhedrosis virus of Heliothis zea (Boddie) and Heliothis virescens (F.). II. Biology and propagation of diet reared Heliothis. J. Invert. Pathol. 7(2):218-226.

Ishii, S. and Urushibara, H. 1954. On fat soluble and water soluble growth factors required by the rice stem borer, Chilo simplex Buttler, Bull. Natn. Inst. Agric. Sci. Tokyo, Ser. C. 4:109-133.

Kulkarni, U. V. and Amonkar, S. V. 1988. Microbial control of Heliothis armigera (Hb.): Part I-Isolation and characterization of a new strain of Bacillus thuringiensis and comparative pathogenicity of three isolates of B. thuringiensis against H. armigera. Indian J. Experimental Biology 26:703-707.

Navon, A., klein, M. and Braun, S. 1990. Bacillus thuringiensis potency bioassays against Heliothis armigera, Erias insulana and Spodoptera littoralis larvae based on standardized diets. J. Invertebr. Pathol. 55:387-393.

Rock, G. C., Glass, E. H. and Patton, R. L. 1964. Axenic rearing of the redbanded leaf roller Argyrotaenia velutinana on meridic diet. Ann. Ent. Soc. Am. 57:617-621.

Shorey, H. H. 1963. A simple artificial rearing medium for cabbage looper. J. Econ. Entomol. 56(4):536-537.

Shorey, H. H. and Hale, R. L. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Ent. 58(3):522-524

Vanderzant, E. S., and Rieser, R. 1956. Studies on the nutrition of the pink bollworm using purified casein media. J. Econ. Ent. 49:454-458.

Vanderzant, E. S., Richardson, C. D. and Fort, S. W. Jr. 1962. Rearing of bollworm on artificial diet. J. Econ. Ent. 55:140.

Susceptibility of larval instars of *Helicoverpa* (*Heliothis*) *armigera* to HD-1-S-1980 and relative toxicities of commercial *Bacillus thuringiensis*

Khalique Ahmed, Feeroza Khalique, Bashir Ahmed Malik, and David N. Ferro*

Pulses Programme
National Agricultural Research Center (NARC)
P.O. NARC, National Park Road, Islamabad, Pakistan

Abstract

Dosage-mortality responses of different instars of *Helicoverpa armigera* (Hübner) were assessed using the *Bacillus thuringiensis* subsp. *kurstaki* Berliner standard (HD-1-S-1980) and commercial *B.t.* products (Dipel 2X WP, Dipel ES, Biobit XL, China *B.t.*, MVP, Florbac FC, and Echotech Pro FC). First, second, and third instars were highly susceptible to HD-1-S-1980 as compared with the fourth instar. Dipel 2X was the most toxic formulation tested against third instar larvae. Probit analysis showed the lowest LC_{50} 21.0 µg/ml and potency 52,000 IU/ml for Dipel 2X as compared with the other commercial products. Percentage mortalities from L_1 to L_4 increased gradually with the increase in toxin concentration per ml of diet. The study warrants field application of *B.t.* and initiation of similar studies for other major pests.

Key words: *Helicoverpa* (*Heliothis*) *armigera* (Hübner); *Bacillus thuringiensis* Berliner; reference standard (HD-1-S-1980); diet *B.t.* bioassay; potency; relative potency.

Introduction

The chickpea pod borer *Helicoverpa* (*Heliothis*) *armigera* (Hünber) is a serious pest of chickpea in Pakistan, particularly during the spring season. Moreover, because of its polyphagous nature, it also causes economic losses to maize, tobacco, tomato, sunflower, cotton, vegetable and fodder crops (Ahmed et al., 1989). In some years, chickpea yield losses due to this insect have reached 90% in Northern Pakistan (Ahmed et al., 1986, 1990).

In recent years, because of the regular and indiscriminate use of broad-spectrum insecticides, many lepidopterous pests, including *H. armigera*, have developed resistance to a number of insecticides. Therefore, it has become imperative to use alternate methods for the control of *H. armigera*. Microbial control involving the use of *Bacillus thuringiensis* is one of alternatives to conventional insecticides for use in the integrated pest management.

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* Department of Entomology, University of Massachusetts, Amherst, MA 01003, USA

Microbial control of insect pests has shown promising results all over the world during the last two decades, as a result, several potent commercial preparations of B.t. have appeared for control of a variety of lepodopterans (Khalique et al., 1989). Most current formulations of B.t. contain the HD-1 strain of the subspecies kurstaki (serovar H3a:3b)(van Frankenhuyzen et al., 1992).

In addition to its general environmental safety, the use of B.t. spares insect parasites and predators which, under favorable conditions, cause up to 41.8% parasitization of younger larvae of H. armigera on chickpea during November (early crop stage), thereby putting a natural check on forthcoming population of this pest (Ahmed et al., 1989). McDonald et al. (1990) demonstrated that the insect parasite Cotesia rubicula can successfully emerge and pupate from B.t.-intoxicated host cabbageworm (Pieris rapae).

Many investigators have conducted studies on the susceptibility of larval instars of various insects and bioassays of B.t. commercial products (Angus and Norris, 1968; Morris, 1973, 1986; Mcgaughey, 1978; Fast and Dimond, 1984; Trottier et al., 1988; Kulkarni and Amonkar, 1988a, 1988b; van Frankenhuyen and Fast, 1989; Navon and Klein, 1990; Brownbridge and Onyango, 1992; van Frankenhuyzen et al., 1992; and Aboul Ela et al., 1993)

However, the susceptibility of early and late first, second, third and fourth instar larvae of H. armigera to bacterial toxin in Pakistan has not been reported. This paper presents results on the relative toxicities of seven commercial B.t. products, comparison of the susceptibility of H. armigera larval instars with the U.S. Department of Agriculture (USDA) reference standard HD-1-S-1980.

Materials and methods

Larvae

Bioassays were carried out on different larval instars of H. armigera (within 24 h of moult). The artificial diet and the rearing techniques used in the experiments were as described by Ahmed and Khalique (1993), with the exception that formaldehyde was omitted from the diet, causing a reduction in the potency of B.t. as reported by Beegle et al. (1981).

This insect develops cannibalism from the late third to the fifth instar. We have developed a technique that allows rearing H. armigera in groups of 90 to 100 larvae per petri dish (17.0 x 3.0 cm), with a thin layer of diet at the bottom and in the cover of the plate. After infestation of the petri dish with about 110 first instar larvae, sterilized two ply tissue paper was folded and placed in the petri dish to soak up excessive diet moisture and allow the developing larvae to move into the tissue paper folds, thereby reducing chances of possible larval cannibalism. In this way, aseptic rearing up to the early third instar larvae for use in

second or third instar larval bioassays was done. This rearing technique significantly reduced the cost of development of second or third instar larvae as compared with the individual rearing technique (one larva/ container).

The rearing of late third and fourth instar larvae was done individually in diet containing glass capsule vials (Ahmed and Khalique, 1993). The rearing was carried out at 25±3 °C. All the bioassays were done at room temperature 25±3 °C, RH 60-80% and 15:9 light and dark cycle. A 25±3 °C rearing temperature is close to the maximum daytime temperature (23-29 °C) typically encountered by *H. armigera* during high infestation in chickpea fields.

Bacterial Toxins

Commercial preparations of *Bacillus thuringiensis* were used: Biobit XL (*B.t. kurstaki*, serotype H-3a,3b) 12,600 IU/µl (eqv. 1.6% active ingredient) and Florbac FC (*B.t. aizawai*, serotype H-7) 8,500 IU/mg, Novo Nordisk, Denmark; MVP (genetically engineered encapsulated delta-endotoxin) (*B.t. kurstaki*, serotype H-3a,3b), Mycogen Corp., USA; Echotech Pro (Condor), Hoechst Pakistan Limited; China *B.t* (*B.t. kurstaki*, serotype H-3a,3b) 16,000 IU/mg, Hubai Academy of Agric. Sciences, Wuhan, China; Dipel 2X (*B.t. kurstaki*, serotype H-3a,3b) 32,000 IU/mg and Dipel ES (*B.t. kurstaki*, serotype H-3a,3b) 17,600 IU/mg, Abbott Laboratories, North Chicago, Illinois, USA; and a 6-year-old refrigerated sample of USDA's reference standard *B.t.* HD-1-S-1980 procured from Sub-Tropical Crops Insect Research, Agriculture Research Service, USDA, Brownsville, Texas, USA.

Preparation of toxin dilutions

Six serial dilutions of each bacterial toxin (spore-δ-endotoxin) were prepared using USDA's serial dilution technique as described by Anonymous (1982). The dilutions were prepared in fresh phosphate buffer (7.0 pH). The dilutions were kept in 250 ml capacity sterilized flasks. From each dilution, 10.0 ml of bacterial toxin suspension was removed by pipette and mixed into 390.0 ml freshly prepared diet, making the final diet-*B.t.* mixture a volume of 400.0 ml. The diet was kept at 60 to 70 °C to prevent it from solidification. The final diet-*B.t.* serial dilutions were 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 µg/ml diet (Khalique et al., 1989; Ahmed et al., 1988).

The diet formula (under consideration for publication) used for preparation of a 4.0 liter batch was agar 25.0 g, Cowpea powder (*Vigna unguiculata* L. Walp) 600.0 g, ascorbic acid 7.0 g, sorbic acid 3.0 g, vitamin E 0.02 g, dried active yeast (granule) 20.0 g, methyl-para-hydroxybenzoate 10.0 g, vitamin mixture 5.0 ml, and tap water 3500.0 ml.

The vitamin mixture formula for preparation of a 200.0 ml stock solution contained calcium pentothanate 4.8 g, nicotine acidamide 2.4 g, riboflavin 1.2 g, folic acid 1.2 g, thiamine hydrochloride 0.6 g, pyridoxine hydrochloride 0.6 g, biotin 0.048 g, and vitamin B_{12} 0.0024 g.

Five ml of the liquid diet (kept at 70 °C) from each toxin dilution was poured in sterilized standard size glass capsule vials (2.5 cm in diameter and 5.5 cm high).

Bioassay

For each dilution and each replicate, a batch of 20 vials was filled with the diet containing the respective toxin dilution. Each vial was infested with one H. armigera larva of the appropriate instar (Table 1). The vials were plugged with sterilized cotton wool and inverted to minimize escape of moisture from the diet. Four replications of each concentration were maintained with their respective controls. Average body weight of the larva/instar (within each instar) was also recorded. In each bioassay, HD-1-S-1980 (having an assigned potency of 16,000 IU/mg of powder) was used as the standard (Beegle et al., 1986).

Records

After 7 days of incubation, the numbers of live and dead larvae were counted. The LC_{50} values for each B.t. were established through a Probit Analysis computer programme (Raymond, 1985). The potencies and relative potencies were calculated by the following formulae (Dulmage et al., 1976, 1981; van Frankenhuyzen and Fast, 1989)

$$\text{Potency of test sample} = \frac{LC_{50} \text{ of standard} \times \text{Potency of Standard}}{LC_{50} \text{ of test sample}}$$

$$\text{Relative potency} = \frac{LC_{50} \text{ of standard}}{LC_{50} \text{ test sample}}$$

A range test (DMRT) to compare the LC_{50}'s for different larval instars was calculated on the basis of instar-wise larval mortalities observed in the respective toxin concentrations of each bioassay using the MSTAT-C computer program (version 1.42, developed by Michigan State University, USA)

Results and discussion

The use of artificial diet as food in assessing dosage-mortality response with B. thuringiensis is advantageous from the standpoint of obtaining more precise observations. The food quality

affects the amount consumed by the test insects and artificial media are more easily reproducible than foliage (Morris, 1973).

The results of bioassay on the susceptibility of different larval instars are compared in Table 2. The median lethal concentration for the early first instar (42.2 μg/ml diet) was significantly different from the early and late fourth instar. The slope of the response by the early first instar to B.t. concentrations was greater (slope 4.03) than that of the other instars. The LC_{50}'s from early first to early third instars (ranging from 42.02 to 59.9 μg/ml diet) were significantly different from the LC_{50}'s of the early and late fourth instar larvae (9.91 and 119.9 μg/ml, respectively).

Kulkarni and Amonkar (1988) reported similar results, showing that the first instars of H. armigera were highly susceptible to B.t. strains (ISPC-1, ISPC-4, and ISPC-7), and that the older instars were less susceptible, but took more time to achieve similar results. At 20 °C rearing temperature, younger larval instars (L_3 to L_4) of the bertha armyworm, Mamestra configurata, were more sensitive to Dipel 132 (B. thuringiensis var. kurstaki) than older larvae (Morris, 1986). Fast and Dimond (1984) reported that diet incorporated bioassays detected no difference in the susceptiblity of third, fourth, and fifth instars of the Spruce budworm, Choristoneura fumiferana, exposed to B.t., in terms of either dose, LC_{50}, or LT_{50}. High second instar larval susceptibility to B.t. (Dipel 2X) was found, followed by L_5 while L_3 and L_4 were less susceptible in the case of the spotted stem borer, Chilo partellus (Brownbridge and Onyango, 1992). Our studies showed correspondence with the results of the aforementioned work.

Overall results (Table 2) showed a general instar-wise larval susceptibility to the bacterial toxin in decreasing order from younger to older instar. These results also showed correspondence with the findings of McGhaughey (1978), who reported that first instar larvae of the almond moth, Ephestia cautella (Walker), were more susceptible than second, third, fourth and fifth instars.

Table 3 shows LC_{50}'s, potencies, and relative toxicities of different B.t. commercial products with third instar larvae of H. armigera. Dipel 2X (B.t. var. kurstaki) had the lowest LC_{50} (21.0 μg/ml) and the highest potency (52,000 IU/mg) and relative potency (3.20) when compared with the activity of the other products. This finding corresponds with the finding of Brownbridge and Onyango (1992), who reported that Dipel 2X appeared to be the most toxic formulation against all the instars of the cereal stem borer Chilo pertellus (Swinhoe). Dipel ES, MVP, and Echotech Pro ranked second, third, and fourth, respectively in toxicity, with LC_{50},s 40.4, 45.1 and 48.0 μg/ml diet; potencies 43,000, 38,000, and 30,000; and relative potencies 2.70, 2.40 and 1.9. Novon and Klein (1990) reported that HD-263 (B.t. kurstaki had superior activity (LC_{50} 0.53 μg/g and potency 42,263). The commercial product Florbac FC (B.t. aizawai) showed extremely low activity (LC_{50} 979.8 μg/ml).

Aboul Ela et al. (1993) reported large differences in the susceptibility of the greasy cutworm, Agrotis ypsilon, when exposed to various B.t. varieties.

The overall results in Table 3 indicate that Dipel 2X was the most potent B.t. commercial product followed by Dipel ES, MVP, and Echotech Pro, against third instar larvae of the chickpea pod borer, H. armigera.

Data on percentage mortality, illustrated in Figure 1, indicated a nonsignificant difference among the instars at concentration 2.5 µg/ml, thus showing equal susceptibility to the toxin (HD-1-S-1980). At 5.0 µg/ml, only the mortality of the early first instar was significantly different from that of the other instars. At 10.0 µg/ml, the mortality of the late first instar was significantly higher than that of the late second, early and late third and late fourth instars, indicating a narrow variation in susceptibility to the toxin within instars. Late first instar larvae also showed significantly high mortality at 20.0 µg/ml, while a low mortality percentage was recorded in the late fourth instar.

At 40.0 µg toxin/ml concentration (Fig. 1.), the smaller larvae (first instar) showed significantly higher mortality than that of early second, early and late third and fourth instars. A more or less similar mortality pattern to that at 40.0 µg/ml was observed at 80.0 µg/ml.

In general, the pattern of concentration-mortality response of the insects showed a gradual increase in mortality among all the instars from 2.5 to 80.0 µg toxin/ml diet. This pattern of mortality percentage corresponds with the observations of McGhaughey (1978), who reported a gradual increase in instar-wise larval mortality percentage of the almond moth, Eshestia cautella (Walker), and the Indian meal moth, Plodia interpuntella (Hübner), with an increase in toxin concentration. Moreover, McGhaughey observed significant differences in the mortalities of larval instars of both moths at the highest toxin concentration. Khalique et al. (1982), also reported an increase in larval mortalities of H. armigera with an increase in B.t. toxin concentration.

Conclusion

Laboratory data (bioassays and toxicities of commercial products) indicate that first to third instar larvae of H. armigera are highly susceptibility to bacterial toxin (HD-1-S-1980). The bacteriological insecticides (Dipel 2X and Dipel ES) proved to be highly effective, and can be tested to achieve better results for control of first, second, and third instar larvae of this insect that infests chickpea fields. The dosage-mortality response of the insect also indicates that the bio-insecticides have the scope for application to control major lepidopterous pests of other crops.

In addition, the use of bio-insecticides (*B.t.*) could drastically reduce the amount of broadly toxic chemical insecticides released into the agro-ecosystem of Pakistan. Nevertheless, more research of a similar type on major pests of vegetables and other crops is warranted.

Table 1. *Helicoverpa armigera* larval instar size ranges (reared on diet developed by Ahmed et al., 1993 at 25±3 °C

Instar	Size (mm)	Instar	Size (mm)
1st, early	1.5-2.0	3rd, early	8.5-10-0
late	2.5-3.0	late	12.0-14.0
2nd, early	4.0-5.0	4th, early	15.5-18.0
late	6.0-7.0	late	20.5-23.0

Table 2. Mortality response of *H. armigera* (by stages within each instar) caused by different doses of International Reference Standard HD-1-S-1980

Instar of *H. armigera*	Wt./[1] larva (mg)	LC_{50} µg/ml	95% confidence limits Lower	Upper	Slope ± SE
Early 1st	0.07	42.2 a	37.4	47.5	4.03***±0.48
Late 1st	0.20	37.4 a	30.8	45.8	2.22**±0.31
Early 2nd	0.54	40.9 a	35.7	47.5	2.83**±0.39
Late 2nd	1.40	44.6 a	34.3	59.5	3.00**±0.39
Early 3rd	6.10	59.9 a	52.9	68.4	4.29**±0.68
Late 3rd	27.00	74.1 ab	62.5	96.4	3.09**±0.57
Early 4th	55.00	99.1 b	81.6	154.6	3.57*±0.86
Late 4th	113.00	119.9 c	<----------nsr---------->		

[1] avg. wt. of 50 larvae for late 1st to late 4th instars, 100 larvae for early 1st instar. *** Significant at $p<0.001$; ** Significant at $p<0.01$; * Significant at $p<0.05$;
nsr = nonsignificant regression.

Table 3. Determination of LC_{50} and potencies of *B. thuringiensis* preparations against third instar larvae of *H. armigera*

B. t. preparation	LC_{50} µg/ml	Potency IU/mg	Relative potency	95% confidence limit Lower	Upper	Slope±SE
Biobit XL (12,600 IU/mg)	99.2	7,300	0.54	80.7	153.7	3.20*±0.71
HD-1-S-1980	44.6					
Dipel 2X (32,000 IU/mg)	21.0	52,000	3.20	18.2	24.4	2.46***±0.21
HD-1-S-1980	67.8					
China *B.t.* (16.000 IU/mg)	106.0	10,200	0.64	80.5	181.7	2.29*±0.46
HD-1-S-1980	67.8					
Dipel ES (17,600 IU/mg)	40.4	43,200	2.70	32.3	53.5	1.47**±0.16
HD-1-S-1980	107.9					
MVP (Mycogen	45.1	38,000	2.40	39.2	52.6	2.80**±0.31
HD-1-S-1980	107.9					
Florbac F[illegible] 8,500 IU/[illegible]g	979.8	1,300	<----------nsr---------->			
HD-1-S-19[illegible]0	82.5					
Echotech Pro	48.0	30,000	1.9	42.5	55.1	3.5**±0.42
HD-1-S-1980	90.4					

*** Significant at $p<0.001$; ** Significant at $p<0.01$;
* Significant at $p<0.05$; nsr = nonsignificant regression.

References

Ahmed, K., Khalique, F. and Malik, B. A. 1989. Studies on Helicoverpa (Heliothis) armigera management in Pakistan. Proc. 1st. Intnal. Conf. Econ. Entomol. 2: 153-161.

Ahmed, K; Raja, N. U; Afzal, M., Khalique, F. and Malik, B.A. 1988. Bio-efficacy of four strains of B. thuringiensis Berliner) against hairy caterpillar Diacrisia obliqua Pak. J. Sci. Ind. Res., 31 (9): 637-639.

Ahmed, K., Khalique, F., Afzal, M. and Malik, B. A. 1986. Pulses Entomology Report, Food Legumes Improvement Programme, National Agric. Res. Center, Pak. Agric. Res. Council. pp 31-39.

Ahmed, K.,Lal. S.S, Morris, H., Khalique, F. and Malik, B.A.1990. Insect pest problems and recent approaches to solving them on chickpea in South-Asia. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Chickpea in the Nineties: proceeding of the Second International Workshop on Chickpea Improvement, 4-8 Dec 1989, ICRISAT Center, Patancheru A.P. India pp165-168

Ahmed, K. and Khalique, F.1993. Mass rearing of chickpea pod borer, Helicoverpa armigera on artificial diet. Annual Report "Bio-control of chickpea pod borer research project (PAK-CS-PB-14), Food Legumes Improvement Programme, National Agricultural Research Center, Pakistan Agricultural Research Council, Islamabad, Pakistan pp 9-11

Aboul Ela, R., Salama, H. and Ragai, M. 1993. Assay of Bacillus thuringiensis (Berl.) isolates against the greasy cutworm, Agrotis ypsilon (Rott.) (Lep: Noctuidae). J. Appl. Ent. 116:151-155.

Angus, T. A. and Norris, J. R. 1968. A comparison of the toxicity of some varieties of Bacillus thuringiensis Berliner for silk worm larvae. Jr. Invertebr. Pathol. 11, 289-295.

Anonymous. 1982. Investigations on new strains of Bacillus thuringiensis (B.t.) affecting Lepidopterous Pests. (B.t. Research Project), Department of Microbiology, University of Karachi. pp 66-78.

Beegle, C. C., Cauch, T. R., Alls, R. T., Versoi, P. L. and Lee, B. L. 1986. Standardization of HD-1-S-1980: US standard for assay of lepidopterous-active Bacillus thuringiensis. Bull. Entomol. Soc Am. 32: 44-45.

Beegle, C. C., Lewis, L. C., Lynch, R. E. and Martinez, A. J. 1981. Interaction of larval age and antibiotic on the susceptibility of three insect species to Bacillus thuringiensis. J. Invertebr. Pathol., 37: 143-153.

Brownbridge, M. and Onyango, T. 1992. Laboratory evaluation of four commercial preparations of Bacillus thuringiensis (Berliner) against the spotted stem borer, Chilo partellus (Swinhoe) (Lep., Pyralidae). J. appl. Ent., 113: 156-167.

Dulmage, H. T., Martinez, A. J. and Pena, T. 1976. Bioassay of Bacillus thuringiensis (Berliner) δ-endotoxin using the tobacco budworm. Technical Bulletin No. 1528, USDA. ARS pp:1-15.

Dulmage, H. T. and Cooperators. 1981. Insecticidal activity of isolates of Bacillus thuringiensis and their potential for pest control. In Microbial control of insect pests and plant diseases 1970-80 Edited by H. D. Burges, Academic Press, New York, pp 193-222.

Fast, P.G. and Dimond, J. B. 1984. Susceptibility of larval instars of spruce budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae), to Bacillus thuringiensis Can. Ent. 116: 131-137.

Khalique, F., Ahmed , K., and Afzal, M. 1989. Evaluation of Bacillus thuringiensis Berliner against chickpea pod borer. Pak. J. Sci. Indust. Res., 32(2):114-116.

Khalique, F., Ahmed , K., Khan, A. F., Shaikh, M. R., and Shaikh, D. 1982. Effect of spore-δ-endotoxin of Bacillus thuringiensis on the development of corn earworm, Heliothis armigera (Hübn.). Pakistan J. Sci. Indust. Res. 25(1-2): 28-30.

Kulkarni, U. V. and Amonkar, S. V. 1988a. Microbial control of Heliothis armigera (Hb.): Part II-Relative toxicities of spore and crystals of Bacillus thuringiensis varieties to H. armigera and their efficacy in field control. Indian J. Experimental Biology 26: 708-711

Kulkarni, U. V. and Amonkar, S. V. 1988b. Microbial control of Heliothis armigera (Hb.): Part I-Isolation and characterization of a new strain Bacillus thuringiensis and comparative pathogenicity of three isolates of B. thuringiensis against H. armigera. Indian J. Experimental Biology 26: 703-707.

McDonald, R. C., Kok, L. T. and Yousten, A. A. 1990. Response of fourth instar Pieris rapae parasitized by the braconid Cotesia rubecula to Bacillus thuringiensis subsp. kurstaki δ-endotoxin. J. Invertebr. Pathol. 59:422-423.

McGaughey, W. H. 1978. Effects of larval age on the susceptibility of almond moths and Indian meal moths to Bacillus thuringiensis. J. Econ. Entomol. 71: 923-925.

Morris, O. N. 1986. Susceptibility of the Bertha army worm, Mamestra configurata (Lepidoptera: Noctuidae), to commercial formulations of Bacillus thuringiensis var. Kurstaki Can. Ent. 118: 473-477.

Morris, O. N. 1973. Dosage-mortality studies with commercial Bacillus thuringiensis sprayed in modified potter's tower against some forest insects. J. Invertebr. Pathol. 22:108-114.

Novon, A., Klein, M. and Braun, S. 1990. Bacillus thuringiensis potency bioassays against Heliothis armigera, Erias insulina and Spodoptera littoralis larvae based on standardized diets. J. Invertebr. Pathol. 55:387-393.

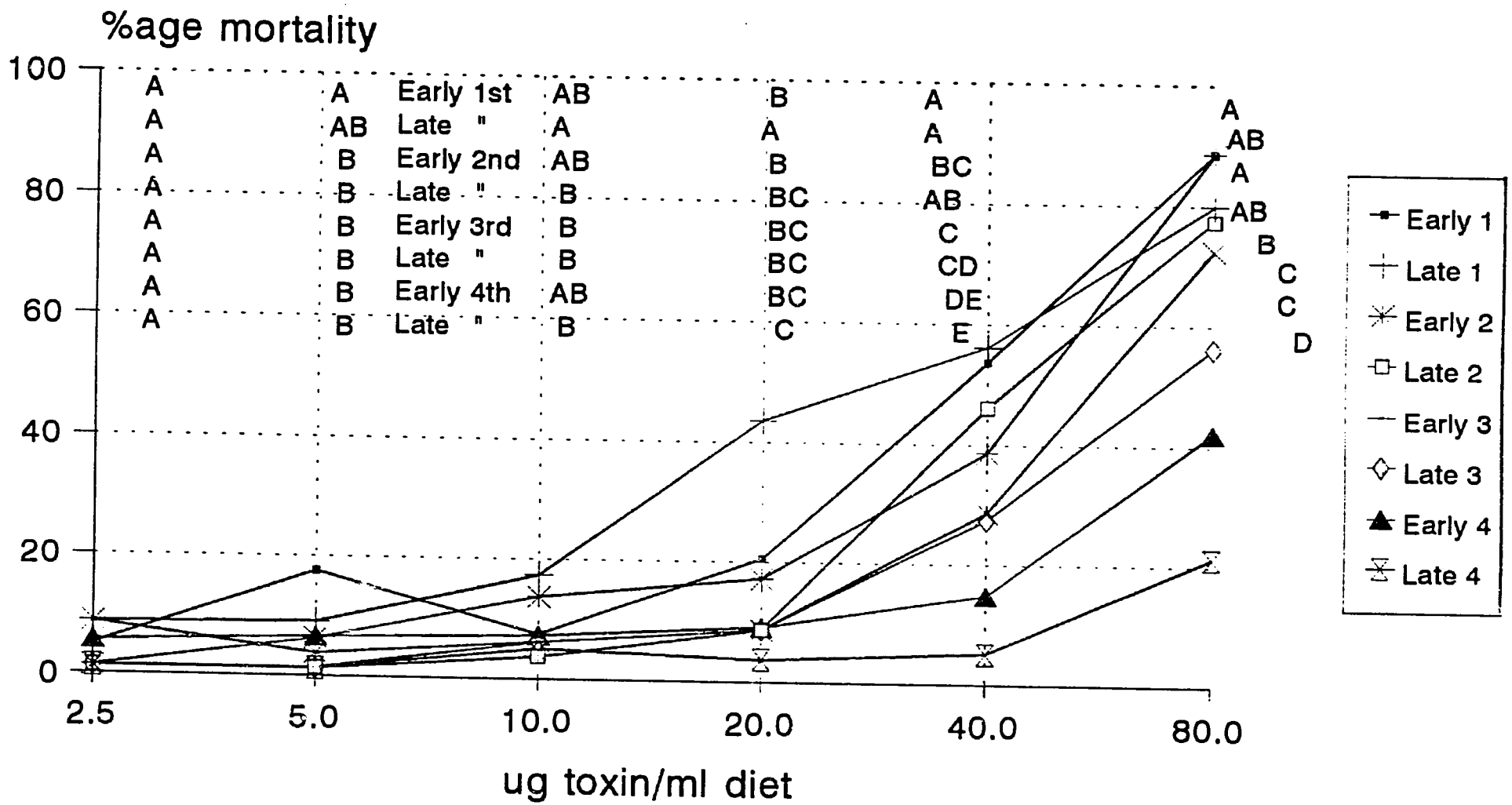
Raymond, M. 1985. Presentation d'un porgramme d'analyse log-porbit pour micro-ordinnateur. Cah. ORSTOM, Ser. Ent. med et Parasitol., 22(2):

Trottier, M. R., Morris, O. N., and Dulmage, H. T. 1988. Susceptibility of the bertha armyworm, Mamestra configurata (Lepidoptera, Noctuidae) to 61 strains from 10 varieties of Bacillus thuringiensis Berliner. J. Invertebr. Pathol. 51:242-249.

van Frankenhuyzen, K., Milne, R., Brousseau, R. and Masson, L. 1992. Comparative toxicity of the HD-1-S-1980 and NRD-12 strains of Bacillus thuringiensis subsp. kurstaki to defoliating forest lepidoptera. J. Invertebr. Pathol. 59:149-154.

Van Frankenhuyzen, K; and Fast, P. G. 1989. Susceptibility of three coniferophagous Choristoneura species (Lepidoptera: Tortricidae) to Bacillus thuringiensis var. Kurstaki. J. Econ. Entomol. 82 (1): 193-196.

Fig 1. Percentage mortalities of *H. armigera* L_1 to L_4 and within each instar due to various concens. of HD-1-S-1980.



Points which do not share common letters are significantly different at $P<0.05$

Repellency and Growth-Inhibitory Effects of Indigenous Plant/Marine Animal Extracts Against Red Flour Beetle, *Tribolium castaneum* (Herbst) Coleoptera: Tenebrionidae

Noor Ullah, Syed Asim Rehan Kazmi, Farzana Ibrahim and Rahila Nazli

Grain Storage Research Laboratory
Tropical Agricultural Research Institute
PARC, Karachi University Campus
Karachi-75270, Pakistan.

ABSTRACT

Extracts of two plant and two marine animal species were tested as repellents and growth inhibitors against red flour beetle, *Tribolium castaneum* (Herbst). n-Hexane extract of *Zoanthid* species (600 and 300 $\mu g/cm^2$) provided 63.1% repellency over an 8-week period, which was significantly higher than the repellency of *Curcuma longa* extract (a reference botanical repellent) at these dose levels. Extract of *Artemisia dubia* and *Aplysia juliana* provided 53 and 41.6% average repellency at a dose rate of 600 $\mu g/cm^2$, respectively. Ethanol extracts of the same materials significantly reduced numbers of larvae, pupae, and F_1 adults when test insects were reared on treated rice grain. Weights of the larvae feeding on treated rice were significantly lower. However, pupal and adult weights remained unaffected. These materials have shown promising repellent and growth-inhibiting effects, and can be used in development of botanical grain protectants.

Key words: Repellency, Growth inhibition, Plant extracts, Marine animal extracts, *Tribolium castaneum*

INTRODUCTION

Ecological sustainability has become a key consideration in all aspects of technology development, including insect pest control. Most insecticides currently in use are synthetic, nonselective, or toxic chemicals that have had serious social and environmental repercussions. The poisoning of livestock, fish, wildlife, other beneficial organisms, and humans is well known and has been linked with increased insecticide use, especially in developing countries.

Pest resurgence and a widespread increase in the development of insecticide resistance in insects are other inherent problems associated with the use of synthetic insecticides. There is a great need to develop new insecticides that are pest-specific, nontoxic to humans and beneficial organisms, biodegradable, less prone to insect resistance and resurgence, and less expensive. Development of insecticides from indigenous plants or marine animals may be a solution to these problems.

Traditional use of plant materials to protect stored grains and woolen clothes from insect attack has been documented (Golob and Webley, 1980). Grainge and Ahmed (1988) have prepared a database of more than 1800 plant species occurring worldwide that possess anti-insect properties. Jacobson (1958, 1975) has listed several naturally occurring plants having insecticidal properties. A bibliographic database of various botanical materials has been prepared, indicating their pest control potential (Rees and Dales, 1993). Several plant species indigenous to Pakistan have been tested for their repellency, toxicity, and feeding-deterrent properties against insect pests of stored grains. Fewer and underweight larvae, pupae, and adults of *Tribolium castaneum* (Herbst) were produced when parent adults were fed on wheat flour treated with turmeric (*Curcuma longa* L.) oil at a rate of 200 ppm (Jilani et al., 1988). Similarly, turmeric oil exhibited repellent and feeding-deterrent activities against lesser grain borer (*Rhizopertha dominica* F.) adults (Jilani and Saxena, 1990).

Repellency of several plant materials against *T. castaneum* adults has been reported (Jilani et al., 1989; Ullah et al., 1990;

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Jilani et al., 1991). In addition to plant sources, marine fauna indigenous to Pakistan can be studied for their anti-insect properties since some have exhibited medicinal activity (Rahman et al., 1989; Tischler et al., 1991). The study reported here was undertaken to screen some new materials from plant and marine animal sources for their repellent and growth-inhibitory effects against a major insect pest of stored grain.

MATERIALS AND METHODS

Preparation of Materials

Plant materials and marine animals were collected from areas surrounding Karachi and Karachi coast, respectively. The extracts were prepared by H.E.J Research Institute of Chemistry, University of Karachi. First, 1 kg of natural material was ultra-turraxed (high-speed maceration) in 80% ethanol. The resulting mixture was then filtered and the filtrate evaporated under vacuum to obtain solid material. This solid gum was dissolved in n-Hexane, and the portion soluble in n-Hexane was removed by filtration. The hexane-insoluble portion was dissolved in ethanol. Both the n-hexane and ethanol portions were concentrated separately under vacuum to obtain their respective extracts. These final extracts were used to prepare stock solutions in acetone, which were diluted to the desired concentrations for application on filter paper or rice grain. The n-Hexane extracts were used for repellency evaluation, and the ethanol extracts for evaluation of growth-inhibitory effects.

Rearing of Insects

Adults of *Tribolium castaneum* (Herbst) were reared in the laboratory on whole wheat flour containing brewer's yeast (98:2) in 1 lb glass jars under controlled conditions of 29 ± 1 °C and 60 ± 5% r.h.

Repellency Evaluation

The repellency of n-Hexane extracts of test materials was evaluated using the method described by McDonald et al. (1970). Whatman No. 1 filter paper strips (10 x 8 cm) were dipped individually in solutions of the test materials in acetone to get deposits of 150, 300, and 600 $\mu g/cm^2$. Acetone-treated strips served as controls. Solvent was evaporated from these strips in a fume hood for complete dryness. Each strip was then attached lengthwise, edge-to-edge, to a control strip using cellulose tape on the reverse side. A glass ring (2.5 cm high, 7 cm dia) was placed over the two matched strips so that the joined edge bisected the ring.

Ten adult insects (1-2 weeks old) were then released in the middle of the level arena within the glass ring at 0800 hr. The individuals settled on treated and control halves were counted at 0900 and 1500 hr daily for 5 consecutive days. Tests were repeated with the same strips during the second, fourth, and eighth weeks after treatment, using fresh insects each time. The average insect count of each 5-day period was converted to percent repellency using the formula of Leonard and Ehrman (1976):

$$\text{Percent Repellency} = \frac{Nc - Nt}{NT} \times 100$$

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where:

Nc = number of insects found on untreated side
Nt = number of insects found on treated side
NT = total number of insects found on both sides

The overall repellency of the different treatments was compared statistically. Each treatment was replicated four times.

Growth-Inhibition Studies

For these studies, 150 gr of polished rice grain (IR-6 variety) was treated with ethanol extract of plant or marine animal at three dose rates -- 0.1, 0.05 and 0.025% (wt/wt)-- in 1 lb glass jars. The materials were pipetted onto grain while the jar was shaken for uniform application. Each jar was incubated with 50 adult beetles (mixed sexes) under the same conditions used for rearing of insects. Adult beetles were allowed to oviposit for 7 days, then removed. The numbers and weights of larvae, pupae, and F_1 adults that emerged from the treated and untreated samples were recorded. Control samples were treated with acetone only while master controls were untreated samples. Data were statistically analyzed. Each treatment was replicated three times.

RESULTS AND DISCUSSION

Results on the repellency of two marine animal and three plant extracts against adults of *Tribolium castaneum* are provided in Table 1. Extract of *Curcuma longa* was used as a reference repellent material since it has already exhibited promising repellent effects against the test insects under laboratory conditions. Extract of *Zoanthid* Spp. at 600 and 300 $\mu g/cm^2$ dose rates provided an average

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of 63.1% repellency, which was significantly higher than the repellency of *C. longa* extract at the same dose rates. At a dose rate of 150 $\mu g/cm^2$ this extract provided 58.6% repellency, which again was significantly higher than the repellency of *C. longa* extract. Extract of *Artemisia dubia* also provided significantly higher repellency values than *C. longa*, but significantly lower values than *Zoanthid* Spp. extract at all dose rates tested. Extract of *Aplysia juliana* exhibited almost the same repellency as that of *C. longa*. These results indicate a promising repellent effect of *Zoanthid* Spp., which is a microscopic marine animal found in and around the Karachi coast. Extracts of *A. dubia* and *A. juliana* followed in repellency, indicating their potential as repellents as well. These three materials can be further fractionated chemically to isolate and identify pure fractions/compounds responsible for their repellent action.

Extract of *Sesbania sesban* provided an average repellency of 32.38% at a dose rate of 600 $\mu g/cm^2$, which is significantly lower than the repellency of *C. longa* (Table 1). The repellency of this material at lower doses was also lower. The data indicate relatively less potential for this material as a repellent.

The growth-inhibitory effects of ethanol extracts of these materials against *T. castaneum* were also studied. Mean numbers of F_1 progeny (larvae, pupae, and adults) and their weights are provided in Tables 2 and 3, respectively. Rice grain treated with *Zoanthid* spp. extract at all dose rates provided significantly fewer numbers of F_1 progeny than did the control and master control (Table 2). Similarly, the numbers of F_1 progeny that emerged from

rice grain treated with *A. dubia* and *A. juliana* were significantly lower than in the *C. longa*, control, and master control samples. The numbers of progeny that emerged from the *C. longa*-treated rice were not different from those of the controls, a result that would be expected since this material has been shown to be a strong repellent (Su et al., 1982).

The weights of larvae that emerged from rice grain treated with *A. juliana*, *Zoanthid* spp., and *A. dubia* extracts were significantly lower than with the *C. longa*, control, and master control (Table 3), indicating less feeding activity of the larvae in treated grain. Although the weights of pupae and adults were not significantly different among the treatments, their numbers were significantly lower in the treated grains than in the controls (Table 1).

The repellent activity of *C. longa* has been attributed to the presence of two sesquiterpene compounds, turmeron and ar-termeron (Su et al., 1982). Similarly, *Artemisia* spp. contain some monoterpene compounds, such as camphor, borneol, and cineol, that are strong repellents to stored-product insects (Ullah, 1990). It is envisaged that n-Hexane extracts of the materials tested in these studies may contain similar chemical components that are responsible for repellent activity. Ethanol extracts of several plant species have exhibited growth-inhibitory effects against some stored-grain insects (Jilani et al., 1988; Jilani and Saxena, 1990). The activity has been attributed to the presence of some antifeedant and toxic components, such as β-Asaron from *Acorus calamus* oil. Further chemical fractionation of the extracts found

promising in the present study is required to identify their active principles so they can be used in developing pesticides based on indigenous resources of Pakistan.

CONCLUSIONS

The present screening of new materials has indicated that *Zoanthid* Spp., *Artemisia dubia*, and *Aplysia juliana* are promising repellents that have exhibited growth-inhibitory effects against the red flour beetle, *Tribolium castaneum*. These materials can be used for further chemical fractionation, isolation, and identification of the active component(s)/compound(s) through various chromatographic techniques. This process may lead to the development of new insecticide(s) based on indigenous resources that are economical, readily available, and environmentally safe.

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REFERENCES

Golob, P. and Webley D.J. (1980) The use of plants and minerals as traditional protectants of stored products. TPI report G 138, 32 pp.

Grainge, M. and Ahmed S. (1988) Handbook of plants with pest control properties. John Wiley & sons, 470 pp.

Jacobson, M. (1958) Insecticides from plants: a review of literature. 1941-1953. USDA Agric. Handb. 134, 299 pp.

Jacobson, M. (1975) Insecticides from plants: a review of literature. 1954-1971. USDA Agric. Handb. 464, 138 pp.

Jilani, G. and Saxena R.C. (1990) Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). J. econ. Ent. 83, 629-634.

Jilani, G. Saxena R.C. and Rueda B.P. (1988) Repellent and growth inhibitory effects of turmeric oil, sweetflag oil, neem oil and "Margosan O" on red flour beetle (Coleoptera: Tenebrionidae). J. econ. Ent. 81, 1226-1230.

Jilani, G., Ullah N. and Ghiasuddin (1989) Repellency of some plant extracts against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)-I. Pak. Entomol. 11, 18-22.

Jilani, G., Ullah N., Ghiasuddin and Khan M.I. (1991) Repellency of some plant extracts against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)-II. Pak. Entomol. 13, 5-8.

Leonard J.E. and Ehrman L. (1976) Recognition and sexual selection in *Drosophila*: Classification, quantification and identification. Science 193, 693-695.

McDonald L.L., Guy R.H. and Speirs R.D. (1970) Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects-1. USDA Mktg. Res. Rpt. 882, 8 pp.

Rahman A., Alvi K.A., Abbas S.A., Chaudhary M.I. and Clardy J. (1989) Zoanthaminone, a new triterpenoidal alkaloid from marine *Zoanthid*. Tetrahedron Lett. 30, 6825.

Rees D.P. and Dales M.J. (1993) Alternative methods for the control of stored product insect pests: A bibliographic database. Chatham. Natural Resources Institute, England.

Su H.C.F., Robert H. and Jilani G. (1982) Isolation, purification and chemical characterization of insect repellents from *Curcuma longa* L. J. Agric. Food Chem. 30, 290-292.

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Tischler M., Andersen R.J., Chaudhary M.I. and Clardy J. (1991) Terpenoids from the sponge *Aplysilla glacialis* and specimens of the Nudibranch *Cadilina luteomarginta* found on the sponge. J. Org. Chem. 56, 42.

Ullah N. (1990) White sagebrush, *Artemisia ludoviciana* Nutt. (Asteraceae) flower extract as a protectant of wheat against stored-product insects. Ph.D. dissertation, Kansas State University, Manhattan, Kansas, USA. 141 pp.

Ullah N., Jilani G., Khan M.I. and Ghiasuddin (1990) Repellency of turmeric and its derivatives applied to rice against red flour beetle, *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae). Pak. Entomol. 12, 38-41.

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Table 1. Repellency of two marine animal and three plant extracts against adults of *Tribolium castaneum* at three dose levels over 8 weeks.

Extract	Dose ($\mu g/cm^2$)	Mean* percent repellency after				Average Repellency
		1 week	2 weeks	4 weeks	8 weeks	
Aplysia juliana	600	35.5	44.3	55.5	31.0	41.6 ef
	300	33.0	25.5	42.5	46.0	36.0 efg
	150	37.5	23.0	26.5	21.5	27.1 ghi
Zoanthid Spp.	600	54.0	58.0	73.0	67.5	63.1 a
	300	50.5	70.5	62.0	69.5	63.1 a
	150	45.0	60.5	60.5	68.5	58.6 ab
Sesbania sesban	600	33.5	25.0	35.5	35.5	32.4 fgh
	300	16.5	17.5	39.0	30.5	26.0 hi
	150	28.0	23.5	36.0	35.0	30.6 gh
Artemisia dubia	600	58.5	52.0	47.0	54.5	53.0 bc
	300	51.0	55.0	49.0	49.0	51.0 bcd
	150	53.0	35.0	28.5	54.5	42.9 de
Curcuma longa	600	60.0	27.5	43.0	48.0	44.6 cde
	300	41.5	29.0	45.0	25.8	35.3 e-h
	150	40.0	14.0	27.5	35.5	29.3 ghi
Control	-	7.0	21.5	20.5	33.3	20.6 i

* Each value is a mean of four replicates. Values followed by the same letters are not significantly different at alpha = 0.05. LSD = 9.4

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Table 2. Mean Numbers of F_1 progeny of *Tribolium castaneum* on rice grain treated with two marine animal and three plant extracts at three dose levels.

Extract	Dose (% w/w)	Numbers* of different stages		
		Larvae (18 days)	Pupae (25 days)	Adults (45 days)
Aplysia juliana	0.100	42.00 cde	40.00 de	38.00 cde
	0.050	64.00 b-e	61.33 cde	48.00 cde
	0.025	74.00 b-e	62.00 cde	50.00 cde
Zoanthid Spp.	0.100	24.00 e	32.00 e	18.00 e
	0.050	36.00 de	30.00 e	28.00 de
	0.025	72.00 b-e	66.00 cde	62.00 cde
Sesbania sesban	0.100	109.00 a-d	96.33 b-e	85.00 b-e
	0.050	168.67 a	158.67 ab	152.67 ab
	0.025	119.67 abc	111.67 a-d	106.33 abc
Artemisia dubia	0.100	74.00 b-e	50.00 cde	46.00 cde
	0.050	124.00 abc	114.00 a-d	100.00 a-d
	0.025	84.00 b-e	72.00 cde	62.00 cde
Curcuma longa	0.100	180.33 a	162.00 ab	151.67 ab
	0.050	136.33 ab	120.67 abc	103.33 abc
	0.025	168.33 a	154.67 ab	137.33 ab
Control	-	174.33 a	166.33 ab	157.33 ab
Master Control	-	183.33 a	175.00 a	163.67 a
LSD		83.37	77.04	73.34

* Each value is a mean of three replicates. Values in columns followed by the same letters are not significantly different at alpha = 0.05.

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Table 3. Mean weights (mg) of F_1 progeny of *Tribolium castaneum* on rice grain treated with two marine animal and three plant extracts at three dose levels.

Extract	Dose (% w/w)	Weights* of different stages		
		Larvae (18 days)	Pupae (25 days)	Adults (45 days)
Aplysia juliana	0.100	0.20 b	0.71 a	0.46 a
	0.050	0.43 b	1.37 a	0.84 a
	0.025	0.55 b	1.44 a	0.92 a
Zoanthid Spp.	0.100	0.20 b	1.14 a	1.07 a
	0.050	0.26 b	1.42 a	0.75 a
	0.025	0.37 b	2.08 a	1.04 a
Sesbania sesban	0.100	1.78 a	1.52 a	1.57 a
	0.050	1.83 a	1.89 a	1.40 a
	0.025	1.79 a	1.55 a	1.50 a
Artemisia dubia	0.100	0.29 b	1.40 a	0.59 a
	0.050	0.56 b	2.05 a	1.25 a
	0.025	0.38 b	1.91 a	1.23 a
Curcuma longa	0.100	1.69 a	1.46 a	1.23 a
	0.050	1.72 a	1.58 a	1.51 a
	0.025	1.72 a	1.67 a	1.36 a
Control	-	1.60 a	1.44 a	1.41 a
Master Control	-	1.62 a	1.68 a	1.28 a
LSD		0.40	NS	NS

* Each value is a mean of three replicates. Values in columns followed by the same letters are not significantly different at alpha = 0.05.

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Evaluation of Some Plant Extracts/Products against White-Backed Plant Hopper, *Sogatella furcifera* (Horvath) Infestation on Rice

Syed Asim Rehan Kazmi, Noor Ullah and
Mohammad Ramzan Hakro[1]
Grain Storage Research Laboratory
Tropical Agricultural Research Institute
PARC, Karachi University Campus
Karachi-75270, Pakistan.

ABSTRACT

Ethanol and hexane extracts of three plants, viz., sweetflag (*Acorus calamus*), neem (*Azadirachta indica*) and turmeric (*Curcuma longa*), were tested against white-backed plant hopper (WBPH) infestation on rice. The hexane extracts of *A. calamus* and *A. indica* performed better than that of *C. longa* after 24 hours of initial treatment in suppressing the WBPH population. However, the hexane extract of *C. longa* performed better than the other extracts after 48 and 72 hours. The efficacy of the extracts was comparable to that of the synthetic pesticides Padan and Karate. Neem Bond-A, a commercial pesticide from Thailand, was also found effective against WBPH. However, insect resurgence was observed during this treatment. The neem (ethanol) treatment had the least infested hills up to 2 weeks, followed by the neem (hexane) and sweetflag (ethanol) treatments. No statistically significant difference was observed in plant height, number of tillers per hill, panicle size, number of seeds per panicle, thousand seed weight, or crop yield under the various treatments.

Key Words: Botanical products, White Backed Plant Hopper, Rice (*Oryza sativa*, L.)

INTRODUCTION

In developed and developing countries alike, the loss of crops to pests is a significant problem (National Research Council, 1992). Rice is cultivated in 111 countries of the world (Moomaw and Vergara, 1965; IRRI, 1978). In Pakistan, besides being an important food crop, rice also serves as a major source of foreign exchange earnings. Rice production has increased around the world since the

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[1] Entomologist, Rice Research Institute, Dokri, Sindh

introduction of modern high-yielding varieties. The shorter growth duration of these varieties and the development of irrigation systems have allowed increased cropping intensity.

Despite these agronomical advances, rice production still faces numerous constraints (De Datta, 1986). The increased cropping intensity, along with factors such as high tillering, closer spacing, and more intensive use of fertilizer, is believed responsible for an increase in severity of damage by rice insects. For example, the introduction of insect-resistant varieties led to the development of biotypes (Varca and Feuer, 1976; Saxena and Barrion, 1985, 1987) not only in the brown plant hopper, *Nilaparvata lugens* (Stal), but also in other pests, including the white-backed plant hopper (WBPH), *Sogatella furcifera* (Horvath), and green leaf hopper, *Nephotettix virescens* (Distant). The insect pest complexes that had thus achieved different status showed high reproductive potential and are r-strategists (van Emdem, 1977), with more numerous generations (1-4) per year, and the capability to migrate over long distances (Kisimoto, 1976).

A multitude of insect pests feed on all plant parts of rice, from the roots to the foliage and grains (Grist and Lever, 1969; PANS, 1976). Actually, rice is food for more than 1,400 species of insect herbivores (Walker, 1962). A number of insect species vector diseases to rice (Ling, 1972; Ou, 1979). Of this total, approximately 1-2% (12-30 species) are considered major pests (Yasumatsu et al., 1981; Ressing et al., 1986; van Vreden and Ahmadzabidi, 1986), and these are scattered into five orders--Hemiptera,

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Lepidoptera, Coleoptera, Orthoptera, and Diptera. Yield losses due to rice insect pests have been estimated to be about 30% in Asia (Cramer, 1967).

As awareness of the economic costs and environmental consequences of the indiscriminate use of pesticides and other chemicals has grown, alternative agricultural practices have received increasing attention (National Research Council, 1992). There is now a great need to develop new insecticides that are pest-specific, nontoxic to humans and beneficial organisms, biodegradable, less prone to insect resistance and resurgence, and less expensive.

Analysis of traditional farming techniques reveals that highly efficient natural regulators exist and often are still being used by farmers (Kotschi et al., 1989). Grainge and Ahmed (1988) have prepared a database of more than 1800 plant species occurring worldwide that possess anti-insect properties. Jacobson (1958, 1975) has listed several naturally occurring plants having insecticidal properties. Yang and Tang (1988) have also reviewed 267 plants used for pest control in China. Thus, evaluation of extracts from indigenous plants for pesticidal value could provide solutions for rice pest control.

In the studies described here, ethanol and hexane extracts of three plants, viz., sweetflag *(Acorus calamus)*, neem (*Azadirachta indica)*, and turmeric (*Curcuma longa)*, as well as Neem Bond-A (A commercial neem-based product of Thailand), were evaluated against WBPH on rice crop. Two commercial pesticides, Padan (4G) and Karate (2.5% EC), were used at the recommended rate for comparison.

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MATERIALS AND METHODS

Experimental Design

Rice variety IR-6 was sown on June 27, 1993, at Rice Research Institute Dokri, to raise the nursery. The experimental field was prepared according to the method described by Xuan and Ross (1976), Gomez and Gomez (1976, 1988), and Heinrichs et al. (1981) using randomized complete block design, with three replicates for each treatment. The rice nursery was transplanted on July 27, 1993, in subplots of 12 x 4 m (48 m^2) per replicate. Fertilizer was applied in three splits at the rate of 135 kg nitrogen and 67 kg phosphorus/ha.

Preparation of Extracts

Plant materials, i.e., neem seed and sweet flag and turmeric rhizomes, were collected or purchased from Karachi. The extracts were prepared by H. E. J. Research Institute of Chemistry, University of Karachi. First, 1 kg of natural material was ultra-turraxed (high-speed maceration) in 80% ethanol. The resulting mixture was then filtered, and the filtrate evaporated under vacuum to obtain solid material. This solid gum was dissolved in n-hexane, and the portion soluble in n-hexane was removed by filtration. The hexane-insoluble portion was dissolved in ethanol. Both the n-hexane and ethanol portions were concentrated separately under vacuum to obtain their respective extracts. Each extract (25% EC) was prepared separately by mixing 4.8 ml of respective material with 2 l of water and a half-teaspoon of detergent (Surf, Lever Brothers Pakistan) for the field application.

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Application of the Test Materials

Each test material (25% EC) was sprayed at the dose rate of 4 l/ha using a knapsack sprayer, with subsequent washing with detergent between treatments and before pesticide application. Neem bond-A was applied at 1 l/ha, Karate 2.5% EC at 625 ml/ha, and Padan 4G at 25 kg/ha. Control plots were kept untreated.

Observations

The WBPH population was recorded using the method of Heinrichs et al., (1981). Twenty hills were selected at random from each experimental plot having adequate standing water. Each hill was shaken, and hoppers that fell on water were counted. Observations of nymphal population before application of the test materials and 1, 2, 3, 7, and 15 days following application were recorded.

Resurgence of WBPH following application of the treatments was assessed through comparison of reproductive rates on the treated and untreated plants by the following formula, described by Chelliah and Heinrichs (1980 a, b).

$$\text{Resurgence ratio} = \frac{\text{Number of insects on treated plants}}{\text{Number of insects on untreated plants}}$$

The data were transformed by $\sqrt{\text{count} + 0.5}$ to account for missing values. Other observations included number of tillers/hill, height of the plant, % burnt leaves, panicle size, thousand-grain weight, and final yield.

RESULTS AND DISCUSSION

Data on the attack of WBPH on rice fields under the experiment at Rice Research Institute, Dokri, are presented in Table 1. The

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natural WBPH population was unevenly distributed in the different plots, as indicated by the high standard error of means (Table 1), and but the differences were not significant. The insect population had decreased significantly within 24 hours following initial treatment, except for the turmeric hexane (TH) treatment, which had a population comparable to that of the control. The best performance was exhibited by Karate (2.5% EC), followed by Neem Bond-A, sweetflag ethanol (SE), neem hexane (NH), and sweetflag hexane (SH).

The population continued to decrease, even after 48 hours, most prominently with Padan and TH. The uptake of Padan took more time since it is a systemic insecticide. Continued suppression of the population after 48 hours was similar among the other treatments.

Post-treatment observation on day 3 (after 72 hours) indicated a significant decline in overall population from day 1, with the performance of the treatments still significantly different from that of the control. NE, TH, and Karate were found to be the best treatments after this interval.

On days 7 and 15, no insect population was observed for most of the treatments as compared with the control, where a gradual population buildup was recorded. Thus the plant extracts exhibited an efficacy comparable to that of the commercial pesticides used.

The resurgence ratio (Table 2) was calculated to rule out all possibilities of stimulation of insect reproduction by the test materials. No resurgence was recorded for any of the treatments, though some increase in the Neem Bond-A treatment was noted.

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However, observations recorded on days 7 and 15 showed population buildup under NE, followed by SH and Karate, suggested a decline in the persistence of these treatments.

Observations on plant height, number of tillers per hill, and panicle size showed that none of the test materials had a phytotoxic effect (Table 3). No burnt leaf was noted at the doses used. Mean numbers of paddy grains per panicle were higher in all the treated plots except Karate; however, the difference was not significant. Similarly, no difference in thousand-grain weight (wet basis) was recorded. It is interesting to note that all the treatments produced more yield as compared with the control, except TH. The highest yield was recorded for the TE treatment as compared with the control, and the lowest yield was recorded for the TH treatment. Ethanolic extract of turmeric seems to have had some plant growth-promoting effect, as indicated by the high yield in spite of the high percentage of infested hills (41.64%). This effect was not observed for the TH treatment. NH had the second highest yield, followed by SE, Padan, and NE. However, the mean yield for all the treatments was higher than for the control, although the significance was masked by the high yield in one of the control plots.

CONCLUSIONS

Using plant materials to control rice pests may alleviate the burden of heavy reliance on synthetic pesticides. Neem extracts/ products have been thoroughly evaluated for controlling rice crop pests in many parts of the world, and have been found effective

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against more than 200 insect pest species (Grainge and Ahmed, 1988). Rajasekaran et al., (1988) tested neem products against stem borer (*Scirpophage incertulus*), leaf folder (*Cnaphalocrocis medianalis*), whorl maggot (*Hydrella sasaki*), spider (*Lycosa pseudoannulata*), earhead bug (*Leptocrosia oratorius*), and green leaf hopper (*Nephotettix* species). Their results showed significant control of rice insects, but the treatments did not harm the spider population. Shukla et al., (1988) evaluated neem oil against green leaf hopper and WBPH, and found it on a par with monocrotophos and chloropyriphos. Jilani et al., (1988) and Jilani and Saxena (1990) reported the repellent, feeding-deterrent, and growth-inhibiting effects of turmeric, sweetflag, and neem oil against Lesser grain borer, *Rhyzopertha dominica* (F.), and red flour beetle, *Tribolium castaneum* (Herbst). However, the use of sweetflag and turmeric extracts for the control of insect pests of rice crops in the field has rarely been reported. In China, water extracts of sweetflag rhizome/leaves were used to control rice leaf hopper and rice borer (Yang and Tang, 1988) about four decades ago. Hexane extracts of turmeric have been used to control stored grain pests (Jilani, 1985). The quality of turmeric as a condiment in food is not affected by the extraction of oil used for pest control (Ullah et al., 1990). Therefore, these products can be employed in protecting rice from insect damage.

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REFERENCES

Cramer, H.H. (1967). Plant Protection and world crop production. Pflanzenschutz-Naehr Dayer, Levenkusen, Germany, 20(1): 1-524.

De Datta, S.K. (1981). Principles and practices of rice production. John Wiley and Sons, Inc. New York. 618 pp.

Gomez, K.A. and A.A. Gomez (1976). Statistical procedures for agricultural research with emphasis on rice. International Rice Research Institute. Los Banos, Philippines. 294 pp.

Gomez, K.A. (1972). Technique for field experiments with rice. International Rice Research Institute, Los Banos, Philippines.46pp.

Gomez, K.A. and A.A. Gomez (1984). Statistical procedures for agricultural res. John Wiley and Sons Inc., Singapore. 2Ed. 679 pp.

Grainge, M. and Ahmed S. (1988). Handbook of plants with pest control properties. John Wiley & sons, 470 pp.

Grist, D.H. and R.J.A.W. Lever. (1969). Pests of rice. Longmans, Green & Co. Ltd., London. 520 pp.

Heinrichs, E.A., S. Chelliah, S.L. Valenica, M.B. Arceo, L.T. Fabeller, G.B. Aquino and S. Pickin (1981). Manual for testing insecticides on rice. International Rice Research Institute, Los Banos, Philippines. 134 pp.

IRRI (1978). Rice research and production in China: an IRRI team's view. Los Banos, Philippines. 119 pp.

Jacobson, M. (1958). Insecticides from plants: a review of literature. 1941-1953. USDA Agric. Handb. 134, 299 pp.

Jacobson, M. (1975). Insecticides from plants: a review of literature. 1954-1971. USDA Agric. Handb. 464, 138 pp.

Jilani, G (1985). Quality improvement in basmati rice in aging process. Proceedings of regional field workshop on rice grading, inspection and analysis, organized by FAO and Pakistan Agricultural Research Council.. p. 198-211.

Jilani, G., R.C. Saxena and B.P. Rueda (1988). Repellent and Growth-Inhibiting Effects of Turmeric oil, Sweetflag oil, Neem oil, and Margoson-O on Red Flour Beetle (Coleoptera: Tenebrionidae). J. of Eco. Entoml. Vol. 81(4); p. 1226-1230.

Jilani, G. and R.C. Saxena (1990). Repellent and Feeding Deterrent Effects of Turmeric oil, Sweetflag oil, Neem oil, and a Neem-based Insecticide Against Lesser Grain Borer (Coleoptera: Bostrychidae) J. of Eco. Entomol. Vol. 83(2); p. 629-634.

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Kisiomoto, R. (1976). Synoptic weather conditions inducing long distance migration of plant hoppers Sogatella furcifera (Horv.) and Nilaparvata lugens (Stal). Ecol. Entomol. 1: 95-109.

Ling, K.C. (1972). Rice virus diseases. International Rice Research Institute, Los Banos, Philippines. 142 pp.

Moomaw, J.C. and B.S. Vergara. (1965). The environment of tropical rice production. In the mineral nutrition of the rice plant. Proceedings of a Symposium at IRRI, February 1964. The Johns Hopkins Press, Baltimore, Maryland. pp. 3-13.

National Research Council (1992). Toward Sustainability: An Addendum on Integrated Pest Management as a Component of Sustainability Research. Washington, D.C.: National Academy Press. 35 pp.

Ou, S.H. (1979). Rice plant diseases. In Rice: production and utilization. Westport, Connecticut. pp. 235-259.

PANS (Pest Articles News and Summaries). (1976). Pest control in rice. Manual #3: 117-246.

Rajasekaran, B., S. Jayraj, R. Saroja and Raju, N. (1988). Final Workshop on Botanical Pest Control in Rice-Based Cropping System, IRRI, Philippines, 12-16 Dec.

Reissig, W.H., E.A. Heinrichs, J.A. Litsinger, K. Moody, L. Fielder, T.W. Mew, and A.T. Barrion. (1986). Illustrated guide to integrated pest management in rice in tropical Asia. International Rice Research Institute. Los Banos, Philippines. 411 pp.

Saxena, R.C. and A.A. Barrion. (1985). Biotypes of the brown plant hopper *Nilaparvata lugens* (Stal). and strategies in deployment of host plant resistance. Insect Sci. Appl. 6(3): 271-289.

----------. (1987). Limitations of host plant resistance: insect biotypes. Paper presented in the eleventh International Congress of Plant Protection, PICC, Manila, Philippines. 30 pp.

Shukla, B.C., S.K. Shrivastava and R. Gupta (1988). Final Workshop on Botanical Pest Control in Rice-Based Cropping System, IRRI, Philippines, 12-16 Dec.

Ullah, N., G. Jilani, M.I. Khan and Ghiasuddin (1990). Repellency of turmeric and its derivatives applied to rice against the red flour beetle, *Tribolium castaneum* (HBST.) (Coleoptera: Tenebrionidae). Pak. Entomol., Vol. 12, No. 1-2: pp. 38-41.

van Emdem, H.F. (1977). Insect pest management in multiple cropping systems - a strategy. pp. 325-343. In proceedings, Symposium in cropping systems research and development for the Asian rice farmer. International Rice Research Institute, Los Banos, Philippines. 454 p.

554

Van Vreden, G. and A.1. Ahmadzabidi. (1986) Pests of rice and their natural enemies in Peninsular Malaysia. Pudoc Wageningen, Netherlands. 230 pp.

Varca, A.S. and R. Feuer. (1976). The brown plant hopper and its biotypes in the Philippines. Plt. Prot. News 5:38-41.

Walker, H.G. (1962). Preliminary list of insects and mites recorded on paddy rice. Mimeograph. Food Agr. Organ. United Nations, Rome. 63 pp.

Xuan, Vo-Tong and V.E. Ross (1976). Training manual for rice production. International Rice Research Institute, Los Banos, Philippines. 140 p.

Yang, R.Z. and C.S. Tang (1988) Plant used for pest control in China: A literature review : Economic Botany; 42(3): pp 376-406

Yasumatsu, K., T. Wongsiri, C. Tirawat, N. Wongsiri, and A. Lewvanich. (1981). Contributions to the development of integrated rice pest control in Thailand. Japan International Cooperation Agency. 204 pp.

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Table 1. Mean number of white backed plant hoppers per hill of rice treated with botanicals and pesticides at Rice Research Institute, Dokri (Larkana) Sindh

Treatment	Pretreatment	Posttreatment data (day)				
		1	2	3	7	15
Neem (hexane)	6.33 ± 2.74	0.63 ± 0.46 B	0.83 ± 0.23 B	0.34 ± 0.09 BC	0.00 B	0.00 B
Neem (ethanol)	4.93 ± 2.63	0.92 ± 0.21 B	0.56 ± 0.27 B	0.15 ± 0.08 C	0.67 ± 0.33 B	0.67 ± 0.33 B
Turmeric (hexane)	4.20 ± 0.76	4.08 ± 2.37 A	0.43 ± 0.17 B	0.20 ± 0.08 C	0.00 B	0.00 B
Turmeric (ethanol)	12.86 ± 2.79	1.28 ± 0.50 B	0.78 ± 0.16 B	0.92 ± 0.35 B	0.00 B	0.00 B
Sweetflag (hexane)	6.67 ± 0.79	0.65 ± 0.53 B	0.45 ± 0.05 B	0.33 ± 0.20 BC	0.33 ± 0.33 B	0.33 ± 0.33 B
Sweetflag (ethanol)	6.00 ± 2.00	0.55 ± 0.28 B	0.63 ± 0.23 B	0.40 ± 0.10 BC	0.00 B	0.00 B
Neem Bond-A	6.20 ± 3.40	0.50 ± 0.20 B	0.46 ± 0.29 B	0.52 ± 0.19 BC	0.33 ± 0.33 B	0.33 ± 0.33 B
Karate (2.5 % EC)	9.67 ± 3.20	0.43 ± 0.28 B	0.76 ± 0.25 B	0.22 ± 0.14 C	0.00 B	0.00 B
Padan (4G)	9.13 ± 3.23	0.92 ± 0.44 B	0.35 ± 0.22 B	0.40 ± 0.21 BC	0.00 B	0.00 B
Control	14.40 ± 2.03	4.70 ± 1.08 A	3.17 ± 0.60 A	2.38 ± 0.11 A	2.67 ± 0.88 A	3.33 ± 1.453 A
LSD	NS	2.628	0.833	0.609	0.983	1.458

Means followed by the same letter in a column are not significantly different at $P = 0.05$

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Table 2. Mean resurgence ratio (transformed values) of white backed plant hopper population per hill of rice crop treated with botanicals and pesticides at Rice Research Institute, Dokri (Larkana) Sindh.

Plant extract /treatment	Day									
	1		2		3		7		15	
Neem (hexane)	0.81 ± 0.04	B	0.87 ± 0.03	B	0.89 ± 0.05	ABC	0.71 ± 0.00	C	0.71 ± 0.00	C
Neem (ethanol)	0.85 ± 0.06	B	0.84 ± 0.09	B	0.86 ± 0.13	BC	0.83 ± 0.06	B	0.81 ± 0.06	B
Turmeric (hexane)	1.22 ± 0.10	A	0.81 ± 0.05	B	0.81 ± 0.03	C	0.71 ± 0.00	C	0.71 ± 0.00	C
Turmeric (ethanol)	0.86 ± 0.04	B	0.88 ± 0.07	B	1.20 ± 0.27	AB	0.71 ± 0.00	C	0.71 ± 0.00	C
Sweetflag (hexane)	0.79 ± 0.05	B	0.80 ± 0.02	B	0.93 ± 0.12	ABC	0.78 ± 0.07	BC	0.78 ± 0.07	BC
Sweeflag (ethanol)	0.77 ± 0.03	B	0.86 ± 0.07	B	0.93 ± 0.11	ABC	0.71 ± 0.00	C	0.71 ± 0.00	C
Neem Bond-A	0.76 ± 0.02	B	0.89 ± 0.08	B	0.97 ± 0.10	ABC	0.71 ± 0.00	C	0.71 ± 0.00	C
Karate (2.5% EC)	0.77 ± 0.03	B	0.88 ± 0.08	B	0.86 ± 0.10	BC	0.76 ± 0.05	BC	0.74 ± 0.03	BC
Padan (4G)	0.82 ± 0.04	B	0.79 ± 0.03	B	0.82 ± 0.06	C	0.71 ± 0.00	C	0.71 ± 0.00	C
LSD	0.142		0.178		0.353		0.093		0.093	

Means followed by the same letter in a column are not significantly different at $P = 0.05$

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Table 3: Effects of different plant extracts and pesticides on white back plant hopper infestation and some agronomic parameters of rice.

Plant extract /treatment	% WBPH 2 weeks	% infested hills 2 weeks	plant height (cm)	tillers/ hill	panicle size (cm)	number of seeds/ panicle	TGM (wet basis) (gr)	Yield (kg/hectare)
Neem (hexane)	0.00	15.00	114.13 ± 3.54	16.20 ± 0.65	27.17 ± 0.78	103.33 ± 5.46	22.460 ± 0.24	1861.28 ± 349.60
Neem (ethanol)	40.54	11.64	113.93 ± 1.07	14.93 ± 0.32	25.17 ± 0.27	97.00 ± 4.04	22.747 ± 0.67	1704.85 ± 362.67
Turmeric (hexane)	0.00	24.99	113.40 ± 2.19	17.00 ± 0.06	24.70 ± 1.80	107.67 ± 8.09	22.373 ± 0.38	1487.50 ± 207.45
Turmeric (ethanol)	0.00	41.64	109.20 ± 2.20	15.37 ± 0.07	26.93 ± 0.48	110.00 ± 6.51	22.820 ± 0.39	1923.61 ± 324.54
Sweetflag (hexane)	15.01	19.98	110.27 ± 5.24	15.30 ± 0.38	25.13 ± 1.42	100.00 ± 12.86	22.390 ± 0.42	1562.50 ± 300.69
Sweetflag (ethanol)	0.00	18.33	109.73 ± 5.16	17.57 ± 1.47	26.03 ± 0.58	104.33 ± 4.84	21.553 ± 0.53	1788.19 ± 366.68
Karate (2.5% EC)	10.34	31.05	115.07 ± 1.13	14.80 ± 1.04	24.13 ± 1.22	86.33 ± 8.69	22.427 ± 0.79	1649.31 ± 172.33
Padan (4G)	0.00	30.00	112.07 ± 3.64	15.63 ± 0.97	24.90 ± 0.87	100.00 ± 8.72	22.100 ± 0.77	1568.75 ± 309.08
Neem Bond-A	0.00	34.98	113.93 ± 3.14	15.20 ± 1.11	26.30 ± 1.74	106.00 ± 16.07	22.253 ± 0.14	1787.51 ± 365.03
Control	69.44	43.42	111.13 ± 2.14	16.50 ± 1.04	26.03 ± 0.98	98.67 ± 6.49	22.867 ± 0.75	1505.03 ± 440.29

Farm level evaluation of some botanical products as protectants of stored rice against insect attack

Noor Ullah, Syed Asim Rehan Kazmi, and Ghulam Jilani*

Grain Storage Research Laboratory
Tropical Agricultural Research Institute
Pakistan Agricultural Research Council
Karachi University Campus
Karachi-75270

ABSTRACT

Neem (*Azadirachta indica*) seed oil and turmeric (*Curcuma longa*) and sweetflag (*Acorus calamus*) rhizome oils were evaluated as protectants of stored rice against insect infestation on farm storage sites in Pakistan. Neem oil applied to bags and Neem Bond-A (a commercial pesticide from Thailand) applied to bags and grain together were found to be the most effective treatments in reducing the *Oryzaephilus surinamensis* (L) population at Thatta. At Dokri, turmeric oil applied to bags was best against the same species. All neem, turmeric, and sweetflag oil treatments and Coopex best controlled *Tribolium castaneum* (Herbst) at Dokri. The moisture content of the stored rice at Thatta was 1% higher than at Dokri. No significant difference in numbers of weeviled grains (%) and TGM was observed among the treatments and the two sites because of the low insect population.

Key words: Botanicals, Stored rice, Protectants, Insects, Farm level storage

INTRODUCTION

Indiscriminate use of synthetic pesticides to control various pests has increased environmental pollution. These chemicals are not only toxic to target organisms, but also harmful to humans, beneficial insects, wildlife, and sometimes crop plants. Moreover, they are the major cause of pest resurgence and resistance in target organisms as a result of inappropriate use. Alternate methods and strategies that are more safer, more economical, and readily available are being developed to control insect pests. Use of

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*Present Address : International Institute of Biological Control, PARC, Murree Road, Rawalpindi.

botanical products for this purpose has been shown to be a promising alternative strategy.

It has been an age-old practice in rural Pakistan to mix dried neem leaves with stored grain, and to place the leaves in the fold of woolen clothes to ward off insects. In some south Asian countries, rice or wheat is stored by mixing with 2% turmeric powder (Chatterjee, 1980). People in Punjab, Pakistan apply small amounts of turmeric powder, along with mustard oil and table salt, to Basmati rice for aging (Jilani, 1984). Similarly, sweetflag rhizomes are used in China as an insectifuge and insecticide (Yang and Tang, 1988).

More than 100 plant species indigenous to Pakistan have been evaluated for their repellent and insecticidal effects (Jilani et al., 1984, 1989, 1991) under laboratory conditions. The chief components of turmeric oil (sesquiterpene ketones in the form of turmerons) were shown to be strong repellents against red flour beetle adults (Su et al., 1982). Sweetflag rhizome powder mixed with rice at 1% exhibited strong repellency against *Tribolium castaneum* (Herbst) (Jilani et al., 1984). Fewer and underweight larvae, pupae, and adults were produced when parent adults were fed on wheat flour treated with turmeric oil, neem oil, sweetflag oil, and margosan-O at the rate of 200 ppm (Jilani et al., 1988). Similarly, these oils exhibited repellent and feeding-deterrent properties against lesser grain borer *Rhizopertha dominica* F. adults (Jilani and Saxena, 1990).

It is required that any such potential materials be tested for their effectiveness under farm-level and natural storage

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conditions. The studies described here was undertaken to evaluate three plant extracts/products as protectants of stored rice under natural conditions: viz. neem, turmeric, and sweetflag oils, all of which have exhibited promising anti-insect properties under laboratory conditions.

MATERIALS AND METHODS

The experiment was conducted at two sites in Sindh: Rice Research Institute, Dokri (Larkana), and Rice Research Station, Thatta. Polished rice grain, IR-6 variety (20 kg), and jute bags (45 x 60 cm size) in three replicates were treated with different botanical products and stored in a storage room at each site. The treatments were as follows: neem oil to bag (NOB), neem oil to grain (NOG), neem oil to bag + grain (NOBG), turmeric oil to bag (TOB), turmeric oil to grain (TOG), turmeric oil to bag + grain (TOBG), sweetflag oil to bag (SOB), sweetflag oil to grain (SOG), sweetflag oil bag + grain (SOBG), neem Bond-A to bag (NBAB), Neem Bond-A to grain (NBAG), Neem Bond-A to bag + grain (NBABG), Coopex dust (CD), polyethylene enclosure and phosphine fumigation (PEPF), control (phosphine fumigated grain), and master control (untreated).

Neem (*Azadirachta indica* A juss) seeds and turmeric (*Curcuma longa* L.) and sweetflag (*Acorus calamus* L.) rhizomes were purchased from a local market in Karachi. These materials were ground to a fine powder (30-mesh particle size) and dipped in commercial n-Hexane or petroleum ether for 7 days. The solvent containing oil was filtered and concentrated on a rotary vacuum evaporator.

Neem Bond-A (NBA) a neem-based pesticide manufactured in Thailand, was purchased from Bangkok. Coopex (Permethrine 0.5%

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dust, Wellcome Pakistan Limited) and Phostoxin (Aluminum phosphide, AG Services Pakistan) were purchased from a local market in Karachi.

Ten bags of rice grain, each containing 100 kg of IR-6 variety having 5% broken grain, were purchased from a local market in Thatta. The same amount, variety, and quality of rice grain was purchased from Bada, Dokri (Sindh).

The jute bags were treated with the different oils at a dose of 1000 $\mu g/cm^2$ of surface area. To cover the entire surface area of a bag, 5.4 ml of an oil was dissolved in 64.6 ml acetone and applied with a hand-held atomizing sprayer ("Windex" sprayer). The Neem Bond-A treatment was prepared by adding 5.4 ml of Neem Bond-A to 20 ml of water and 44.6 ml of acetone. For the neem oil treatment, 5.4 ml of neem oil was dissolved in 30 ml of petroleum ether, and 34.6 ml of acetone was added to make a 70 ml volume. For the control treatments, 70 ml of acetone was used. The PEPF treatment was prepared by inserting a jute bag containing rice in a polyethylene envelop and placing 1/3 part of Phostoxin pallet (66 mg phosphine) in each bag. The open side of the polyethylene envelop was folded and sealed using masking tape. Master controls were untreated.

The rice grain (20 kg lots) was treated with the different oils at a dose of 500 ppm. Ten ml of each oil was dissolved in 40 ml acetone and sprayed onto the grain with the help of the "Windex" sprayer as the grain tumbled in a device. The tumbling device was rotated an additional 50 turns after spraying for uniform

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distribution of the oil on the rice grain. For the Neem Bond-A and neem oil applications, water and petroleum ether were used to dissolve the materials as described in the previous section. The grain protectant, 20 gr of Coopex dust (0.5%) was admixed with 20 kg rice grain in a tumbler.

A total of 48 bags of rice were placed on wooden dunnage in a storage room at Rice Research Institute, Dokri, and Rice Research Station, Thatta. The bags were randomly stacked on the wooden dunnage replicate-wise.

Grain samples of 250 gr rice were drawn from each bag every 2 months using a spear sampler. The moisture content (%), numbers of insects (larvae, pupae, and adults, live/dead), numbers of weeviled grain (%), and thousand-grain weight (grams) were recorded.

RESULTS AND DISCUSSION

Mean numbers of adult insects in different stored product species averaged over 12 months of storage at Thatta and Dokri are given in Tables 1 and 2, respectively. In the rice grain stored at Thatta, *Oryzaephilus surinamensis* (L) was the most dominant species, followed by *Tribolium castaneum* (Herbst) and *Rhizopertha dominica* (F) (Table 1). At Dokri, however, *T. castaneum* was found to be higher in number than *O. surinamensis* and *R. dominica* (Table 2). A very low population of *Sitophilus oryzae* (L) adults was recorded at both the sites.

At Thatta, neem seed oil applied to bags (NOB) and Neem Bond-A applied to bags and rice grain (NBABG) were quite effective

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treatments against *O. surinamensis*, since numbers of adults of this species were found to be significantly lower in these treatments than in the rest of the treatments and the controls (Table 1). Turmeric oil applied to bags and grain (TOBG) and polyethylene enclosure and phosphine fumigation (PEPF) treatments provided good control of *R. dominica*, while neem seed oil applied to grain (NOG) and in combination to bag (NOBG), turmeric oil to grain (TOG) and in combination to bag (TOBG), Neem Bond-A to grain (NBAG), and Coopex proved promising in reducing *T. castaneum* adults (Table 1). Against *S. oryzae*, no significant difference was found among the various treatments since its overall population was very low.

Rice grain stored at Dokri had comparatively lower numbers of adult insects than that stored at Thatta; however, the prominence of the different species of insects was similar. At this site, *T. castaneum* was the dominant species followed by *O. surinamensis*, *R. dominica* and *S. oryzae* (Table 2). NOB, NOG, TOG, TOBG, and Coopex were promising in reducing numbers of *T. castaneum* adults. TOB resulted in significantly fewer numbers of *O. surinamensis* than the other treatments and the controls. In addition, NOB, NOBG, sweetflag oil applied to bag and grain together (SOBG), NBAG, and NBABG also had some effects in controlling the *O. surinamensis* population. Against *R. dominica*, TOB, NBABG, and Coopex were effective treatments (Table 2). No treatment was effective against *S. oryzae* since numbers of this species were nonsignificant.

Results reported here indicate promising effects of neem seed oil, turmeric oil, and Neem Bond-A in keeping the population of

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different insect species very low. The effects exhibited by these botanical oils were comparable to those of Coopex dust, a chemical grain protectant commercially available. However, sweetflag oil showed no appreciable effects under natural storage conditions. In laboratory trials, this material has been reported to exhibit toxic effects against *T. castaneum* and *R. dominica* (Jilani et al., 1988; Jilani and Saxena, 1990). The chemical basis for the effectiveness of neem and turmeric oils is well known (NRC, 1992; SU et al., 1982). In addition, the present study has indicated their potential as protectants of stored rice under natural conditions. It is therefore suggested that these botanical materials be standardized and formulated for commercial use in Pakistan.

Some quality characteristics, such as grain moisture content, numbers of weeviled grains, and thousand-grain weight were also recorded bimonthly during this study. No significant difference was found among the treatments and controls for these quality parameters, indicating no adverse effect of the tested materials during storage. Since these materials have shown strong protective action against insect attack in storage, their development and commercialization as botanical pesticides is envisaged.

CONCLUSIONS

After 12 months of storage, rice grain treated with neem seed and turmeric oils contained significantly fewer numbers of adult insects of three stored product species. The data indicate the strong protective action of these materials against insect infestation in stored rice. Moreover, these botanicals had no

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adverse effects on the quality of the stored rice. The results suggest that neem and turmeric oils should be standardized and formulated as botanical pesticides in Pakistan.

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REFERENCES

Chatterjee, P.B. (1980) Correspondence contribution at p.50 in P. Golob and D.J. Webley's book. Tropical Product Institute, London, 32 pp.

Compton, J.A.F., Tyler, P.S., Hindmarsh, P.S., Golob, P., Boxall, R.A. and Haines, C.P. (1993) Reducing losses in small farm grain storage in the tropics. Trop. Sci. 33: 283-318.

Jilani, G., Ullah N., Ghiasuddin and Khan M.I. (1991) Repellency of some plant extracts against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)-II. Pak. Entomol. 13, 5-8.

Jilani, G. (1984) Use of plant materials as grain protectants against stored grain insects- A review. Proc. Planning Workshop on the use of Plant materials for Pest Control by Resource Limited Farmers. IRRI, Manila, Philippines. 6-10 August, 1984, 33 pp.

Jilani, G., Ullah N. and Ghiasuddin (1984) Studies on repellent properties of some indigenous plant materials against the red flour beetle, *Tribolium castaneum* (Hbst.). Pakistan Entomologist 6(1-2): 121-130.

Jilani, G., Ullah N. and Ghiasuddin (1989) Repellency of some plant extracts against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)-I. Pak. Entomol. 11, 18-22.

Jilani, G. Saxena R.C. and Rueda B.P. (1988) Repellent and growth inhibitory effects of turmeric oil, sweetflag oil, neem oil and "Margosan O" on red flour beetle (Coleoptera: Tenebrionidae). J. econ. Ent. 81, 1226-1230.

Jilani, G. and Saxena R.C. (1990) Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). J. econ. Ent. 83, 629-634.

National Research Council (1992). Neem: A tree that could help the world. National Academy Press, Washington, D.C. 149 pp.

Su, H.C.F. Robert H. and Jilani G. (1982) Isolation, purification and characterization of insect repellents from *Curcuma longa* L. J. Agric. Food. Chem. 30: 290-292.

Yang, R.Z. and Tang C.S. (1988) Plants used for pest control in China: A Literature Review. Economic Botany, 42(3): 376-406.

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Table 1. Mean numbers of adult insects of different species recorded in 250 gr samples of stored rice over 12 months of storage at Rice Research Station, Thatta.

Treatment**	Mean numbers* ± SEM of adult insects			
	O. surinamensis	*R. dominica*	*T. castaneum*	*S. oryzae*
NOB	8.7 ± 1.2 f	2.0 ± 0.6 bcdef	3.0 ± 0.6 cde	1.7 ± 1.2
NOG	15.0 ± 0.6 abcd	3.7 ± 0.7 ab	2.3 ± 0.3 e	1.3 ± 0.3
NOBG	12.3 ± 2.0 cde	4.0 ± 1.5 a	2.7 ± 1.2 de	2.0 ± 1.0
TOB	15.7 ± 0.9 abc	2.7 ± 0.3 abcd	4.0 ± 0.6 cde	2.3 ± 1.3
TOG	17.0 ± 1.2 ab	1.7 ± 0.3 cdef	2.7 ± 0.3 de	0.3 ± 0.3
TOBG	15.0 ± 1.2 abcd	0.7 ± 0.3 ef	2.7 ± 0.3 de	0.7 ± 0.3
SOB	14.0 ± 1.5 bcd	1.3 ± 0.3 def	5.3 ± 2.3 bcde	2.0 ± 0.6
SOG	17.0 ± 0.0 ab	2.3 ± 0.9 abcde	6.0 ± 2.0 abc	1.0 ± 0.6
SOBG	18.3 ± 1.8 a	1.7 ± 0.3 cdef	5.7 ± 1.2 abcd	2.0 ± 0.6
NBAB	16.0 ± 1.0 ab	1.0 ± 0.6 def	3.0 ± 0.6 cde	1.0 ± 0.6
NBAG	11.7 ± 1.2 def	1.3 ± 0.3 def	2.3 ± 0.3 e	1.7 ± 0.7
NBABG	9.3 ± 1.9 ef	2.7 ± 1.2 abcd	5.0 ± 0.6 cde	2.3 ± 0.3
COOPEX	12.3 ± 0.9 cde	3.3 ± 0.9 abc	2.7 ± 0.8 de	1.3 ± 0.9
PEPF	14.3 ± 1.2 bcd	0.3 ± 0.3 f	4.3 ± 0.7 cde	1.7 ± 0.3
CONTROL	18.3 ± 1.5 a	4.0 ± 0.6 a	8.7 ± 1.2 a	1.0 ± 0.9
MASTER CONTROL	16.7 ± 0.3 ab	2.7 ± 0.3 abcd	8.3 ± 2.0 ab	1.0 ± 0.0
LSD	3.61	1.99	3.30	NS

* Each value is a mean of three replicates. Values in columns followed by the same letters are not significantly different at $P = 0.05$.

** NO = Neem oil TO = Turmeric oil SO = Sweetflag oil NBA = Neem Bond-A
B = Bag treatment G = Grain treatment BG = Treatment to bag and grain both

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Table 2. Mean numbers of adult insects of different species recorded in 250 gr samples of stored rice over 12 months of storage at Rice Research Institute, Dokri.

Treatment**	Mean numbers* ± SEM of adult insects			
	O. surinamensis	*R. dominica*	*T. castaneum*	*S. oryzae*
NOB	3.3 ± 0.3 cd	2.7 ± 1.7 cd	4.7 ± 1.2 d	0.6 ± 0.3
NOG	5.0 ± 0.6 bcd	8.0 ± 2.0 a	4.7 ± 0.3 d	1.0 ± 0.0
NOBG	3.0 ± 0.6 cd	2.7 ± 1.2 cd	6.0 ± 0.0 cd	1.0 ± 0.0
TOB	2.3 ± 0.3 d	2.0 ± 0.6 d	5.0 ± 0.0 d	1.0 ± 0.0
TOG	5.3 ± 0.3 abcd	3.3 ± 0.9 cd	6.7 ± 0.3 cd	0.0 ± 0.0
TOBG	4.7 ± 0.3 bcd	3.0 ± 0.6 cd	5.3 ± 1.9 d	0.7 ± 0.3
SOB	4.7 ± 1.2 bcd	3.3 ± 1.3 cd	8.3 ± 1.2 bcd	1.0 ± 0.6
SOG	5.7 ± 0.3 abc	5.0 ± 1.0 bc	12.7 ± 3.2 a	0.7 ± 0.3
SOBG	3.0 ± 0.6 cd	3.0 ± 0.0 cd	7.7 ± 0.9 bcd	1.0 ± 0.0
NBAB	4.3 ± 0.9 bcd	2.3 ± 0.3 cd	7.7 ± 0.7 bcd	0.7 ± 0.7
NBAG	3.0 ± 0.6 cd	3.7 ± 0.9 cd	5.7 ± 1.2 cd	0.7 ± 0.3
NBABG	3.7 ± 1.5 cd	2.0 ± 0.6 d	6.7 ± 0.9 cd	1.3 ± 0.3
COOPEX	7.0 ± 1.5 ab	2.0 ± 0.6 d	5.3 ± 0.9 d	0.0 ± 0.0
PEPF	8.3 ± 2.9 a	2.7 ± 0.3 cd	9.3 ± 0.9 abc	0.0 ± 0.0
CONTROL	4.7 ± 0.9 bcd	7.0 ± 1.2 ab	11.0 ± 2.5 ab	0.7 ± 0.7
MASTER CONTROL	5.7 ± 1.8 abc	5.0 ± 1.2 bc	10.7 ± 1.0 ab	1.0 ± 0.6
LSD	3.27	3.00	3.91	NS

* Each value is a mean of three replicates. Values in columns followed by the same letters are not significantly different at $P = 0.05$.

** NO = Neem oil TO = Turmeric oil SO = Sweetflag oil NBA = Neem Bond-A
B = Bag treatment G = Grain treatment BG = Treatment to bag and grain both

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SYNERGISTIC EFFECT OF CULTIVAR TYPE AND FUNGICIDE ON SOUTHERN LEAF BLIGHT AND YIELD IN CORN

A.SHABEER, Associate Professor, Department of Plant Pathology and M.SALIM, Associate Professor, Department of Plant Breeding & Genetics, NWFP Agricultural University, Peshawar, Pakistan.

ABSTRACT

Two foliar fungicides, propineb and mancozeb, each at 2.2 kg/ha, were applied weekly to five cultivars of corn -- Azam, Dehqan, Kissan, Sarhad white, and a local variety, and were evaluated for their effect in reducing the infection of southern corn leaf blight and increasing corn yield. The application of mancozeb was more effective in decreasing thr area under disease progress curve (ADPC) in Dehqan than in the other cultivars. Grain yield and number of kernels per ear were substantially increased in some of the improved cultivars where the disease resistance was inadequate in the absence of fungicides. However, fungicide application did not result in significant yield increase with the local variety. These fungicides, if used at appropriate time and concentrations, can enhance productivity in corn.

Additional key words: SCLB, cultivars, fungicides, yield components, Pakistan.

INTRODUCTION

Corn in the North West Frontier Province (NWFP) of Pakistan suffers heavy yield losses from several diseases, including

southern corn leaf blight (SCLB). The planting of resistant varieties minimizes these losses. However, where such varieties are not available, fungicides may function as an alternative means of disease control. Ten fungicides were tested against Drechslera maydis (syn. Bipolaris maydis = Cochliobolus heterostrophus) in culture and on corn in the field. The fungicides dinocap at 0.07% (w/v) and carbendazim at 0.1% (w/v) showed the best control of the disease and greatest increase in yield (Saxena et al., 1985). Similarly, the foliar fungicides zineb + maneb, chlorothalonil, and dyrene reduced the disease incidence and increased yield (Comstock et al., 1974). In another study, out of 12 fungicides, captafol was the most effective against isolate 1 of B. maydis. (Kumar and Lal, 1985). Several other researchers have evaluated different fungicides for the control of SCLB and Northern leaf blight (Kachapur and Hedge, 1988; Miller, 1972; Scott and Akers, 1983; Sharvelle, 1970; White, 1983). Because most of the corn cultivars planted in the NWFP are moderately to highly susceptible to SCLB, and because growers hesitate to use fungicides for disease control, this study was undertaken to research an integrated approach through cultivar x fungicide interaction to encourage use of such an approach amongs the growers.

MATERIALS AND METHODS

Three short-duration (85-100 days) and one long- duration (110-120 days) improved cultivars -- Azam, Dehqan, and Kissan, and Sarhad white, respectively -- were tested against one local

cultivar used as a check. The yield potential of improved cultivars ranges from 4-7 tons/ha, whereas the local cultivars have appreciably lower yields.

Two fungicide formulations were used: (1) propineb, zinc propylene-bisdithiocarbamate (Antracol, Bayer AG), and (2) mancozeb, coordinated product of zinc ion + manganese ethylene-bisdithiocarbamate (Dithane-M45, Rohm & Hass Co). Both were applied at 2.2 kg/ha with a knapsack sprayer, beginning at the first appearance of the disease and continuing weekly until growth stage (GS)-8. Control plots were sprayed with water in order to detect inoculum from outside the plot area. Test plots consisted of two rows, 5 m long and 0.75 m apart. Each plot was separated by two border rows of the same cultivar to reduce interplot effects. The experiment was laid out at four agroecologically different sites in the NWFP with a split plot arrangement in a randomized complete block design. The fungicides were kept in the main and cultivars in the sub-plots.

Artificial epiphytotics of the disease were created by placing a few grains of wheat infested with B. maydis in the whorl of each plant at the 4-5 leaf stage of the crop. A second inoculation was made a week after the first. Disease ratings were recorded every 7-10 days, beginning with GS-5 (Hanway, 1963). The disease severity was rated on an entire plot basis as follows: 1 = one to few scattered lesions on lower leaves; 2 = moderate number of lesions on lower leaves; 3 = abundant lesions on lower leaves, few on middle leaves; 4 = abundant lesions on lower and middle leaves,

extending to upper leaves; and 5 = abundant lesions on all leaves, plants prematurely dried or killed by the disease (Sharma, 1983). The data were analyzed statistically by analysis of variance.

Upon physiological maturity of the crop, the experimental plots were hand harvested and mechanically shelled. Data on seed weight and moisture content were collected. Yield was expressed in kg/ha at 15.5% grain moisture. Number of kernels per ear and 1000-kernel weight were recorded. Fischer's least significant difference test was used to determine differences among the treatment means. The correlation between disease severity and each of the yield component was determined.

RESULTS AND DISCUSSION

Area under disease progress curve

Fungicide application decreased the area under disease progress curve (ADPC) more in the improved cultivars than in the local variety (Table 1). Within the improved cultivars, the fungicide mancozeb was more effective in reducing ADPC in Dehqan than in the other cultivars. Differences within the cultivars or fungicides were significant $P < 0.05$). However, the interaction was not significant at some of the locations.

Seed weight

Significant differences ($P < 0.05$) in seed weight were observed among the different treatments (Table 2). The seed weight recorded fop Dehqan treated with antracol was 28.7% higher than that of the local cultivar treated with the same fungicide. The other three

improved cultivars also showed higher grain yields than the local cultivar. The type of fungicide affected seed weight as well. Proponeb treatment resulted in greater yield increases than mancozeb treatment. A significantly negative correlation ($r = -0.77$) was found between disease severity and seed weight reflecting the adverse effect of the disease on yield.

Number of kernels/ear

Mean values for number of kernels per ear showed significant differences (Table 2). Fungicide x variety interaction was significant indicating that fungicide application increased the kernel number. The disease severity was, however, negatively correlated ($r = -0.57$) with number of kernels.

1000-kernel weight

The treatments did not differ significantly ($P < 0.05$) in 1000-kernel weight (Table 2). The improved cultivars had larger kernels but this trend was not significant. Similarly, in most instances, fungicide application increased the 1000-kernel weight over the no-fungicide treatment. The correlation between the two was negative and nonsignificant ($r = -0.07$).

Stalk rot

The incidence of stalk rot was recorded only at the Cereal Crops Research Institute (CCRI), Pirsabak. The averaged treatment values were not significantly different from each other ($P < 0.05$) (Table 2). However, the correlation between SCLB and stalk rot was calculated to be positive ($r = 0.6$). This suggested that the leaf

blight predisposed the corn to stalk rot attack.

CONCLUSIONS

The negative correlation between disease severity and yield underlines the importance of SCLB in inflicting severe losses on corn crop. Neverthless, the extent of the damage is usually underestimated by growers as they plant the same local material year after year. Better comparisons can be made when tests include both local and improved cultivars; this was one of the objectives of the present study. Improved cultivars were found superior to the local variety in disease resistance and yield performance.

The synergistic effect of fungicide application was reflected more in the improved cultivars than in the local variety in significant yield increases. However, the disease resistance of the improved cultivars was inadequate in the absence of the fungicides. The increased yield resulted primarily from an increase in number of kernels per ear and not in kernel weight. This indicates that early fungicide applications were most beneficial and early disease development the most detrimental for yield. Late season fungicide applications had little effect on the disease, except to limit the number of aborted kernels.

The high financial expense involved in the use of fungicides in the NWFP region precludes their use on a wide scale. However, the low resistance to SCLB in the presently cultivated varieties necessitates that fungicides be used, with refinement in rates and

application procedure, to minimize crop losses. This is also true because some of the improved varieties can endure the disease attack and show profitable yield if treated with the promising fungicides. Without any fungicide, cv. Dehqan had only a 5.8% higher yield than the local variety. However, treatment of the improved cultivars with propineb resulted in about a 22% increase in yield. Large- scale application and evaluation of these results may provide an integrated method for ensuring higher productivity of corn in Pakistan.

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REFERENCES

1. Comstock, J.C., C.A.Martinson., and A.H. Epstein. 1974. Fungicidal control of Southern corn leaf blight of Texas-male sterile corn. Plant Dis.Rep.58:104-107.
2. Hanway, J.J. 1963. Growth stages of corn (Zea mays L.). Agro. J. 55:487-492.
3. Kachapur,M.R., and R.K.Hedge.1988. Studies on turcicum blight of maize(Zea mays L.) caused by Exserohilum turcicum (Pass) leonard & Suggs with special reference to crop loss assessment. Plant Patholog Newsletter 6(1-2):33-35.
4. Kumar,P., and S.Lal. 1985. Relative tolerance of Drechslera maydis isolates to fungicides. Ind. J. Mycol. & Plant Pathology 15(2):195-198.
5. Miller,P.M. 1972. Fungicidal control of Helminthosporium maydis and three other species of Helminthosporium. Plant Dis. Rep. 56:612-614.
6. Saxena,A.,R.C. Gupta., and I.D.Singh. 1985. Fungicidal control of leaf spot disease of maize. Madras Agric. J.

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72(6):357-360.

7. Scott,D.H.,and D.P.Akers. 1983. Evaluation of fungicides for control of southern corn leaf blight. Fungicide-Nematicide Test Results, The American Phytopathological Soc. 38:70.
8. Sharma, R.C. 1983. Maydis leaf blight of maize. Pages 5-15 in:Techniques of scoring resistance to important diseases of maize. All India Coordinated Maize Improvement project. Ind. Agric. Res. Instt., New Dehli. pp.133.6.
9. Sharvelle,E.G. 1970. Fungicides for southern leaf blight. Plant Dis. Rep. 54:1112-1113.
10. White,D.G. 1983. Control of northern leaf blight and southern leaf blight with various fungicides, 1982. Fungicide-Nematicide Test Results, The American Phytopathological Soc. 38:70-71.

Table 1. Area under disease progress curve (ADPC) for spread of *B. maydis* as affected by synergistic effect of cultivar and fungicide at different locations

Treatment	DIKhan	Peshawar	Pirsabak	Swat
1. Azam x Antracol	1388.0 a	655.0 de	687.5 abcd	90.0 b
2. Azam x Dithane	1221.0 a	289.8 efg	437.5 cd	86.3 b
3. Azam x No fung.	583.0 a	236.5 fg	725.0 abcd	90.0 b
4. Dehqan x Antracol	1654.0 a	502.0 efg	700.0 abcd	98.8 b
5. Dehqan x Dithane	1042.0 a	143.8 g	375.0 d	78.8 b
6. Dehqan x No fung.	528.0 a	198.0 fg	450.0 cd	86.3 b
7. Kissan x Antracol	1697.0 a	514.5 efg	787.5 abc	90.0 b
8. Kissan x Dithane	989.5 a	289.0 efg	712.5 abcd	90.0 b
9. Kissan x No fung.	813.5 a	408.3 efg	612.5 bcd	90.0 b
10. S/white x Antracol	1083.0 a	999.0 cd	837.5 ab	98.8 b
11. S/whitexDithane	1730.0 a	467.0 efg	987.5 a	90.0 b
12. S/whitexNo fung.	874.5 a	575.8 ef	837.5 ab	90.0 b
13. LocalxAntracol	1933.0 a	1756.0 a	825.0 ab	416.3 a
14. LocalxDithane	1440.0 a	1400.0 ab	600.0 bcd	265.0 ab
15. LocalxNo fung.	775.0 a	1045.0 bc	975.0 a	291.3 ab
LSD (0.05) =	4758.0	387.5	361.8	198.3

* Values followed by the same letter were not significantly different from one another at 0.05 level according to the least significant difference (LSD) test.

Table 2. Effect of cultivar x fungicide on yield components of corn

Treatment	Seed weight (kg/ha)	Number of kernels/ear	1000 - kernel weight (g)
1. Azam x Antracol	3863 abc	336 ab	270.7 a
2. Azam x Dithane	3917 abc	340 ab	260.3 a
3. Azam x No fung.	3681 bcde	333 b	259.0 a
4. Dehqan x Antracol	4179 a	358 ab	257.0 a
5. Dehqan x Dithane	4088 ab	372 a	254.5 a
6. Dehqan x No fung.	3557 cde	355 ab	254.0 a
7. Kissan x Antracol	3996 abc	332 b	253.2 a
8. Kissan x Dithane	3649 bcde	339 ab	249.0 a
9. Kissan x No fung.	3633 bcde	338 ab	248.2 a
10. S/white x Antracol	4062 ab	339 ab	245.3 a
11. S/white x Dithane	3982 abc	344 ab	241.1 a
12. S/white x No fung.	3759 abcd	328 b	237.3 a
13. Local x Antracol	3247 e	328 b	237.1 a
14. Local x Dithane	3800 abcd	357 ab	234.6 a
15. Local x No fung.	3363 de	323 b	233.1 a
LSD (0.05) =	493.1	36.6	39.11

* Values followed by the same letter were not significantly different from one another at 0.05 level according to the LSD test.