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Collection, Evaluation , Maintenance, and Utilization of Sesame Germplasm for Breeding Adapted High Yielding Cultivars

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1. Executive Summary

Collection of dwindling germplasm resources, study of their morpho-physiological and genetic variation, utilization in hybridization, mutation breeding and anther culture programs were undertaken to establish a wide genetic and infra-structural base for sesame (*Sesamum indicum* L.) improvement. Over 1400 exotic and 35 local germplasm accessions collected from different regions represent the second largest genetic collection in Sri Lanka after rice and one of the largest genetic collections of sesame in the world encompassing accessions with useful agronomic characters such as determinate growth, indehiscent capsules, short maturation period, low seed shattering and reaction to day length, pest, disease and drought resistance etc. New genetic characters that could solve cultivation and processing problems such as disease resistance, photo-insensitivity, easy dehulling, low shattering have been combined with high yielding adapted cultivars through mutation induction and cross breeding. Crosses with high heterosis and combining ability have been identified. Natural cross pollination in sesame under field conditions in Sri Lanka was found to be relatively high (4-5.1%). Possibility of reducing contamination by sampling seeds from lower capsules of early-flowering varieties and upper capsules of late-flowering varieties has been shown. Possibility of overcoming narrow adaptation of pure lines due to genotype-environment interaction by using multi-lines has been demonstrated. Initial work in the development of a protocol for using doubled haploid technology for increasing the efficiency of sesame breeding was undertaken and high rates of callus induction was achieved.

2. Research Objectives:

2.1 Advantages of sesame

Sesame (*Sesamum indicum* L.) is an oil seed crop grown largely in the less developed countries (LDC) under "marginal conditions" by subsistence farmers. Out of 6.67 mln. hectares of sesame grown throughout the world in 1985, 6.666 mln. hectares or 99.9% was located in LDC. India (2.2 mln. ha), China (0.9 mln. ha), Burma (0.6 mln. ha) are the major producers in Asia. Sudan (1.1 mln. ha), Nigeria (0.25 mln. ha), Somalia (0.15 mln. ha) and Mexico (0.19 mln. ha) are important in other parts of the world (FAO, 1986). The crop has important agricultural advantages. It grows well in tropical to temperate climates, it can grow and set seeds under fairly high temperatures giving satisfactory yields in harsh climates, it can grow on stored soil moisture without (or very little) rainfall or irrigation. The land races have evolved under low input conditions and give satisfactory yields under such conditions. Sesame is a good crop is rotation (Joshi, 1961, Weiss, 1971, Brar and Abuja, 1979, Ashri, 1988).

The major advantage of sesame for poorer sections of the farming community is the availability of a ready market for sesame seed as the demand is ever increasing in the international trade due to its wide use in baking and confectionery industries and also the value of oil and cake. Therefore the price fluctuation according to production is minimal in sesame and farmers are assured of selling their product (Salunkhe & Desai, 1986).

In Sri Lanka, coconut is the single largest traditional source of edible oil, occupying 0.45 mln. ha in the Wet and Intermediate Zones of the low and mid country. However due to industrial development, rapid urbanization, development of tourism etc, in this part of the country, the area under coconut has shown a tendency to decrease, thus reducing the surplus available for export. The Wet Zone and the wetter part of the Intermediate Zone have been planted to the three major plantation crops - tea, rubber and coconut. Therefore the potential area for increasing the production of annual field crops lies in the Dry Zone and drier parts of the Intermediate Zone which account for more than 75% of the land area of the Island.

Sesame has been a traditional crop in this area and is grown by the subsistence farmers. Any increase in yield through varietal improvement which does not cost additionally to the farmers will therefore benefit the poorer sections of farming community.

2.2 Problems of cultivation

The most important annual oilseed crop in Sri Lanka is sesame with an estimated 33000 ha producing 15000 MT of seed at an average yield of 455 kg ha^{-1}. This yield is fairly high when compared to the world average of 353 kg ha^{-1} or to India, the main producer (205 kg ha^{-1}) (FAO, 1985).

Although the mean yields of sesame are low, potentially they could be considerably higher. The main reason for the low yields

is the lack of improved cultivars. The yield fluctuation across environments is high due to narrow adaptation of existing cultivars. Sensitivity of existing cultivars to day length is one reason for this narrow adaptability (Ghosh, 1955, Weiss, 1971, Tonnar and Bhargava, 1980, Pathirana, 1984). The existing varieties have poor response to added inputs such as fertilizer, irrigation etc, as the land races have been selected under 'marginal conditions'.

Pests and diseases are partly responsible for the low yields. In Sri Lanka, blight caused by *Phytophthora nicotianae* var *Parasitica* is the single most yield reducing disease, particularly in the South and Southeastern in parts. Other important diseases are also soil borne, caused mainly by *Macrophomina*, *Fusarium* and *Rhizoctonia* although powdery mildew is also widespread during some seasons. It was envisaged to develop high yielding cultivars with increased resistance to *Phytophthora* blight and to identify other diseases of major importance for future breeding programmes.

Seed shattering due to dehiscence of capsules at maturity and indeterminate growth habit resulting in non-uniform ripening of the capsules can result in severe yield losses, particularly if harvesting is delayed or mechanized (Weiss, 1971, Ashri, 1988, Khalid, 1988). The only indehiscent genotype available to breeders and found in 1946 by Langham has other disadvantage (Ashri, 1989) and has not resulted in any commercial varieties.

It is obvious that these defects of the sesame crop are due to the lack of concerted breeding efforts, partly because it is a

crop of the LDC countries and the poorer sections of farming community, working on 'marginal' lands. Sesame has not been included in the breeding programs of any of the CGIAR network of International Agricultural Research Institutes. As a result the improvement has been slow due to the lack of widely based germplasm resources, research on breeding, genetics, selection methods etc. Due to the emphasis placed on cereal (and pulse crops in the last decade) improvement in the past, oil crops in general and sesame in particular has been neglected by the national research programs of the LDC countries.

Inspite of poor adaptability, low yield, seed shattering, susceptibility to diseases, lack of seeds of improved cultivars sesame can not be replaced from many of its growing areas due to the lack of other suitable oil or food crops which are adapted to the climatic and edaphic conditions and to the cropping systems (Ashri, 1998). It is also indispensable in some popular bakery products and confectioneries.

2.3 Germplasm studies

Prerequisite for any breeding program that can have an impact is the availability of a collection of germplasm accessions representing the diversity and genetic variability of the crop. One of the major objectives of the present project was to collect, evaluate and maintain the cultivars of different origin. Empleasis was to be placed on collection of traditional land races that are being lost with the introduction of new varieties or other crops.

These collected materials were screened for yield and other desirable characters that can meet the new demands for intensive cultivation.

2.4 Cultivar improvement

The sesame plant has to be restructured and reconstructed to suit the modern cropping systems and agricultural practices if it is to become a more remunerative crop in the future. The only available means of achieving this is to include the genotypes of diverse origin in a crossing program and to induce mutations to increase variability for characters not present in the collected germplasm (Anon, 1981, Anon, 1985, Ashri, 1987, 1988). The hybridization program was aimed at combining *Phytophthora* resistance (Pathirana, 1992) with day neutrality (Pathirana, 1984) and determinate growth habit to produce cultivars adapted to intensive cropping systems. Oil yields per unit area was to be increased through a crossing programme involving high yielding genotypes with high oil cultivars, particularly Anuradhapura local sel. 1.

With improved yield, it was expected that sesame will become a component of the intensive cropping systems. Thus in India for example, the mean yield of sesame has steadily increased during the 1980's due to introduction of the crop to irrigated areas or to summer crop with assured irrigation using newly improved varieties (Rai and Bhatnagar, 1988).

The next stage would be to exploit the heterosis in a hybrid seed production programme. Although it was proposed to induce cytoplasmic male sterility in the original proposal, the panel of reviewers of the project proposal advised against embarking on this program as it could dilute the efforts of other more urgent needs of improvement. Hence the programme was directed at estimating heterosis in different combinations of crosses so that it could be exploited in future when the time is right.

2.5. Multi lines

Another aspect that need the attention of researchers is the concern about the danger of genetic vulnerability of cultivars as a result of increase in homozygozity and homogeneity of varieties. The land races are generally mixtures of different genotypes and therefore have a natural buffering whereas breeder selected homozygous lines could be extremely vulnerable to diseases, pests as well as environmental changes.

Therefore the possibility of using multilines instead of pure lines was also investigated.

2.6. Doubled haploid method in sesame improvement

Use of diploidized haploids through anther or microspore culture has great potential in increasing efficiency selection, particularly for stress conditions and also in decreasing the time of the breeding process (Morrison & Evans, 1988; Snape, 1989; Szarejko et al., 1991).

Attempts were undertaken in the latter stages of the project to develop a protocol for haploid production.

3. Methods and Results

3.1 Methods

3.1.1 Germplasm collection & studies

Exotic germplasm was introduced through correspondence with sesame breeders throughout the world. The local material was collected in collection expeditions and from local markets during the harvesting period. The collected germplasm was grown in batches with the recommended cultivar MI 3 at 10 row intervals. The experiments were replicated twice in most cases. The characterization was done according to IBPGR sesame descriptors (IBPGR, 1984). About 5-10 flowers per entry were selfed by using an adhesive or by fastening the tip of unopened corolla with thread. Seeds from these capsules were used for preservation and in the experiments. Three taximetric methods were used to analyse the data generated from scoring for twenty eight morphological characters.

3.1.2 Hybridization, heterosis, combining ability and selection

Cultivars were selected for hybridization on the basis of their geographical origin, differences in characters and yield components and presence of desirable characters. In F_1 the heterosis for yield and components was measured on the mid-parent and high parent basis.

In the segregating populations the progenies of successful crosses were advanced by different methods such as bulk, pedigree, single seed descent and early generation testing procedures (Details in appendix 1). The breeding lines generated from crosses in the early part of the project are now in advanced breeding nurseries. Some have undergone farmers' field testing successfully.

Three diallel crosses were carried out in addition to the normal hybridization program. These included two 6 x 6 diallels without the reciprocals and one 8 x 8 complete diallel. The field trials with diallels were planted in 3-4 replications and data analysis carried out according to Griffing (1956), considering the parent as a fixed sample (for details see appendix 2).

The advanced breeding lines showing promise were evaluated for yield and agronomic characters at two or three locations per season in plots of 10-15 m^2 in randomized complete block design using MI 4 variety as the control.

Where possible, all the experiments were statistically analyzed using GLM, ANOVA, CORRE, REG, CLUSTER, DISCRIM and other procedures of the SAS statistical package in an IBM personal computer (SAS, 1987).

3.1.3 Multi lines

The multilocational testing of breeding lines emanating from the breeding program showed high response to environment in some of them. Therefore three breeding lines selected from the same population were mixed in different proportions and tested at three locations with the original breeding lines, MI 3 (control) and MB 29W (a white seeded brittle seed coat line).

The lines 83-9, 83-10 and 83-14 from the cross Suduthala 102 x (MI 3 x AI) cross were selected for the study. Given below are the proportions of mixtures:

Mixture 1	-	33-3% of every breeding line
Mixture 11	-	83-9 - 25%
		83-10-25%
		83-14-50%
Mixture 111	-	83-9-60%
		83-10-20%
		83-14-20%
Mixture 1V	-	83-9-20%
		83-10-40%
		83-14-40%

At each location the experiment was planted in four replications in Randomized Complete Block Design.

3.1.4 *Studies on natural cross pollination*

Germplasm collected need to be preserved in its original form as far as possible. Natural cross pollination is the major source of contamination of genetic collections of sesame. Studies were undertaken to estimate the degree of out-crossing under field conditions at two locations using three cultivars with contrasting morphological characters. Seeds were sampled from upper, middle and lower portions of plants of the three varieties having a few days difference in time taken to flowering. The degree of inter-genotypic out-crossing was estimated by scoring the number of plants with dominant characters in the recessive genotype (Details in appendix 3).

3.1.5 *Induced mutations*

Induction of mutations using physical and chemical mutagenic agents was attempted with the aim of improving the existing adapted cultivars and to improve the indehiscent cultivars (*id id* genotype) which always have several undesirable characters such as corrugated leaf, semi-sterility of pollen leading to fewer seeds per capsule etc. Gamma rays, diethyl sulphate and ethyl methane sulphonate were used as the mutagenic agents. Preliminary studies were undertaken to estimate the optimal doses of mutagens as the previous studies have given a wide range of LD_{50} values (see appendix 4).

In controlled selection experiments, it was attempted to estimate the variability and heritability of quantitative characters in irradiated populations and to compare the results with broadsense heritabilities and variability in the germplasm collection (see appendix 5). The promising mutants were included in variety testing program along with the promising breeding lines from the hybridization program (see appendix 6 and 7).

3.1.6 Drought resistance studies

Drought in the early stages of growth and at flowering can reduce the yield inspite of the well known drought resistant quantities of sesame (Weiss, 1983). Studies were undertaken with improved genotypes emanating from the breeding program to assess the degree of resistance to simulated drought conditions and other characters associated with drought tolerance. The studies involved the following aspects:

(a) Seed germination in mannitol and polyethylene glycol at different osmotic pressures.

(b) Behaviour of stomata under artificially induced water stress by detaching leaves.

(c) Stomatal density

(d) Shoot / root ratio in plants grown in 18 l buckets.

(e) Other morphological characters such as leaf area, pubescence.

(f) Evapotranspiration and dry matter accumulation under varying water regimes in pot experiments in the green house.

3.1.7 Doubled haploid method for sesame improvement

The future of sesame crop will largely depend on the ability of breeders to develop new varieties with improved characters for changing requirements of modern agricultures. .The classical breeding methods require relatively long periods to develop new cultivars. The Doubled Haploid (DH) method requires short period for the development of cultivars if efficient protocols for haploid production, diploidization and *in vitro* screening procedures are available (Morrison & Evans, 1988; Snape, 1989; Szarejko et al., 1991). Murashige and Skoog (1976) medium with different concentrations of 2,4-D, IAA and BAP was used to induce callus and for regeneration studies of anthers from MI 3 cultivar. Effects of explant age and of different light regimes (total darkness and 12 hrs light per day) were investigated for callus induction (see appendix 7).

3.2 Results

3.2.1 Germplasm collection and evaluation

The collection of germplasm represents sixty two countries in the American, African, European, Asian and Australian continents. Except for the 35 morphologically different varieties identified in Sri Lanka the largest collections were from Korea (155), Turkey (128), USA (106), Egypt (55), China (58), India (54), Pakistan (45).

Twenty eight morphological characters were studied and attempts were made to establish patterns of similarities in the wide variation observed by means of three faximetric methods. The variability studies indicated wide genotypic variation of characters indicating the ancient nature of the crop and its wide ecological adaptation. The degree of environmental variability of the same biometrical character fluctuated with in certain limits in different cultivars, whereas different characters showed highly significant differences even in the same genotype.

The taximetric analysis yielded five major groups of cultivars and five characters accounting for 85% of the variance.

3.2.2 Hybridization and selection

Over 250 different combinations of crosses were made successfully. Selections were made using different methods in the segregating populations, Five segregating populations originating from geographically diverse parents were subjected to five methods of selection for yield. There were no significant differences among methods of selection but the genotypes selected had significant differences among themselves for yield. Genotype-environment interaction of the selected genotypes was more pronounced in crosses developed from exotic parent material. More high yielding lines with less genotype - environment interactions could be selected in crosses involving atleast one locally adapted high yielding cultivar (see appendix 1). Therefore much attention

was paid to these crosses for developing lines with high yield and stability of performance. Lines with *Phytophthora* resistance (Fatmiani, 1992), low-reaction to day length such as Australian Introduction, high oil content and large seeds were included in many crosses and some of the advanced promising lines have these varieties as parents.

3.2.3 Heterosis and combining ability

The F_1 generation of many crosses was more vigorous than the parents. This effect was apparent in plant height, branching, number of capsules and leaves and in seed yield. High heterosis was apparent in crosses involving either high x low or low x low yielding parents, confirming observations of previous workers (Gupta, 1981; Ibrahim et al., 1983; Krishnaswami et al., 1985; Lichuan, 1988). The heterosis ranged from 5%-65% in our studies but the high heterotic crosses need to be checked once more.

The combining ability was studied in three diallel crosses. The yield and many related characters recorded significant GCA and SCA mean squares except 1000 seeds weight. GCA and SCA components were equally important in determining the characters. SCA component had a great influence in yield indicating presence of non-additive gene action in determining yield in sesame (appendix 2).

3.2.4 *Natural cross-pollination in sesame*

The results of natural cross-pollination (NCP) studies indicated 4-5.1% intergenotypic out-crossing in three varieties and at two locations. Unlike in previous studies (Langham, 1944; Muhammed et al., 1965; van Rheenen, 1968; Khidir, 1972; van Rheenen, 1980) the seeds were sampled from upper, middle and lower portions of the plant in three varieties differing in time taken to flowering and maturity. This enabled to conclude that the lower portion of early-flowering genotype and upper portion of the late-maturity genotype carry extremely low proportion of seeds derived from out-crossing (appendix 3). The results indicated the need for controlled self- or sib-pollinations for purity maintenance in germplasm and breeding plots. However, contamination due to NCP can be reduced by sampling seeds from the lower capsules of early-maturity and medium-maturity genotypes and from upper capsules of late-maturity genotypes in plots where pollination control is not practiced. The results obtained in these studies will be important in future considerations of methods for germplasm maintenance and pollination control (appendix 3).

3.2.5 *Induced mutations*

Results of our studies on radio-sensitivity of sesame confirmed the notion that its seeds are extremely resistant to irradiation. Even after irradiation with 1750 Gy gamma rays, the seeds of HI 3 variety germinated. Nevertheless the optimum doses

for inducing useful mutations was found to be in the range of 500-700 for MI 2 and 600-750 for MI 3. Varietal differences in radiation sensitivity were also found (appendix 4).

Selection experiments indicated that lower doses are more useful for inducing micro-mutations involving yield and other quantitative traits (appendix 5). More genetic variation and therefore effective selection could be practiced in M_3 rather than in M_2 as indicated by higher heritability of M_3-M_4 regression than in M_2-M_3 regression (appendix 5).

A number of mutants with changed morphological, physiological and anatomical characters have been isolated and confirmed in high yielding, locally adapted varieties (appendix 6). The early-flowering and early-maturity mutants, those with increased number of capsules per leaf axil (in MI 2 variety), branching in MI 3 variety etc. are useful in future breeding programs and some have recorded higher yield than their parent varieties recommended for cultivation (appendix 6). The brittle seed coat mutant MB 29 of MI 2 variety will be useful in the post-harvest processing of sesame (Annexure 6). By back-crossing with white seeded varieties, different colour variants and white seeded breeding lines with mutant character have been isolated. The original mutant MB 29w was tested in farmers fields in the Hambantota District and it has recorded 24.2% higher yield than MI 3 variety in ten farmers' fields.

The seeds of variety UCR 82-203DS having indehiscent capsules were irradiated with the hope of breaking the linkage of the indehiscent character with undesirable effects of *id id* genotype. In a population of more than 150000 M_2 plants representing about 40000 M_1 plants, two mutants without undesirable characters were found. At maturity it was found that the indehiscent character was also lost. This indicates that the *id id* locus has pleiotropic effects and there is a need for search of alternate sources of indehiscence or better seed retention.

3.2.6 *Advanced yield testing, superiority of mixtures*

Promising sesame lines developed through selection in segregating populations and by mutation breeding were tested in multilocational trials. These trials revealed the superiority of several breeding lines and mutants developed from MI 2 variety (Appendix 6). The breeding lines 83-9, 83-10 and 83-14 isolated in the same segregating population from the cross Sudu Thala 102 x (MI 2 x AI) were all outstanding but showed high Genotype-Environment interaction. The seeds of the three breeding lines when mixed with 60% of 83-9 and 20% each of 83-10 and 83-14 recorded the highest yield and stability. These lines were selected in F_3 and inspite of the presence of some degree of heterogeneity in each line, the performance improved when mixed. This indicates the high genetic vulnerability of pure lines and therefore early-generation selection should be practiced in sesame. Possibility of releasing mixture of lines with similar morphological and phenological characters is being considered.

In 1991 two advanced breeding lines were tested in ten farmers' fields in Monaragala District, in the Southeast of Sri Lanka. The two lines 83-14 and 83-10 recorded 41.4% and 29.5% higher average yield. In the Hambantota District the 'brittle seed coat mutant' HB 29w recorded 24.2% higher yield than MI 3 variety in 10 farmers fields.

3.2.7 Investigations on diseases of sesame

The research on identification and isolation of diseases was confined to the Southern Region because higher incidence occurs there due to cultivation in wet season. Pathogenicity tests revealed the presence of *Alternaria sesamicola*, *Cercospora sesami*, *Fusarium oxysporum* and *Phytophthora nicotianae* var. *Parasitica*. High incidence of *Alternaria sesamicola* at the University Research Farm and of *Phytophthora* blight at the research farm of the Regional Agricultural Research Station, Angunakolapelessa was observed. Testing for sensitivity to fungicides *in vitro* showed that 10 g ai ml^{-1} of Ridomil (84% Mancozeb and 16% metalaxyl) completely inhibited mycelial growth of *Alternaria sesamicola*.

3.2.8 Drought resistance studies

The characters associated with the drought resistance qualities of selected sesame genotypes were studied in different series of experiments. The genotypes with more leaves such as 83-10 and 83-14 recorded less evapotranspiration (ET) and vice versa. Seed germination in solutions with high osmotic potential have been used to study the drought resistance at seedling stage (Table 1).

Results indicated that accession No. 131 from Thailand and MI 3 had a higher capacity of germination under high osmotic potentials. When artificial drought was imposed under green house conditions, accession No. 132 (Thailand) was the earliest to wilt and MI 3 and 83-14 took the longest time. Fastest to recover was MR 29w. Accession 132 which wilted faster then other varieties recorded the longest period to recover (Table 1).

Table 1 - Some characters associated with drought resistance in sesame breeding lines and accessions compared with MI 3.

Genotype	No of leaves at flowering	ET per cm² of leaf at flower--ing	Seed Germination %		Time to wilt	Time to recover
			Mannitol, 6 atm.	Polyethene glycol, 2.3 atm.		
131	32 de	0.36 b	33 ab	34 ab	133 b	46 ab
132	19 e	0.40 b	25 c	40 a	99 b	66 a
135	58 b	0.20 a	26 bc	20 cd	126 b	41 ab
83-10	72 a	0.20 a	13 d	33 ab	128 b	48 ab
83-14	85 a	0.17 a	14 d	28 bc	186 a	52 ab
MB 32	35 cd	0.27 ab	ni	ni	ni	ni
MB 29w	48 bc	0.22 a	ni	ni	108 b	32 b
MI 2	42 cd	0.29 ab	ni	ni	ni	
MI 3	32 de	0.39 b	34 a	33 ab	191 a	35 ab

ni - not included in the experiment.

Values denoted by the same letter are statistically not significant according to DMRT.

Sesame shows heterophylly. Therefore the leaf area measurements are complicated without automatic measuring devices. Therefore we developed regression equations for leaf area based on the length of leaf for three different leaf shapes, namely lanceolate, ovate and tripartite. Further experiments with these formulae showed that the leaf area derived by these formulae highly correlate with the actual leaf area, with correlation coefficients ranging from 0.87 to 0.96 in different varieties and among different leaf shapes.

3.2.9 Doubled haploids - callus induction from anthers

As the first stage of anther culture for using doubled haploids for sesame breeding, callus tissue has been induced at high percent of success in the MS 5 medium used. This medium includes 2,4-D, IAA and BAP in concentrations of 10, 2 & 2 mg l^{-1} respectively with the basal salts of Murashige and Skoog (1976). The stage of the ex-plant, light and temperature optimal for the induction of callus in MS 5 medium have been found (appendix 8).

4. Impact, Relevance and Technology Transfer

The exotic and local germplasm collection made during the project is the second largest (by number of accessions) genetic collection after rice in Sri Lanka. By the number of countries of origin, it is the most comprehensive. This material represents a wide range of variability for morphological, physiological and

genetic characters and will serve the sesame breeders in Sri Lanka and throughout the world.

The breeding material developed in the hybridization and mutation induction programs represent new sources of genetic variability and have adaptive features required for Sri Lankan conditions. The advanced, more promising breeding lines will be included in the National Coordinated Trials in sesame conducted by the Department of Agriculture (DOA) for release to farmers. This will be the first instance when the DOA will include breeding lines developed by breeders other than from DOA.

The DOA extended its support during the project period by allowing the evaluation of breeding lines and germplasm at its Research Stations in Girandurukotte, Angunakolapelessa and Maha Illuppallama. This enabled the establishment of collaborative arrangements resulting in new project activities. Council for Agricultural Research Policy (CARP) has granted Rs. 680000/= to the Principal Investigator for further studies with the collected germplasm. The Pathology Division of Central Agricultural Research Institute, Gannoruwa, Peradeniya and Division of Oilseeds, Agricultural Research Station, Angunakolapelessa are collaborating in this project.

Sarvodaya Rural Enterprises Development Services, (REDS), an NGO, participated in the village level testing of promising breeding lines. Farmer families in Moneragala and Hambantota Districts participated. Promising breeding lines have already got

into the hands of farmers through this activity. In LDC's where certified seeds of crops are often not available, farmers' field testing in growing areas will enable the farmers to select and use the seeds of the cultivar of his preference. Growing of the test varieties using relatively advanced technology (row seeding fertilizer application, weeding etc.) in the farmers' fields convinced the farmers of the advantages.

Another project proposal for the development of a dehuller for 'brittle seed coat mutant' and to organize village level growing and dehulling is being considered by the Hambantota Intergrated Rural Development Project (NORAD funded). Once this project is implemented, dehulled seeds will be supplied by farmers to oil mills. Harischandra Mills, Matara has already indicated its willingness to buy dehulled seeds at a premium price. The dry dehulling will be possible with the new mutant, thus enabling the processing of the seeds in the areas of production, generating employment and income in the rural areas.

The Principal Investigator was able to meet a number of sesame researchers from different parts of the world during his attendance at conferences, resulting in international collaboration. Already one proposal has been prepared and submitted to the UNDP for an international research network in sesame. The proposal was prepared at a group consultation in Vienna convened jointly by FAO and IAEA. Other international funding sources have also been approached for financial assistance in collaborative research projects.

A tissue culture facility at the Agricultural Biology Section of the Department of Agronomy has been established with partial funding from project. Initial work on doubled haploids for sesame improvement has been started. This will pave the way for future research and training in tissue culture and plant biotechnology, a field with the potential of a second cycle of 'green revolution' to overcome hunger and poverty in LDC's. The facility is already in use for demonstrating tissue culture techniques for under-graduate students. Several graduates have already acquired skills in tissue culture during their work in the project.

5. Project Activities / Out puts

The P.I. attended several meetings and conferences with full or partial financial assistance from the project. The abstract or full paper presented is listed with the names of authors.

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03. Pathirana, R., Perera, R.A.S., Heepagala, H., and Pemadasa, H.A. (1988). Photoperiod response studies in sesame (*Sesamum indicum* L.) Proc. Int. Conf. Res. in Plant Sce. and It's Relevance to Future, Univ. of Delhi, New Delhi, India. pp. 80.

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11. Pathirana, R. (1992). Yield and quality improvement of sesame through induced mutations and cross breeding. In : The second Int. Symp. on New Genetical Approaches to Crop Improvement. 15-20 Feb., 1992, Karachi, Pakistan (Full paper in press).

The publications resulting from the project are given as appendices.

Dr. Rohan Rajapakse visited Field Experiment Station in Ubon Ratchathani Thailand in 1987 and P.I. visited several sesame research stations in Thailand during 1992. The P.I. also visited the Faculty of Agriculture, Hebrew University of Jerusalem and the Israel Gene Bank. During the visit of IAEA expert, Dr. M.A.Q. Shaikh, the P.I. participated in organizing a workshop in Mutation Breeding for Crop Improvement held at the Institute of Fundamental Studies, Kandy. About twenty five plant breeders participated.

Several farmer training workshops were held at Moneragala and Hambantota Districts in collaboration with REDS.

The B.Sc. (Agric.) degree at the Faculty of Agriculture, University of Ruhuna, requires the submission of a research report during the final year for specialization subject. Following reports were completed under this project:

01. Vidanagamage, Kusum. 1986. Variability, heritability, correlation and path-coefficient analysis of some quantitative characters in a model population of sesame (*Sesamum indicum* L.) 83 p. Supervisor :-Dr. R. Pathirana.

02. Jayaratne, G.A.S.A., 1986. Evaluation of a collection of sesame (*Sesamum indicum* L.) genotypes for qualitative characters. 89 p.
Supervisor : Dr. R. Pathirana.

03. Wijesekera, G.P.S.C. 1987. Analysis of quantitative characters in sesame (*Sesamum indicum* L.). 70 p.
Supervisor : Dr. R. Pathirana.

04. Meepagala, Mala 1987. Combining ability analysis of characters at flowering and yield components in sesame (*Sesamum indicum* L.) under two photoperiods. 83 p.
Supervisor : Dr. R. Pathirana.

05. Adipola, Meeta 1987. Evapotranspiration in Sesame.
Supervisor : Dr. K.D.N. Weerasinghe.

06. Gammanpila, Sandhys. 1990. Effect of different doses of gamma rays on sesame (*Sesamum indicum* L.) seeds. 32 p.
Supervisor : Dr. R. Pathirana.

07. Siriwardene, Manohari, 1990. Drougnt resistance studies in sesame.
Supervisor : Dr. K.D.N. Weerasinghe.

08. Balachandra, Nayana. 1991 Performance of some breeding lines of sesame (*Sesamum indicum* L.) in mixtures and pure stands. 43 p.
Supervisor : Dr. R. Pathirana.

09. Fernando, Sandhya, 1992. Investigations on drought resistance and variability of quantitative characters in a sesame (*Sesamum indicum* L.) collection. 69 p.
Supervisor : Dr. R. Pathirana.

6. Project Productivity

Most of the objectives of the project were accomplished. However the screening of the germplasm and breeding lines for disease resistance, mainly for *Phytophthora* blight under artificial inoculation could not be accomplished. The frequent change of Research Assistants, disruption of work due to closure of Faculty during the period of civil unrest and the difficulties with regard to methodology were the main reasons. This aspect will be achieved in the CARP project where the Chief Plant Pathologist of the Central Agricultural Research Institute, Peradeniya will be a collaborator.

The establishment of a tissue culture facility and anther culture for sesame improvement was not-envisaged in the initial project proposal. In later correspondence with USAID Office, concurrence for starting this work was obtained and this aspect will be actively persued in future research.

7. **Acknowledgements:** This research was supported under grant 936-5542, Program in Science and Technology Corporation of the U.S. Agency for International Development funded through the National Resources, Energy and Science Authority of Sri Lanka.

Dr. K.D.N. Weerasinghe and Dr. R.H.S. Rajapakse of the Department of Agronomy were involved in evapo-transpiration and disease resistance studies respectively. Dr. K.K.D.S. Ranaweera was associated in anther culture studies. The following Research Assistants worked on different aspects during the period of the project : Mrs. R.A.S. Perera, Mr. G.A.S.A. Jayaratne, Miss. J. Kariyawasam, Mr. A. Samarapala, Mr. W.M.M.A. Sirisena, Miss C. Hewage, Miss. S. Gunaratne Menike and Miss M. Siriwardane. The technical assistants Miss. A. Palangasinghe and Mr. B.G. Wijewanse fulfilled their duties to the best of their abilities and their contribution enabled the successful completion of the project. Mrs. C. Umagaliyage was employed under a grant from the Sri Lanka Atomic Energy Authority and contributed in the mutation research. Work on anther culture and mutation breeding of sesame was partly sponsored by the International Atomic Energy Agency, Vienna under RC 5442 RB. Following final year students of the Faculty of Agriculture completed their research projects in partial fulfillment of the requirements of B.Sc. (Agric.) degree under this research project. Mr. G.A.S.A. Jayaratne, Miss K. Widanagamage, Miss M. Meepagala Mr. G.P.S.C. Wijesekera, Miss M. Adipola, Miss. S. Gammanpila, Miss B.A.A.D.N. Balachandra, Miss M. Siriwardane and

Miss M.S.M. Fernando. Their untiring efforts to complete different aspects of the project are gratefully acknowledged.

Mr. P.B. Jayamanna and Mrs. P. Bandara, Research Officers of the Department of Agriculture helped in the conduct of research work pertaining to project at Angunakolapelessa and Maha Illuppallama Research Stations respectively. Mr. Anura Atapattu of REDS helped in organizing farmers' field trials. Mr. J.P.S. Dissanayake, Mr. Asoka Dharmapriya, Mr. N. Kangaharen and Mr. N. Wijesinghe helped in the work at Kengalla. Dr. L. Obeysekera helped in organising variety testing at Kurunegala. Without the keen interest and timely help by Mrs. S. Prelis and Mr. M. Watson, Officers in charge of the project at Natural Resources, Energy and Science Authority in Colombo and by Mr. S.H. Charles at USAID, Colombo, it would not have been possible to complete the project successfully.

Staff of the Agricultural Biology and Agricultural Engineering Sections of the Department of Agronomy helped in many ways. Allocation of land for Research in the Faculty Farm by Mr. K.A.A. Kumarasiri, Farm Manager and help by the staff of the Faculty Farm is also acknowledged.

Encouragement and interest shown by former Deans of the Faculty, Dr. S.K. Charles and Prof. K.K. Pathirana and by Head of the Dept. of Agronomy, Dr. K.D.N. Weerasinghe is appreciated.

Entering this text in the computer and of many other publications related to project was accomplished by Miss Mangala Karunaratne.

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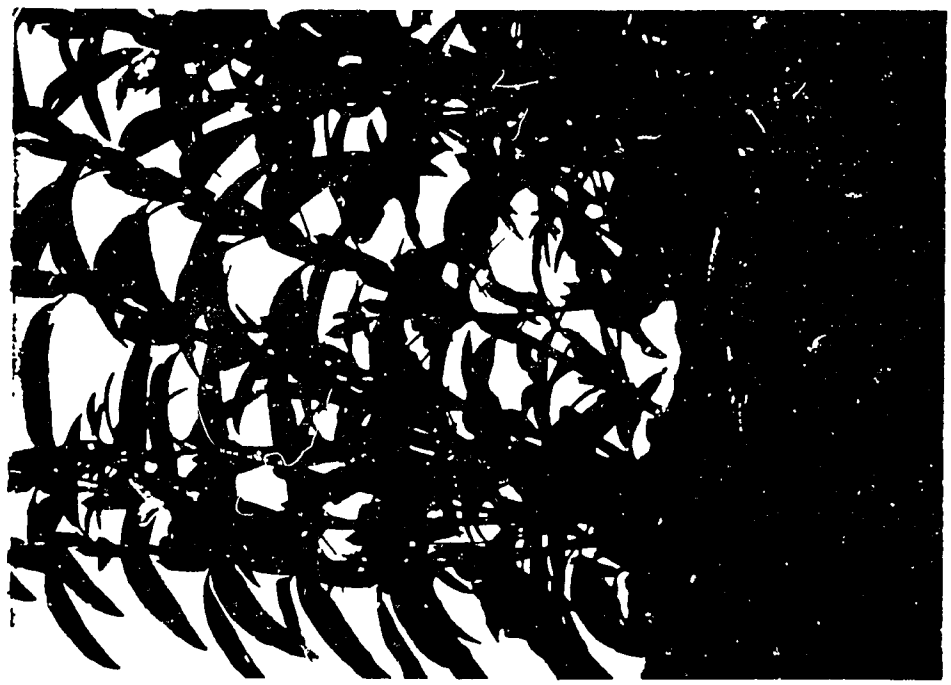
Plate 1 - General view of sesame field at Mapalana Reseach Farm.



Plant 2 - High yielding breeding line 83-10.

Plate 3 - Brittle seed coat mutant MB 29.

Plate 4 - Variety 'Improved Baco' with indehiscent capsules showing associated undesirable characters.

Plate 5 - Heterophylly in sesame.

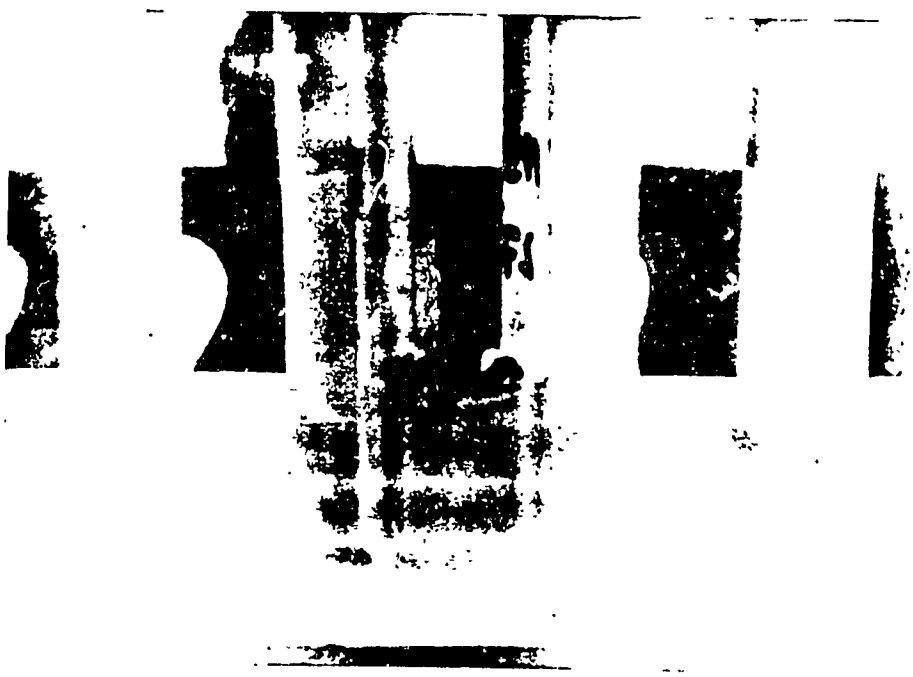


Plate 6 - Callus induced from anthers of MI3 variety.

Appendix I

Paper submitted to 'EUPHYTICA'

Title:

Comparison of Selection Procedures in Breeding for Seed Yield in Segregating Sesame Populations.

Running title:

Selection Procedures in Sesame

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Key Words : Breeding methods, Genotype - environment interaction, Generation advance, Heritability, *Sesamum indicum* (L.), Selection criteria.

Summmary

Sesame (*Sesamum indicum* L.) lines selected by bulk, early generation testing, pedigree and single seed descent procedures were tested for yield and other agronomic characters in F_6 and F_7. There were significant differences among lines for yield and other characters but the differences were not due to method of selection. More high yielding lines were isolated by the bulk method and in the cross involving two local cultivars when two seasons means and performance in each individual season were considered. The cross involving two exotic cultivars did not produce a single line superior to local recommended variety. Genotype-environment interaction was highly significant only for the lines derived from this cross. In lines derived from crosses involving atleast one local cultivar, genotype-environment interaction was either not significant or significant at 5% level. Heritability estimates ranged from 22.1% - 38.1% for yield, 41.8 - 75.7% for plant height and 24.3 - 41.0% for the number of capsules per plant. The results indicate that breeding lines with high yield and better adaptability can be generated by selection in sesame populations derived from crosses involving atleast one high yielding, locally adapted cultivar. Upto 80% breeding lines may be safely discarded after two seasons of testing.

Abbreviations: EGT - Early generation Testing

GxE - Genotype x Environment

SSD - Single seed descent

MSS - Modified single seed descent

BM - Bulk method

PM - Pedigree method

Introduction

Sesame is a major annual oil seed crop of the world and in Sri Lanka. Demand for sesame seed in the international trade is increasing due to the high quality of its oil and wide use in confectioneries and bakery products (Weiss, 1983; Salunkhe & Desai, 1986; Ashri, 1989). Production of sesame is concentrated in the developing, tropical countries and is grown mainly by small holders. As a result the crop has received less attention by breeders (Brar & Ahuja, 1979; Weiss, 1983; Ashri, 1989). In recent years there have been intensive efforts in different parts of the world to assemble the dwindling germplasm resources of this ancient crop. Some collections were more comprehensive and others were rather limited in scope (Thangavelu et al., 1985; Paroda et al., 1987; Ashri, 1990).

Introduction, sometimes accompanied by mass- or single-plant selection proved successful in several countries where this has been practiced. Hybridization programmes have been initiated to generate new variability and/or to combine specific traits (Ashri,

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1985; 1989; Lee & Choi, 1985; Pathirana et al., 1988). There is much scope for this approach in varietal improvement, particularly for increased yield as genetic variability for this trait seems to have been exhausted within local populations due to continuous selection (Rajan, 1981; Weiss, 1983; Anon, 1985; Ashri, 1989). Although many procedures are available for selection and generation advance in segregating populations of self-pollinated crops, their relative efficiency in selecting for yield is not known for sesame. Therefore an attempt was made to compare effectiveness of selection for yield by four standard methods of selection and a modified procedure for single seed descent method which could be applicable to small seeded crops grown under high plant density.

Materials and Methods

Five crosses were selected for the study on the basis of high degree of heterosis in F_1 and good general performance in the F_2. All crosses except one (No. 4) had a parent cultivar originating in Sri Lanka (Table 1). The variety MI 2 is recommended in Sri Lanka, Sudu Thala and MI 3 x AI (a pure line selected from a cross) have performed well in the National Co-ordinated Sesame Trials conducted by the Sri Lanka Department of Agriculture and 180/52 is a *Phytophthora* tolerant mutant isolated recently (Weerasena, 1988, Pathirana, 1992).

The spaced F_2 plants were grown at the Mapalana Research Farm of the Faculty of Agriculture, University of Ruhuna during the

Southwest monsoon season, 1987. At maturity one seed was harvested from each plant per cross for Single Seed Descent (SSD) procedure. One seed from each F_3 and F_4 plant were bulked for generation advance as usually practiced in the SSD procedure. The seeds selected for SSD procedure were space planted in F_3 and F_4 with out removing any extra plants. Three seeds from each F_2 plant were harvested for Modified Single Seed Descent procedure (MSS) and were planted at normal spacing. Ten days after emergence, the weaker seedlings were removed to maintain the number of individuals per cross similar to the original F_2 plant number. This procedure was repeated in F_3. In the F_4 150 best looking plants per population were selected for growing F_5 progeny rows out of which 16 best performing lines were selected for both SSD and MSS procedures for yield testing in F_6 and F_7.

Half the total weight of seeds from each F_2 plant per cross was bulked and grown in 300 m^2 plots. Thereafter total bulks were used for planting until F_4 for the bulk method (BM). The best looking 150 plants were selected in F_4, threshed individually and grown in 3 m row plots. The highest yielding 16 lines were saved for field testing in F_6 and F_7.

The remaining seeds from each F_2 plant were grown in 5 m rows and half of each row was thinned to 10 cm between plants. In the non thinned part, three plants each were selected from the best looking fifty rows for the pedigree method (PM). In the F_3 and F_4 generations 2-5 selections were made from the best looking rows

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and in F_5 generation 16 highest yielding lines were selected for F_6 and F_7 yield tests. In the 50 best looking rows of spaced plants, three plants each were selected for the early generation testing (EGT) method. The F_4 seeds of one hundred and fifty EGT plants per cross were planted in 5m rows, which were divided into 2 m for spaced plant selection and 3 m for yield testing. Three selected plants per family served as the three replications for testing family performance in the 3 m length of each row allocated for yield testing. Three plant selections were made in the space planted section of the highest yielding replications of the best 50 families in F_4. These selections were yield tested in F_5 and the best 16 lines from the highest yielding replications were saved for yield testing in F_6 and F_7 in comparison with lines emanating from other methods.

The 16 best lines from each of the five methods were yield tested with the recommended variety MI 3 as control in a randomized complete block design with four replications. Each population was planted as a separate experiment. Five meter rows were spaced at 30 cm and plants from the central four meters were used for yield testing. Estimates of plant height and number of capsules per plant were taken in F_5 and subsequent generations in the selected plants. Days to flowering and maturity were also observed in F_6 and F_7.

The F_5 generation was grown at the Agricultural Research Station, Giradurukotte. Both growing seasons were used for rapid

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generation advance. The experimental results were analysed by procedures ANOVA, GLM, CORR & REG of the Statistical Analysis Systems (SAS, 1987) package for microcomputers. The heritabilities of yield in different populations were estimated by the standard unit 'b' based on the correlation coefficients (Frey and Horner, 1957).

Results

The analyses of variance showed that there were highly significant differences in yield for the two seasons and for the selections in methods (Table 2). However the differences among selections were not due to the methods of selection. The interaction of genotypes with the seasons was significant in four of the five populations. The interaction effect was highly significant in the cross involving two exotic cultivars, Cola de Borrego and Hnani. The interaction of genotypes with seasons in the selections from the cross 180/52 x Instituto 81 was not significant. The heritability of yield was relatively low. Highest value was recorded for the cross No. 4 involving two exotic cultivars, closely followed by the cross 180/52 x Instituto 81. The lowest heritability was in the cross No. 3 involving two local, Sri Lankan varieties (Table 2).

There were 400 lines derived from five populations selected by five different methods. The number of lines which were among the highest yielding 20% without considering the population is

given in Table 3. The bulk method produced more high yielding lines when two seasons means as well as individual season means are considered. The eighty highest yielding lines recorded a 10.8% increase in yield over the overall mean. Early generation testing method produced the largest number of high yielding lines after bulk method. Other methods recorded almost similar numbers of lines among the highest yielding 20% except for the low number of lines in pedigree method when both seasons are considered individually. The yield of individual lines as a percentage of the yield of MI 3 (control variety in all experiments) was considered for selecting the best 20% lines for yield .

The variation in agronomic characters in breeding lines selected for testing in different populations is compared with the recommended variety MI 3 in Table 4 for the data of more favourable Southwest monsoon season, 1991. Population 3 derived from the cross of two local cultivars produced more lines with high yield and increased number of capsules. The cross number 4 involving two exotic cultivars did not produce a single line exceeding the yield of MI 3 variety. The lines from this cross were late in flowering and maturity. The other three populations produced several lines exceeding the yield of the recommended variety MI 3. They in general produced more capsules per plant and were taller than average. The heritability estimates for plant height were much higher than for number of capsules per plant and for yield (Table 2 & 4).

Discussion

The hybridization programmes in sesame have been followed mainly by mass- or single-plant selection or bulk population procedures (Anon., 1985; Lee & Choi, 1985; Ashri, 1989). No attempts seem to have been made to compare efficiency of different procedures of selection in segregating populations. This could be due to the fact that development of improved varieties of sesame by cross-breeding is still in its early phases in many countries (Ashri, 1989). Results of the present study indicate that visual selection for yield or early generation testing in sesame populations can both lead to lines with increased yield, provided that suitable crosses are used in which atleast one adapted local cultivar is included. Nevertheless use of several yield components having direct relation to yield such as number of capsules, short internodes, increased length of fruiting zone etc. may increase the effectiveness of selection as demonstrated in other experiments (Ibrahim et al., 1983). Regression, path-coefficient and discriminant function analysis in sesame have revealed efficient selection indices (Chavan & Chopde, 1981; Shukla, 1983; Gupta & Chopra, 1984; Reddy, 1984; Saxena & Mathur, 1988) but these have rarely been applied for selection in sesame breeding. Breeders have so far relied on direct selection for yield inspite of its low heritability as revealed in this study and in others (Solanki & Paliwal, 1981; Ibrahim et al., 1983; Pathirana et al., 1988; Pathirana, 1991).

Although visual selection is not considered to be a very accurate method for discriminating yield and should be used purely to discard the poorest lines, this method employed in four selection procedures used in this study seem to be as effective as EGT method. Based on the combining ability studies Rathnaswamy & Jagathesan (1984) successfully applied early generation selection procedure. The poor adaptability of cultivars is a widely encountered problem in sesame, as many cultivars are responsive to day length and temperature (Brar & Ahuja, 1979; Weiss, 1983; Thangavelu et al., 1985; Ashri, 1989). Highly significant G x E interaction recorded for the selections in the cross involving two exotic varieties and its absence or low level of significance in selections from crosses involving local cultivars indicate the importance of inclusion of adapted, high yielding local cultivars in the crossing programmes for developing breeding lines with wider adaptability. Due to high G x E effects in sesame, it is desirable to have more than one years data before discarding breeding lines.

In the future breeding programmes of sesame which is grown mainly under stress environments, the problem of yield stability of pure lines produced by classical breeding methods will be of paramount importance. Two major genetic mechanisms promoting stability are individual buffering and population buffering (Cessarili et al., 1991). Individual buffering being a property of heterozygotes as shown by Allard and Bradshaw (1964), exploitation of population buffering should be aimed in the development of cultivars of self-pollinated diploid species such

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as sesame. Population buffering is a mechanism of stability associated with genetic heterogeneity and therefore multi lines or variety mixtures could provide high yielding populations with increased yield stability. Mixing of breeding lines of sesame selected from the same segregating population has improved yield stability across environments (Pathirana, Unpubl. data).

About half the lines (35 out of 80) selected on the basis of two season's means were among the top 20% each season (Table 3). The bulk method produced more lines with high yield, although the highest yielding lines based on the means of two season's data were produced by pedigree, EGT and SSD producers. This aspect need no further discussions as there were no significant differences among methods of selection in any of the populations studied. Therefore further investigations should be directed at increasing selection efficiency using selection indices as high heritability of some of the yield components such as seed size have been reported (Ibrahim et al., 1983; Pathirana, 1991).

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Table 1 - Parent genotypes used in the crosses and their origin.

Population No.	Cross	Country of origin of parents
1	MI2 x 125 BON	Sri Lanka, Japan
2	180/52 x Instituto 81	Sri Lanka, Mexico
3	Sudu Thala x (MI3 x AI)	Sri Lanka (both)
4	Cola de Borrego x Hnani	Mexico, Burma
5	SI 70 x MI2	India, Sri Lanka

Table 2 - Analyses of variance and heritability estimates for yield in lines derived from five sesame populations/ and five selection methods.

Source of variance	Populations 1	2	3	4	5
Seasons (S)	**	**	**	**	**
Methods (M)	ns	ns	ns	ns	ns
Selections in M	**	**	**	**	**
G^{a} x S	*	ns	*	**	*
Heritability	28.4	36.2	22.1	38.1	29.8

*, ** - Significant at 5 and 1% level, respectively.
ns - Not significant.
[a]G - Indicates genotype which is the combination of methods and selections in methods.

Table 3 - The number of lines among the different methods of selection that were in the top 20% based on 2 seasons' means and in both seasons in five sesame populations.

	Methods						Yield
	SSD	MSS	BM	PM	EGT	Total	% of mean[a]
Top 20% lines based on means of 2 seasons	14	15	20	15	16	80	110.8
Number of lines that were in top 20% both seasons	6	7	9	5	8	35	113.2

[a] - Mean yields were computed on the basis of total 400 lines selected in the five crosses.

Table 4 - Character means and range of selected lines in five sesame populations compared with 'MI 3' variety and heritability estimates (Southwest monsoon season, 1991).

		Population					MI 3
		1	2	3	4	5	
Days to flowering	Mean	29	31	30	32	27	29
	Range	27-33	28-35	27-35	28-38	24-32	
Days to maturity	Mean	88	89	92	94	87	89
	Range	85-94	84-95	85-100	85-102	83-95	
Plant height, cm	Mean	87	93	101	98	89	96
	Range	74-95	79-102	88-119	85-117	80-102	
	Herita-bility	71.3	67.5	41.8	75.7	52.9	
No of capsules per plant	Mean	61	68	74	56	67	70
	Range	49-73	58-81	61-88	41-67	56-79	
	Herita-bility	32.1	37.8	24.3	36.8	41.0	
Yield, g plot^{-1}	Mean	85	97	107	78	94	101
	Range	66-109	76-118	88-122	62-93	78-111	

Appendix 2

Paper submitted to 'Tropical Agriculture' (Trinidad)

Title:

Combining Ability for Yield and Agronomic characters in Sesame cultivars of Diverse Origin.

Running title: Combining Ability in Sesame

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In a diallel of seven sesame varieties originating in different latitudes, heterosis was pronounced for the number of leaves, plant height at flowering and at maturity, dry plant weight, number of capsules and seeds per plant and seed yield. However, hybrids of local high yielding cultivar MI 2 did not record appreciable heterosis for yield. GCA and SCA mean squares were significant for all characters except seed size. GCA and SCA components were equally important in determining the characters. SCA component had the greatest influence on seed yield. Local variety MI 2 recorded positive GCA effects for all characters, including the highest effects for number of capsules and seeds per plant and for yield. Three crosses of late-maturing variety Criollo de Falcon and two of day-neutral cultivar Australian Introduction recorded high SCA effects for seed yield. Simultaneous exploitation of both additive and non-additive genetic components in a biparental mating system involving local high yielding cultivars and early-maturing exotic material followed by recurrent selection may hasten the rate of genetic improvement of yield and its stability in sesame.

Key Words: Diallel analysis, Heterosis, General combining ability, Specific combining ability, Selection of parents, Variance components.

Narrow adaptation of cultivars and land races of sesame (*Sesamum indicum* L.) is considered to be a major reason for the low yields of this important oil crop (Brar and Ahuja, 1979; Weiss, 1983; Ashri, 1989). Cultivars originating in relatively higher latitudes tend to mature early in the tropical zone and are less sensitive to day length providing valuable material for breeding genotypes with wide adaptation (Weiss, 1983). Introgression of genes from cultivars originating in different agroclimatic zones through hybridization could lead to genotypes adapted to a wider

range of environments, increasing the adaptability and stability of sesame yields (Yermanos, 1980; Weiss, 1983; Ashri, 1985).

With the availability of male sterility (Osman and Yermanos, 1982; Ramahatan et al., 1992)) and a polypetalous corolla mutant genotype (Murty and Bhatia, 1990) which can further enhance the observed high levels of out crossing (Yermanos, 1980; Weiss, 1983; Ashri, 1989), interest has developed in the use of F_1 hybrids for the increase of sesame production. High degree of heterosis for different characters including yield and the low seed rate have also encouraged investigations on heterosis and combining ability in sesame. Relatively high estimates of combining ability and heterosis have been observed even among crosses involving local cultivars (Murty, 1975; Osman, 1985; Dora and Kamala, 1986; 1987; Ray and Sen, 1992).

However the lack of successful combinations with good general and specific combining abilities (Krishnaswamy et al., 1985) and non-availability of high heterosis among crosses involving high yielding parents (Ibrahim et al., 1983) has been discouraging in some studies. The objective of the present investigation was to estimate the combining ability and hybrid performance in a diallel cross involving seven promising genotypes from diverse agro-climatic zones, including the only determinate genotype of sesame used widely in hybridization programmes (Ashri, 1985; 1989; Wongyai, 1991).

Materials and Methods

Seven cultivars originating in different latitudes showing relatively good performance in Sri Lanka and their all possible F_1 crosses excluding the reciprocals were grown in a Randomized Complete Block design with four replications at the Mapalana Field Research Station of the Faculty of Agriculture, University of Ruhuna in the Southern Low Country Intermediate Zone of Sri Lanka during Southwest monsoon 1991.

The cultivars used in the crosses are as follows:

1. Australian Introduction (AI)- Introduced from Australia in the early 1960's and showing good adaptation to both monsoon seasons (Pathirana, 1984). Yield potential in Sri Lanka ranges from 800-1100 kg ha^{-1}.

2. Bjal Sadovski (BŚ) - Non branching, early-maturing cultivar with one capsule per leaf axil. Introduced from Bulgaria.

3. III 2 (III) - Profusely branching local cultivar with black seeds. Released in 1960's and has recorded the highest yields in variety trials in Sri Lanka. A selection from a local Sri Lankan land race.

4. Oro Short (OS)- A compact plant with short internodes, early maturing and three capsules per leaf axil. Introduced from USA.

5. Criollo de Falcon (CF). A late flowering cultivar with a profuse initial growth before flowering. Has tolerance to powdery mildew and collar rot. Introduced from Venezuela.

6. Determinate (DM)- An induced mutant of Israeli variety T 65 (Ashri, 1985). The only known sesame genotype having determinate growth habit, with the apical meristem giving rise to a floral bud. Growth continues with the axillary buds.

7. Suweon 21 (SW) - Introduced from South Korea. Has a vigorous growth during the early phase of development.

The twenty eight genotypes (21 crosses and 7 parents) were grown in single row plots of 3 m length and 30 cm between rows. The plants were spaced at 10 cm within rows. The total rainfall of 532 mm received during the growing season was evenly distributed and no irrigation was required.

Fifteen random plants per plot were tagged at the early phase of growth and all the data were recorded on these plants. Data on a plot basis was also recorded for yield. The character measurements or observations were recorded according sesame descriptors (IBPGR,1984).

The number of seeds per plant was counted in an electronic seed counter - totalizer adjusted for sesame seeds. The effects, differences between effects and the corresponding standard errors for the sources, GCA and SCA were estimated according to model 1, method 2 of Griffing (1956), considering parents as a fixed sample. The components of variation for

GCA ($V_{GCA} = 1/6 \; g_i^2$) and

SCA ($V_{SCA} = 1/21 \; S_{ij}^2$) for all traits were also estimated (Griffing, 1956).

Results and Discussion

Significant differences among the means of parents and among the means for the crosses were found for all traits except 1000 seed weight. The grand mean of parents was significantly different from the grand mean of the crosses for the number of leaves at flowering, plant height at flowering and maturity, plant dry weight, number of capsules, number of seeds and seed weight per plant. There were no significant differences for 1000 seed weight.

Several crosses exhibited significant heterosis. The number of crosses with significantly positive high parent heterosis for different characters were as follows: Number of leaves - 4, plant height at flowering and maturity - 4, dry plant weight - 5, number of capsules - 7, number of seeds - 9 and seed yield per plant - 7. The cultivars which produced the majority of crosses with significantly high positive heterosis were those having lower yield

such as Determinate and Bjal Sadovski. Australian Introduction was also involved in three hybrids which recorded high heterosis for seed yield. This cultivar is widely used in the hybridization programmes in Sri Lanka as it is less sensitive to the differences in day length during the two growing seasons (Pathirana, 1984).

None of the hybrids of the local high yielding cultivar MI 2 exhibited appreciable levels of heterosis (at least 5% over high yielding parent). Gupta (1981), Ibrahim et al., (1983), Krishnaswami et al., (1985) and Lichuan et al., (1988) also observed that heterosis of sesame hybrids was higher in low x high yielding varieties than high x high yield varieties. This could be one reason for not having F_1 hybrid cultivars of sesame in widespread use although some of the necessary conditions for their production have been met (Osman and Yermanos, 1982; Ashri, 1988; Murty and Bhatia, 1990; Ramahatan, 1992).

The analysis of variance recorded significant F values for the genotypes in all the characters studied except 1000 seed weight. Therefore combining ability analysis was not performed for 1000 seed weight. Combining ability analysis revealed significant GCA mean square values for all the characters. The SCA had a similar tendency as GCA but the number of seeds and seed yield per plant were significant at 5% level only (Table 1). The combining ability has been found to be significant even in dialled crosses produced from cultivars originating in the same country (Gupta, 1981; Singh et al., 1983; Sharma and Chauhan, 1985; Dora and Kamala, 1987).

In a line x tester analysis using five Indian varieties as testers and lines originating in different agroclimatic zones, Krishnadoss et al., (1987) found that GCA variances were significant for all characters except 1000 seed weight.

Baker (1978) critically assessed the relative importance of general and specific combining ability in Griffing's (1956) fixed and random models. The low level of significance (at 5% probability) of SCA mean square for seed yield and the number of seeds per plant in our study indicate that the best performing progeny for these characters may be produced by crossing the two parents having highest GCA. Nevertheless interactions seem to have an accountable effect in determining the performance of individual crosses and therefore interpretations of GCA should be made with care.

The components of variation showed different patterns according to the characters. Thus, the GCA component was higher for plant height (both at flowering and maturity) and plant dry weight. Apparently additive genetic effects are of greater significance than non-additive effects for these characters. Most of the other characters studied had higher SCA component than GCA component (Table 1) suggesting the importance of non-additive effects. The SCA component had the greatest influence on seed yield. In some previous studies on the combining ability in sesame, the predominance of non-additive or dominance gene action for many characters including seed yield has been reported (Singh

et al., 1983; Krishnadoss et al., 1985; Dora and Kamala, 1987). Nevertheless, greater contribution of GCA is also not uncommon (Gupta, 1981; Sharma and Chauhan, 1985) indicating predominance of additive gene action in some combinations.

The local high yielding cultivar MI 2 recorded positive GCA effects for all the characters, including the highest effect for seed yield as well as the number of seeds and number of capsules which are the two direct yield components included in this study. High positive GCA effects were recorded also in Criollo de Falcon, the late maturing, tall genotype. It's high GCA effects of number of seeds and capsules, plant dry weight, plant height and number of leaves are noteworthy. However the GCA effect of seed yield of Criollo de Falcon was negative (Table 2). The early-maturing cultivar Bjal Sadovski also recorded a positive GCA effect for seed yield. The negative GCA effect of Determinate was negligible and it should be possible to use this genotype effectively in breeding programmes to develop determinate, high yielding cultivars.

The crosses with the largest SCA effects for seed yield were AI x DM, OS x CF, BS x CF and AI x CF. Crosses with low SCA effects (negative) were MI x CF, AI x OS, MI x DM and CF x DM. Thus, three crosses of Criollo de Falcon and two crosses of Australian Introduction recorded high SCA effects and two crosses each of MI 3, Criollo de Falcon and Determinate recorded low and negative SCA effects for yield. The number of capsules per plant also had similar trends except for the high SCA effects of AI x SW,

CF x DM and DM x SW and low SCA effects of MI x DM and CF x SW. Highly positive SCA effects of the crosses BS x CF and DM x SW for plant height, and highly negative SCA effects of MI x CF and AI x MI crosses are also noteworthy.

It is clear from this study that improvement of yield, yield contributing characters and maturity would be possible by the simultaneous exploitation of both additive and non-additive genetic components. Therefore, biparental mating followed by recurrent selection may hasten the rate of genetic improvement for these characters. The local high yielding parents which have good general combining ability for yield and related characters would be utilized in hybridization programme with exotic early-maturing cultivars for improving yield and its stability in sesame.

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Table 1 - Mean squares of the diallel analysis of variance and components of variation for different characters.

Traits	Mean squares GCA	SCA	Components of Variation V_{GCA}	V_{SCA}
Days to flowering	207.7**	52.4**	17.3	49.1
Plant height at flowering	919.4**	68.4**	94.6	51.5
Number of leaves at flowering	486.64**	706.4**	462.2	637.8
Plant height	1598.4**	75.6**	169.2	45.8
Internode length	17.0**	4.1**	0.6	1.17
Dry plant weight	764.4**	81.2**	75.9	52.0
Number of capsules	745.9**	90.9**	72.8	71.3
Number of seeds	1067858.7**	302213.8*	85072	159119
Seed yield	3.98**	3.09*	0.10	2.33

* and ** significant at 5 and 1% levels, respectively.

Table 2 - Estimates of general combining ability effects for parent cultivars.

Parents	Days to flow-ering	Plant height at flowe-ring	Number of leaves	Plant height	Dry plant wei-ght	Number of capsu-les	Number of seeds	Seed yield
AI	0.2	1.1	2.2	3.8	-2.6	-0.3	-50.8	-0.11
BS	-3.6	-9.9	-18.0	-7.9	-5.1	-5.5	-65.3	0.34
HI	2.9	5.8	13.8	9.2	1.3	12.6	596.7	1.37
OS	-2.9	-5.3	-13.9	-9.9	-5.9	-8.5	-371.6	-0.68
CF	9.5	19.7	34.4	24.7	20.1	12.3	115.4	-0.12
DH	-3.5	-5.2	-18.2	-8.3	-1.9	-1.4	181.4	-0.02
SW	-2.6	-6.2	-0.3	-11.5	-5.7	-9.2	-415.8	-0.78
S.E.g_i	0.9	1.9	2.8	2.6	2.5	2.1	178.7	0.41
S.E. (g_i-g_j)	1.4	3.2	4.5	4.7	3.7	3.1	267.4	0.64

Appendix 3

Paper submitted to 'Plant Breeding'

Title:

Natural Cross Pollination in Sesame

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ABSTRACT

Three sesame (*Sesamum indicum* L.) genotypes with contrasting morphological characters were grown in all that rows to estimate the degree of natural cross pollination (NCP) at two locations. Capsules from lower (first formed five capsules), upper (last five fully developed capsules) and middle portions were separately harvested and the frequency of appearence of progenies with dominant markers was used as the criterion for NCP. The degree of intergenotypic NCP was similar in the three genotypes and at two locations (from 4.02% to 5.10% NCP) when the total amount of hybrid seeds (total plant) is considered. The lower portion of the early (1.03-1.31% NCP) and medium (1.51-2.02% NCP) maturity genotypes and the upper portion of the late-maturity (2.27-2.49% NCP) genotype recorded less NCP than the total plant. The NCP in the lower portion of the early-maturity genotype due to pollen from late-maturity genotype was almost nil (0-0.07%). The results indicated the need for controlled self pollination for purity maintenance in breeding plots. However, contamination due to NCP can be reduced by sampling seeds from the lower capsules of early and medium-maturity genotypes and from upper capsules of late-maturing genotypes in germplasm maintenance, breeding nurseries and seed production plots where pollination control is not practiced.

Key words;

Sesamum indicum L., natural cross-pollination, Out-crossing, genetic purity, seed production, germplasm maintenance.

For all practical purposes sesame (Sesamum indicum L.) is considered to be self pollinated although some degree of natural cross pollination (NCP) is present (Brar and Ahuja, 1979, Weiss, 1983, Ashri, 1989). Under many conditions and in many cultivars

the degree of NCP does not exceed 10% (Langham, 1944, Joshi, 1961, Muhammed et al., 1965, van Rheenen, 1968, 1980, Khidir, 1972, Chaudhari and Zope, 1977). Nevertheless, rates as high as 50 - 65% have also been reported (Richharia, 1938/9 as quoted by Joshi, 1961 and Uzo, 1976 as quoted by Osman, 1985, van Rheenen, 1980, Ashri, 1988). This high degree of variation in outcrossing of sesame necessitates studies under particular environmental conditions where variety maintenance, breeding and seed production are practiced. The study reported here was undertaken to obtain information on the extent of natural cross-pollination (NCP) in two popular Sri Lankan varieties and a breeding line. The study was conducted during South - West monsoon season, in which more than 80% of the sesame is produced in the country. The experiment mimicked the conditions under which the early generation breeding lines and the germplasm collections are evaluated and maintained. One of the locations selected for the experiment is the site for the evaluation and maintenance of a duplicate set of the world germplasm collection of sesame made by Prof. A. Ashri (1990).

MATERIALS AND METHODS

Two local varieties 'MI2' and 'MI3' used in the experiment are cultivated in Sri Lanka and have contrasting characteristics. The breeding line '83-14' has successfully undergone the final testing in farmers' fields before release. These genotypes differ in time taken to flowering and maturity with an average difference of 3-5 days. The single capsule per leaf axil and branched stem

characters of 'MI2' and '83-14' are dominant over three capsules and non-branched stem in 'MI3'. The lobed basal leaves of '83-14' are dominant over simple leaf character of the other two varieties. The pink colour of the base of the corolla tube in 'MI3' and '83-14' is dominant over the purple spots in 'MI2'. The coloured (purple) lip of corolla in 'MI2' and 'MI3' is dominant over the white lip of corolla in '83-14'. All the mentioned characters are simply inherited (Brar and Ahuja, 1979) and have often been used as markers for estimating the out-crossing in sesame (Langham, 1944, Joshi, 1961, Martinez and Quilantan, 1963/64, Muhammed et al., 1965, van Rheenen, 1968,1980, Khidir, 1972, Osman, 1985).

The seeds for the experiment were produced by controlled self pollination of typical plants of each genotype multiplied subsequently in isolation with further checking for any mixtures. The selfed seeds of each genotype were sown in the last week of April 1990 in alternate rows with a distance of 30 cm between rows and 10 cm within row. The experiment was conducted during the South-west monsoon season of 1990 at the Kengalla field of the Institute of Fundamental Studies near kandy and at the Mapalana Research Farm of the Faculty of Agriculture, University of Ruhuna where the largest sesame collection in the country is maintained and an intensive breeding programme has also been in progress since 1986 (Pathirana et al., 1988).

At both sites the row length was 12 m and there were 28 rows of each genotype alternating with the other two. At maturity the

five basal capsules (lower part) and last five fully developed capsules (upper part) from each plant were harvested and bulked according to row number and genotype. The rest of the capsules (middle part) were harvested separately discarding the border plants and rows. The seeds thus collected were sown in parts during the three succeeding seasons and the progenies of 'MI2' were scored for pink spots inside the corolla tube and for lobed basal leaves, progenies of 'MI3' variety for one capsule per leaf axil, branched stem and lobed basal leaves and '83-14' progenies for coloured corolla lip.

RESULTS

The variety 'MI 3' flowered first, 3 and 7 days before 'MI 2' and '83-14' respectively. Flowering in all the genotypes lasted for about six weeks. The percentage out-crossing as assessed by the counts of plants with dominant characters in the progenies is given in Table 1. Although the total out-crossing percentage in the three varieties at the two sites was similar, the seeds from the middle portion of the plants recorded more dominant characters than in the upper or the lower portion, indicating higher rates of NCP in the middle portion. The lower portion of the early-flowering variety 'MI3' recorded the least amount of NCP. In the late flowering genotype '83-14' the upper portion had less NCP than in the middle and lower portions (Table 1).

The lobed basal leaves in '83-14' genotype which appear in F_1 hybrids with 'MI2' and 'MI3' cultivars allowed separate estimation of the out-crossing percentage in the latter two varieties with '83-14' (Table 2). The lower portion of 'MI3' plants recorded none (at Kengalla site) or very few (0.07% at Mapalana site) hybrid seeds derived from NCP with '83-14'. The NCP was greater in the middle and upper portions. The lower portion of 'MI2' variety, which also flowers a few days earlier than '83-14' recorded less than average out-crossing with '83-14'. The total amount of out-crossing of 'MI3' (early-maturing variety) with '83-14' (late-maturing variety) was much less than that of 'MI2'(medium maturity variety) at both sites (Table 2).

DISCUSSION

Mechanical admixture and cross-pollination are the two major reasons for contamination of seed crops. The former can be eliminated by good management. Dealing with the latter is rather difficult. Estimates of NCP under different conditions should be available before precautions against it are taken. Although the mode of pollination very largely ensures self-fertilization (Yermanos, 1980, Weiss, 1983, Ashri, 1989) the long flowering period and the abundance of honey bees and other insects in sesame fields (Langham, 1944, Yermanos, 1980, Weiss, 1983, Osman, 1985) expose the plants to cross-pollination.

From the experimental evidence presented it is clear that NCP is sufficiently high for protection to be necessary to maintain

genetic purity at Sri Lankan locations where sesame germplasm maintenance, breeding work and seed production are practiced. The extent of NCP is about the average reported for many countries such as India (Joshi, 1961, Muhammed et al., 1965, Brar and Ahuja, 1979), Nigeria (van Rheenen, 1968) and Sudan (Khidir, 1972). It should be noted that the rates of NCP reported in this study are based on intergenotypic hybrids. The intragenotypic (sib) pollinations have not been accounted for, and the latter could be even greater due to simultaneous flowering of plants of the same genotype. Although the high degree of 65% NCP reported in India by Richharia (1938/9 as quoted by Joshi, 1961 and Osman, 1985) is regarded to be an overestimate as it was based on the number of off-type plants in various cultivars (Osman, 1985), similar high rates (60% and 65%) were reported also by Uzo (1976, as quoted by Osman, 1985) in California. The extent of NCP in Mexico under summer conditions was similar to our results, but was higher (15%) during the winter when insect populations were greater (Martinez and Quilantan, 1963/64). Varietal differences in outcrossing percentage ranged from 2.67% to 51.72% in another experiment in Nigeria (van Rheenen, 1980).

Partitioning of the plant into three portions for the estimation of NCP gave results having important practical implications. The first formed capsules of early-flowering genotype 'MI 3' and the capsules that form last in the late-flowering genotype '83-14' carry less proportion of seeds derived from intergenotypic cross-pollination than other capsules. In the

early-flowering 'MI 3' the first formed capsules carry extremely small proportion (none at Kengalla and 0.07% at Mapalana) of seeds derived from out-crossing with '83-14', the late-flowering genotype.

The three genotypes used in this experiment produce an average of 47-63 capsules per plant. As only the five basal and five terminal capsules were sampled for estimating the NCP in the lower and upper parts of the plant, a larger proportion of capsules were left for the middle part (ca 35-50). Therefore the estimates of NCP for middle part of the plant should be more representative of average cross-pollination than the overall values calculated on the basis of total number of progenies from different parts carrying dominant markers. This is because similar number of plants were grown from seeds belonging to different parts of the plant to score NCP, although a larger proportion of seeds came from the middle part. The NCP in the middle part was greater than the overall NCP and reached 9.11% in 'MI 3' at Kengalla.

In 73% of research stations in 25 countries, the collections of sesame cultivars are maintained under open pollination (van Rheenen, 1980). Controlled self-pollination is generally not practiced due to a large number of accessions in many collections (Thangavelu et al., 1985, Paroda et al., 1987, Ashri, 1990), limitations of manpower at the time of flowering when hybridization has higher priority and because the plant breeders tend to devote their efforts only to a limited number of lines at

any one time (Smith and Duvick, 1989). Considering the high degree of NCP in sesame in many countries, efforts should be taken to maintain the germplasm collections and breeding lines under controlled self pollination. If this is not always possible, sampling of seeds from the first formed capsules of early-maturing cultivars and from capsules maturing last in the late-maturing cultivars can reduce the degree of contamination. Sampling of seeds of first formed capsules in cultivars of intermediate maturity group can also reduce the contamination due to out-crossing. Thus, in the present experiment, the variety 'MI2' of intermediate maturity had a lesser proportion of hybrid seeds in the lower portion of the plant than in the middle or upper portions (Tables 1 and 2).

To decrease the extent of contamination in seed production plots, plantings of seeds from the first formed capsules of early and medium-maturity varieties and from top portions of the late-maturing varieties should be used.

ACKNOWLEDGEMENT :

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Table 1 - Comparison of intergenotypic cross-pollination (%) in different parts of the plants in three sesame genotypes, in two locations and total number of plants tested.

Seeds from	% hybrids in		
	MI 2	MI 3	83-14
Kengalla site			
Lower part	1.51	1.03	3.73
MIddle part	7.16	9.11	7.48
Upper part	3.59	3.98	2.49
Overall	4.47	5.00	5.10
Mapalana site			
Lower part	2.08	1.31	3.67
Middle part	6.56	6.17	6.64
Upper part	3.14	4.07	2.27
Overall	4.23	4.02	4.24
	Overall No. of Plants		
Site			
Kengalla	6821	12070	8140
Mapalana	8804	14295	11226

Table 2 - Comparison of intergenotypic cross-pollination (%) in different parts of plants in MI 2 and MI 3 varieties with line 83-14 in two locations and total number of plants tested.

Seeds from	% hybrids in	
	MI 2	MI 3
Kengalla site		
Lower part	0.44	0
Middle part	4.00	2.10
Upper part	2.64	2.24
Overall	2.61	1.47
Mapalana site		
Lower part	1.11	0.07
Middle part	4.00	2.75
Upper part	2.08	2.08
Overall	2.58	1.72
	Overall No. of Plants	
Site		
Kengalla	6821	12070
Mapalana	8804	14295

Appendix 4

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RESPONSE OF SESAME GENOTYPES TO SEED IRRADIATION

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ABSTRACT :

Due to high degree of variation in radiosensitivity of Sesame (*Sesamum indicum* L.) cultivars observed in the past, two recommended cultivars with different morphological characteristics were studied in detail for sensitivity to seed irradiation. The experiment was conducted in four replications with MI2 and MI3 cultivars. The results clearly indicated that when growth reduction, percent survival and germination were considered as criteria of radiosensitivity, MI3 is more resistant to irradiation than MI2. Fifty percent reduction of different growth parameters ranged from 500 - 700 Gy in MI2, whereas for MI3 variety, the range was from 750 - 1250 Gy. The lower doses in these ranges should be more useful in mutation breeding experiments with these two cultivars. Due to the wide variation in sensitivity of different sesame genotypes to irradiation, it is necessary to estimate the radiosensitivity of cultivars being used for mutation breeding before deciding on the dosage.

INTRODUCTION

The potential role of induced mutations in the improvement of sesame (*Sesamum indicum* L.) is quite high [1-4]. Establishment of the optimal dose levels of the mutagens for a particular crop species is a primary requirement for the success of mutation breeding [5,6].

Resistance of sesame seeds to irradiation has been reported to be generally high [2]. However, there seems to be great variation among genotypes in their response to seed irradiation. Thus, gamma ray doses of 600 and 800 Gy were found to be lethal or semi-lethal for Giza 25 variety and 200 Gy dose was optimal for breeding purposes [7]. In the Korean variety Hanseomggae, doses upto 400 Gy did not reduce germination, plant height, capsule number or seed yield [8]. In another study, five genotypes tested for sensitivity to seed irradiation with gamma rays have shown no reduction in plant height or the number of capsules upto 600 Gy gamma irradiation [9]. Gamma rays in the range of 200 Gy - 1400 Gy have been used in different cultivars for mutation induction in six Indian cultivars [10,11]. Gamma ray doses upto 400 Gy did not adversely affect any of the measured characters of recommended Bangladeshi variety T-6 and of exotic strain SB-S-LP-5, except pollen fertility and the number of capsules per node [12]. In an experiment to induce mutations for field tolerance to *Phytophthora* blight, more lines with tolerance were isolated in 600 Gy dose [13]. For quantitative characters, the greatest variability could be induced using gamma ray doses ranging from 300 - 600 Gy in MI2 cultivar [14].

Considering the range of variation in reaction to seed irradiation observed in cultivars of different origin, it was decided to study in detail, the response of two morphologically dissimilar sesame cultivars MI3 and MI2 to gamma irradiation.

MATERIALS AND METHODS

The cultivar MI2 has recorded the highest yield in multilocational trials in Sri Lanka. This cultivar has basal branching habit, one capsule per leaf axil and black seeds. The only cultivar with white seeds recommended for cultivation in Sri Lanka is MI3. It produces three flowers per leaf axil and has a non branching stem. The phyllotaxy is opposite, resulting in clustered arrangement of capsules on the stem. This variety has lesser yield potential than MI2.

For MI2 variety, ten doses of gamma rays from 100 Gy to 1000 Gy were used for radiosensitivity studies. The variety MI3 was irradiated with 250, 500, 625, 750, 875, 1000, 1125, 1250, 1375, 1500 and 1750 Gy doses. About 5g seeds at 13% moisture were used for irradiation with each dose (1000 seed weight = 3.1g). Higher doses had to be used on MI3 variety because of its relatively greater resistance shown in previous experiments [14]. Seed irradiation was carried out at the Central Agricultural Research Institute, Gannoruwa, Peradeniya. The irradiated seeds were, sown in the experimental field of the Institute of Fundamental Studies at Kengalla in the mid country intermediate zone during the South west monsoon season, 1990.

Two hundred seeds per dose per variety were grown in a randomized complete block design with four replications. Unirradiated seeds served as the control. Seventeen plants per replicate were randomly chosen and used for measurements.

Another batch of 20 plants per replicate were used for uprooting at 15 days after germination to estimate the plant dry weight, shoot and root length and other seedling characters.

RESULTS AND DISCUSSION

The results of seed germination and phenological observations are given in Fig. 1 and 2. Increase in radiation dose did not adversely affect the germination or germination rate in both cultivars. Therefore, the common observation that sesame seed is resistant to irradiation [2] holds true in our experiments with MI2 and MI3 cultivars.

With the increase in radiation dosage, the growth and development of the emerged seedlings were retarded. The flowering and maturity of plants were delayed by about 10 days or more in MI2 variety irradiated with doses greater than 800 Gy and in MI3 variety irradiated with doses higher than 1000 Gy. Although, the germination was not affected, many of the emerged seedlings failed to survive at later stages of development in high dose treatments. There was no appreciable effect on plant survival in treatments with gamma-ray doses lesser than 625 Gy in MI3 and 400 Gy in MI2,

but it was drastically reduced when the dosage was increased further. Survival was about 50% in the 875 Gy treatment in MI3 and 600 Gy in MI2 (Fig. 1 and 2).

Growth reduction in irradiation experiments is very often measured by plant height [5,6]. The plant height in our experiment was recorded every five days and the data upto the 30th day are presented in Fig. 3 and 4. The differences were highly significant statistically, but the measurements taken on the 15th day produced the least coefficient of variation and the best separation into groups. Fifty percent growth reduction (GR 50 value) at every stage of growth of MI3 variety was between 875 and 1250 Gy. For MI2 variety the doses required to cause GR 50 were lower and ranged from 500 - 700 Gy.

The differences in the number of leaves on the stem among treatments were also statistically significant but the group differentiation was not so striking as in the case of plant height (Fig. 5). Even the plants derived from the highest dosage recorded about 70% of the number of leaves of the control. This indicates that the plant height reduction is brought about mainly by the shortening of the internode. Therefore, plant height is a better criterion for growth reduction studies than the number of leaves on the stem.

Fifteen day old seedlings were uprooted and several seedling characters were estimated. The results of the analysis of variance

(Table 1) agree with the data on plant height (Fig. 3). As the trend in the two varieties was similar, only the data for MI3 variety are presented (Table 1). Fifty percent growth reduction in root length was detected in the range 1250 - 1375 Gy for MI3 and 800 - 900 Gy for MI2. The GR 50 values for epicotyl and hypocotyl length of both varieties were lower. For MI3 they were in the range 750 - 875 Gy and for MI2 the range was 500 - 700 Gy as in the case of plant height.

Although the statistical analysis revealed significant results, the weight of the seedlings and dry weight of root or shoot recorded less reliable results due to higher coefficient of variation. Nevertheless, GR 50 was in the range of 750 Gy and 1000 Gy for MI3 and 500 - 700 Gy for MI2 varieties. The high coefficient of variation and difficulties in the estimation of weight with accuracy in small seedlings make height measurements more reliable in estimating growth reduction.

The high level of resistance of sesame seeds to irradiation when germination is considered[2] and the differences in the radiation sensitivity of different sesame genotypes observed by other authors [2,7,8,9,11,12] are substantiated by the present experiment with two morphologically different genotypes. It is clear from these results that the MI3 variety tolerates higher doses of gamma rays than MI2 variety. Based on the growth reduction studies the most suitable doses of gamma-rays for mutation induction using locally recommended sesame cultivars lie in the range of 750-1000 Gy for MI 3 variety and 500-700 Gy for MI

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2 variety. Lower doses within these ranges should be more useful as a greater proportion of emerged seedlings could survive and contribute seeds for selection in subsequent generations. It is also evident from these experiments that seeds of sesame cultivars should be tested for radiosensitivity before deciding on a dose for mutation breeding. This is because the doses which do not produce any effect on some cultivars may be lethal or semi-lethal on another genotype.

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Table 1 :- Effect of gamma radiation on various growth characters at seedling stage (15) days

Dose (Gy)	Root length (cm)	Epicotyl length (cm)	Hypocotyl length (cm)	Fresh weight (cm)	Root dry weight (g)	Shoot dry weight (mg)	1st leaf length (cm)
00	3.75^{a}	8.633^{a}	2.533^{a}	1.327^{a}	9.20^{a}	175.3^{a}	4.50^{a}
250	3.71^{a}	7.100^{b}	2.330^{a}	1.080^{ab}	7.63^{ab}	162.0^{ab}	4.48^{a}
500	3.60^{a}	6.417^{bc}	1.600^{b}	0.823^{bc}	7.70^{ab}	145.4^{ab}	3.33^{b}
625	3.183^{ab}	5.417^{cd}	1.400^{b}	0.700^{cd}	5.70^{bc}	123.7^{bc}	3.18^{b}
750	2.78^{bc}	4.50^{de}	1.267^{bc}	0.417^{de}	5.00^{bcde}	84.00^{cd}	3.08^{b}
875	2.508^{bcd}	3.967^{e}	0.950^{cd}	0.370^{ef}	5.60^{bcd}	83.00^{cd}	2.65^{bc}
1000	2.133^{cde}	3.487^{ef}	0.600^{de}	0.277^{ef}	2.40^{def}	57.00^{de}	2.15^{cd}
1125	2.167^{cde}	3.150^{ef}	0.567^{e}	0.237^{ef}	2.90^{cdef}	49.00^{de}	1.55^{def}
1250	2.33^{cd}	2.100^{fg}	0.433^{ef}	0.197^{ef}	4.10^{cdef}	46.40^{de}	1.75^{de}
1375	1.75^{def}	1.783^{g}	0.100^{f}	0.200^{ef}	4.00^{cdef}	44.50^{de}	1.28^{efg}
1500	1.48^{ef}	1.118^{g}	0.000^{g}	0.070^{ef}	1.70^{f}	16.90^{e}	1.00^{fg}
1750	1.13^{f}	0.773^{g}	0.000^{g}	0.052^{f}	2.20^{ef}	14.90^{e}	0.667^{g}
CV%	17.324	18.401	21.62	38.61	34.05	30.55	16.31
F value	11.94	33.4	49.56	14.88	6.31	14.18	30.44
Pr > F	0.0001	0.001	0.0001	0.0001	0.0001	0.0001	0.0001

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Captions for the figures. Numbers on opposite side of figure.

Fig. 1 - Germination, phenology and survival of gamma ray treated sesame seeds of MI 3 cultivar.

Fig. 2 - Germination, phenology and survival of gamma ray treated sesame seeds of MI 2 cultivar.

Fig. 3 - Plant height of MI 3 variety treated with different doses of gamma rays.

Fig. 4 - Plant height of MI 2 variety treated with different doses of gamma rays.

Fig. 5 - Number of leaves up to 30th day in MI 3 sesame treated with different doses of gamma rays.

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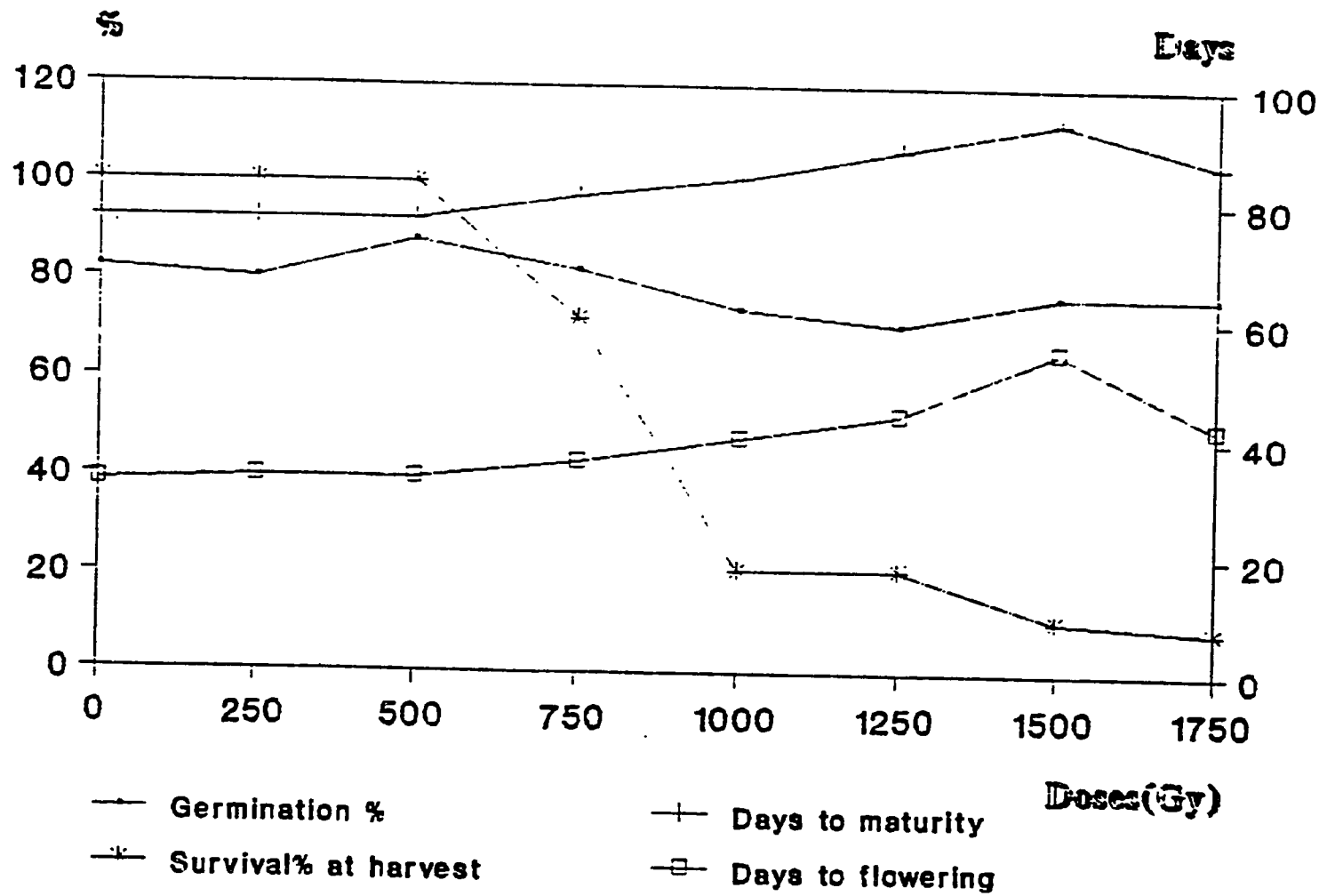
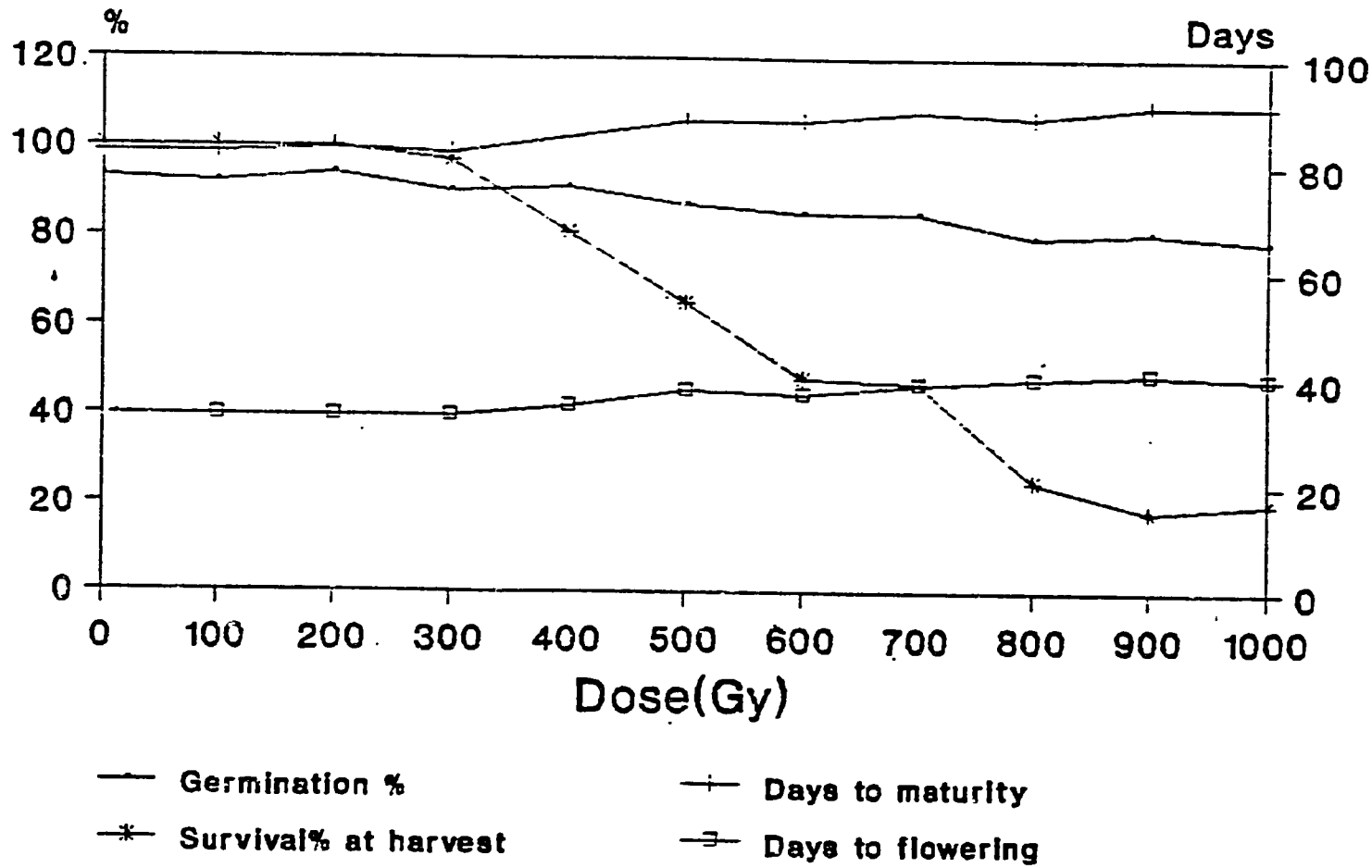

%
Days
120
100
80
60
40
20
0
100
80
60
40
20
0
0
250
500
750
1000
1250
1500
1750
Doses(Gy)
Germination %
Survival% at harvest
Days to maturity
Days to flowering

103


%
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120
100
80
60
40
20
0
100
80
60
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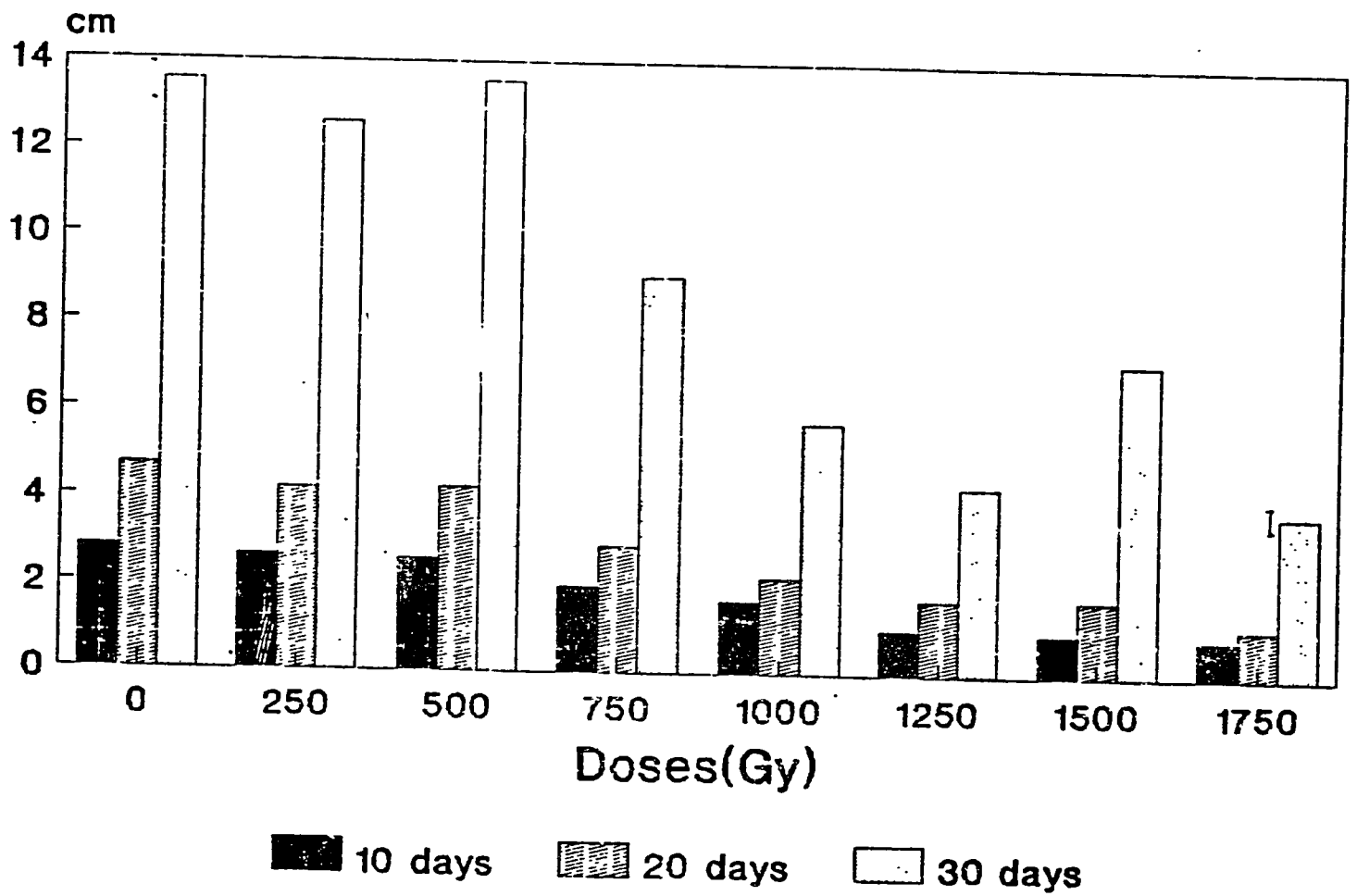
0
100
200
300
400
500
600
700
800
900
1000
Dose(Gy)
Germination %
Survival% at harvest
Days to maturity
Days to flowering

104


cm
14
12
10
8
6
4
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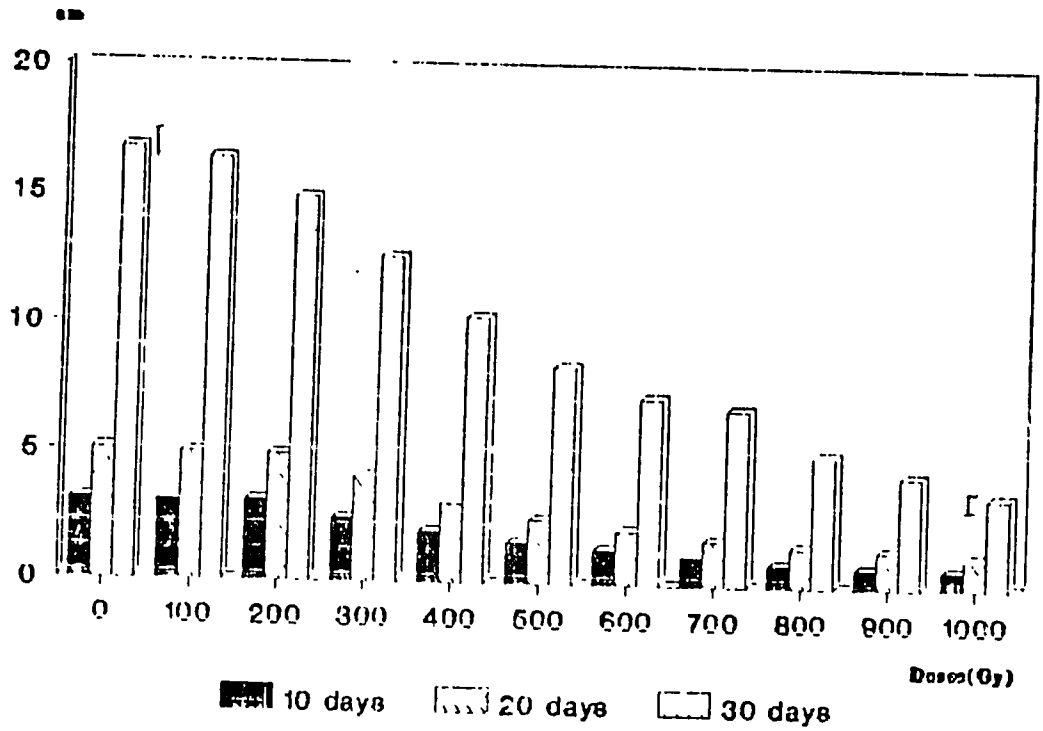
0
0
250
500
750
1000
1250
1500
1750
Doses(Gy)
10 days
20 days
30 days


20
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10
5
0
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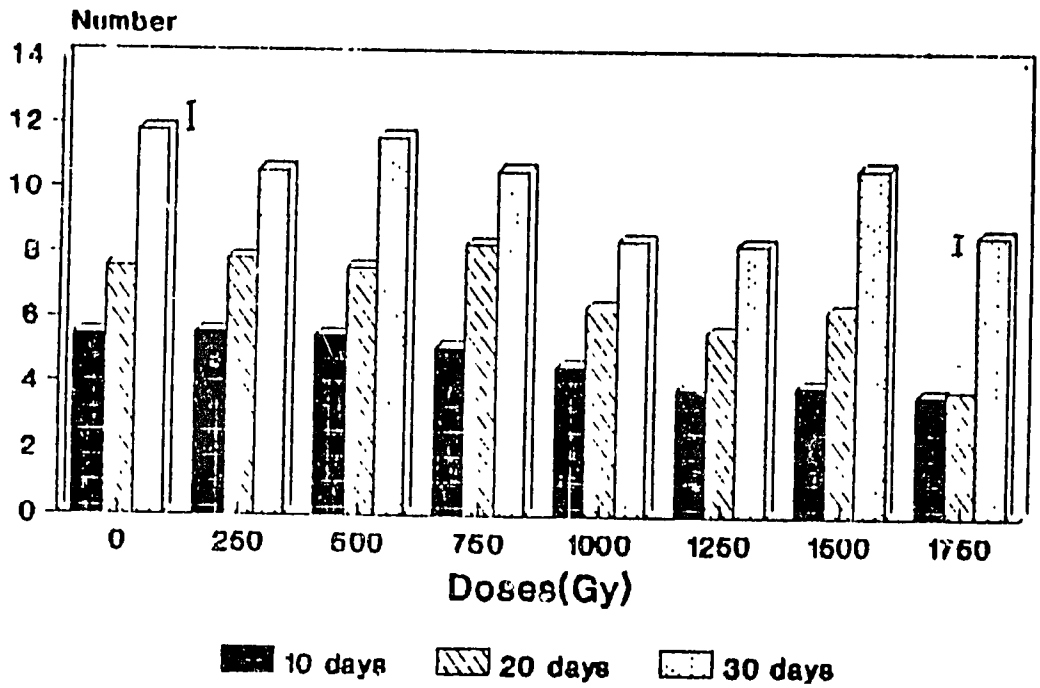
100
200
300
400
500
600
700
800
900
1000
Doses(Gy)
10 days
20 days
30 days

106


Number
14
12
10
8
6
4
2
0
0
250
500
750
1000
1250
1500
1750
Doses(Gy)
10 days
20 days
30 days

101

Appendix 5

Reprint from

PLANT MUTATION BREEDING FOR CROP IMPROVEMENT

PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM
ON THE CONTRIBUTION OF PLANT MUTATION BREEDING
TO CROP IMPROVEMENT
JOINTLY ORGANIZED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
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VIENNA, 1991

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INCREASED EFFICIENCY OF SELECTION FOR YIELD IN GAMMA IRRADIATED POPULATIONS OF GROUNDNUT AND SESAME THROUGH YIELD COMPONENT ANALYSIS*

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Abstract

INCREASED EFFICIENCY OF SELECTION FOR YIELD IN GAMMA IRRADIATED POPULATIONS OF GROUNDNUT AND SESAME THROUGH YIELD COMPONENT ANALYSIS.

The variability parameters of quantitative characters were studied in collections of sesame and groundnut. The genotypic covariances were used to estimate the genotypic correlations and path coefficients (direct effects) of each character on yield by a partial regression procedure. Seeds of two groundnut cultivars (GN-13 and 'Vietnam') and one sesame cultivar (MI 2) were irradiated with different doses of gamma rays and selections were based on the M_2 and M_3 plant performance. The heritability of characters with low environmental variability was high in the collection of both crops. The shelling percentage of groundnut was the only exception. The seed yield of both crops recorded high environmental variability and low heritability, indicating the need to look for alternate characters for yield breeding. The number of pods and the 100 seed weight recorded the highest correlation and direct effect on groundnut yield. The direct effect of the 1000 seed weight in sesame was positive, in spite of the negative correlation. The variability and mutant parent to progeny heritability of the majority of characters were greater in M_3 than in M_2. The yield components recorded higher heritability than the yield in mutant populations. The high yielding groundnut mutants, which outyielded the national check, were selected for seed size and the number of pods in low dose (50-100 Gy) gamma ray treatments. Preliminary results with sesame mutants indicate that selections carried out in the 200-600 Gy treatments for increased capsule number and seed size are better. Proper yield component analysis, and selection based on the results thereof, could increase the efficiency of mutation breeding for yield improvement.

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1. INTRODUCTION

The induction of easily identifiable mutants that could lead to changes in plant architecture associated with increased yield has been practised successfully over several decades [1, 2]. However, less attention has been devoted to changes in the quantitative nature in mutant populations, perhaps because of the difficulty in detecting such mutations (micromutations). Selection of normal looking plants in mutant populations and testing of their progenies for increased mean values for desirable quantitative characters have been proposed in mutation breeding programmes [3, 4]. The present study was undertaken in order to estimate the changes in genetic variability of yield components and other related quantitative characters that can be achieved through gamma irradiation in two important tropical oilseed crops, namely, groundnuts (*Arachis hypogaea* L.) and sesame (*Sesamum indicum* L.), and to investigate the relationship and heritability of such characters in a practical mutation breeding programme. The results are compared with the correlations and broad sense heritabilities estimated in studies of the germplasm collections of the two crops.

2. MATERIALS AND METHODS

2.1. Groundnut

Sixteen quantitatively inherited characters were estimated in 120 cultivars of groundnut adapted to Sri Lanka; they were selected from the 312 cultivars tested. All the cultivars belonged to the Spanish bunch group (variety *vulgaris*) of subspecies *fastigiata*. Ten plants per cultivar were analysed in field experiments. They were grown in a randomized complete block design with two replications at the Agricultural Research Station, Angunakolapelessa, in the semi-arid area of southern Sri Lanka.

The seeds of two cultivars with a wide adaptability to the different agro-ecological zones of Sri Lanka (GN-13 and 'Vietnam') were irradiated with six doses of ^{60}Co gamma rays (ranging from 50 to 450 Gy). The M_1 plants were sampled by harvesting the first four pods and selection was made for approximately 5% of the plants showing the highest yield, seed size, number of pods per plant, number of seeds per pod and shelling percentage in the M_2 bulks and the M_3 progenies. Visual selection was also practised in the remaining populations. For details of the procedures used, see Refs [5, 6]. The selected lines were tested against the parent varieties in M_4 in a randomized complete block experiment and parent to progeny regressions were used to estimate the heritability of different characters in the mutant selection experiment. The mean values of the progenies were computed from a random sample of five plants.

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2.2. Sesame

Eleven quantitative characters were studied in a collection of 60 genotypes grown in a randomized complete block design with two replications at the Research Farm of the Faculty of Agriculture, University of Ruhuna, Mapalana, in the southern intermediate zone of Sri Lanka. Ten plants per plot were randomly selected for data recording, making a total of 20 plants per treatment. The genotypes selected for the study originated in 13 countries in which sesame is widely cultivated.

In the first series of mutation experiments, the seeds of the highest yielding sesame cultivar in Sri Lanka (MI 2) were irradiated with ten doses of gamma rays (ranging from 100 to 1000 Gy at 100 Gy intervals) from a ^{60}Co source at the Central Agricultural Research Institute, Gannoruwa, Peradeniya. Ten grams of seed per dose (1000 seed weight = 3.2 g) were irradiated. In a subsequent experiment, three doses (200, 400 and 600 Gy) were used on 10 g of seed per dose.

Unlike groundnut, selection in segregating M_2 and M_3 populations of sesame was carried out on a single plant basis. The M_2 and M_3 plant selections were tested in subsequent generations on a plant to progeny basis. The characters estimated on five random plants per progeny were used for regression analysis.

2.3. Statistical analysis

The total phenotypic variability observed in the variety screening experiments of the two crops was partitioned into genotypic and environmental variance; on the basis of these values, the coefficients of variation were computed for each character. The genotypic covariance components were used to compute the genotypic correlation coefficients; on the basis of these genotypic correlations, a partial regression method was used to compute the path coefficients (direct effects) of different characters with yield and also the indirect effects [7]. The results of the selection experiments in irradiated populations were used to compute the heritability of characters in the mutant parent to progeny [8].

3. RESULTS

3.1. Model populations

3.1.1. Environmental variability

Studies of the environmental variability of different characters in the model populations (constituted from different genotypes) of the two crops revealed certain regularities. Thus, the environmental variability in seed yield and in closely associated characters such as the number of capsules and seeds per plant in sesame

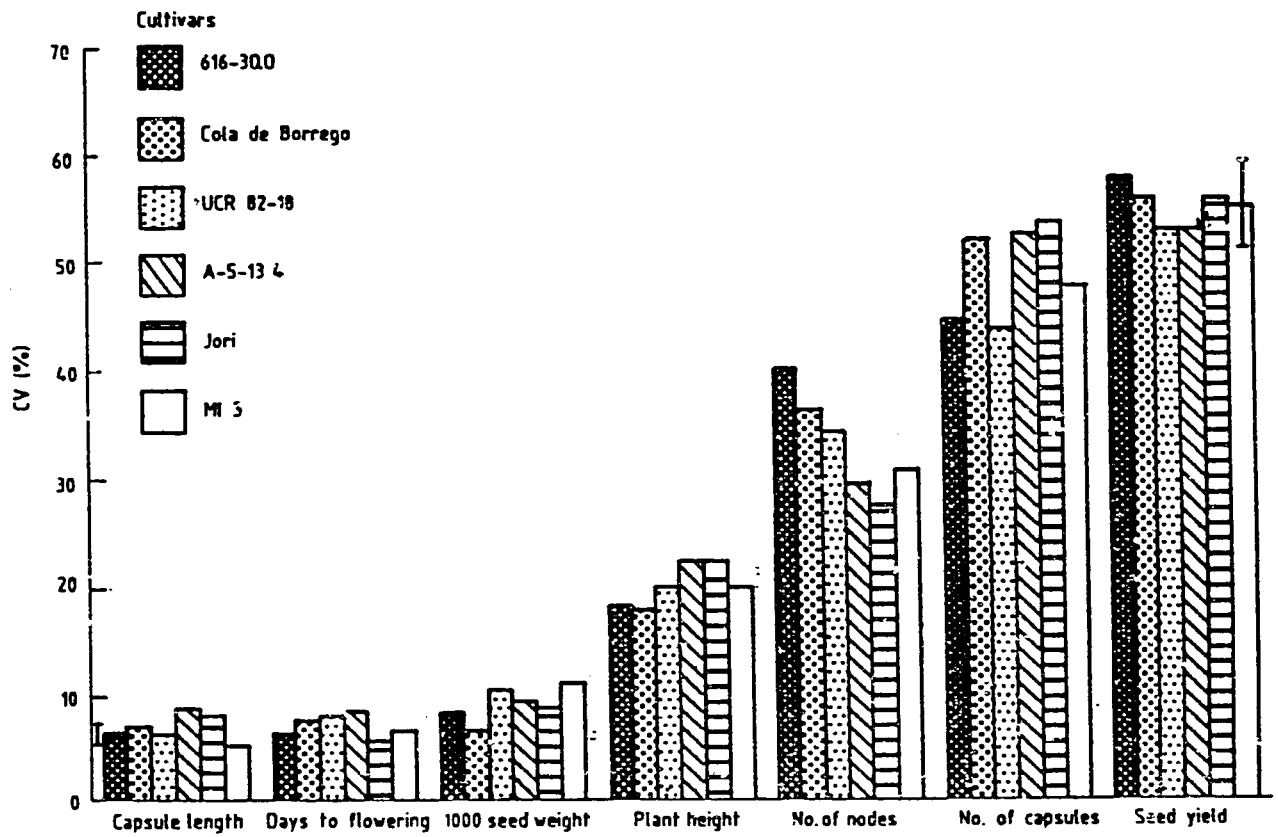

FIG. 1. Environmental variability of quantitative characters in sesame genotypes of diverse origin.

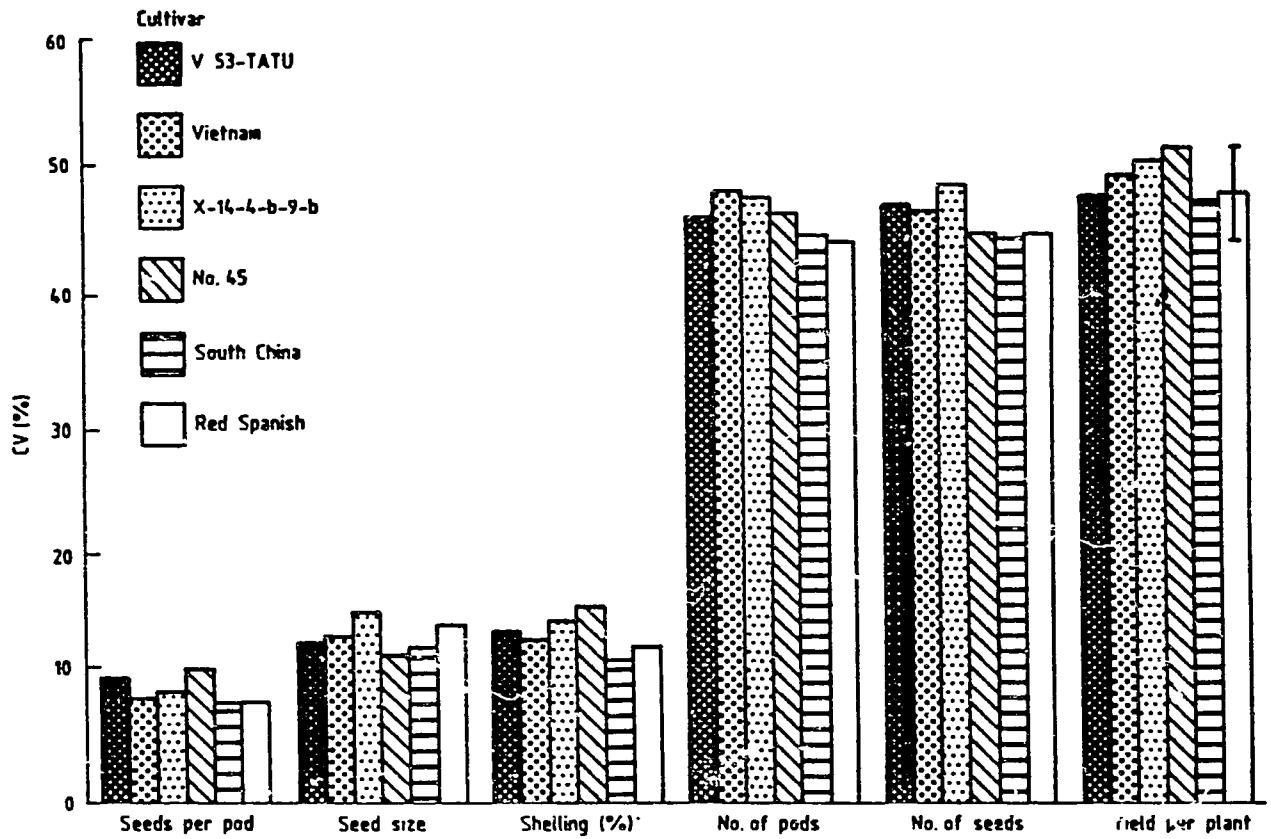

FIG. 2. Environmental variability of quantitative characters in groundnut cultivars.

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TABLE I. VARIABILITY AND HERITABILITY ESTIMATES FOR CHARACTERS IN SESAME

Character	Range	Mean	Range of environmental variability (CV%)	Genotypic variability (CV%)	Heritability (H^2)
Capsule length (cm)	1.84–3.15	2.23	5.6–11.8	10.9	80.2
Days to flowering	27.1–42.4	3.23	3.4–11.1	11.4	70.8
1000 seed weight (g)	2.33–3.77	3.15	5.6–12.2	8.3	68.2
Height to first flowering node (cm)	11.7–45.5	24.9	12.3–27.5	29.3	71.0
Plant height (cm)	22.3–70.1	42.7	14.4–25.2	18.6	65.6
No. of nodes	7–45	17.1	23.5–39.9	38.3	56.7
No. of capsules	4.4–22	9.6	34.5–66.5	19.3	36.2
Seed yield per plant (g)	1.0–4.1	1.9	42.4–78.7	21.2	21.5

were much higher than the plant height, capsule length and 1000 seed weight (Fig. 1). Similarly, in groundnut the pod yield, seed yield, and the number of pods and seeds per plant had a much higher environmental variability than the 100 pod weight, 100 seed weight, number of seeds per pod and shelling percentage (Fig. 2). The two figures reveal that the environmental variability of the same character in contrasting genotypes has insignificant differences. The differences in the level of environmental variability in contrasting characters could be significant.

3.1.2. Genotypic variability and heritability

The genotypic variability and heritability of some of the characters in the sesame and groundnut collections studied are presented in Tables I and II, respectively. The heritability in the sesame collection was high for many of the

TABLE II. VARIABILITY AND HERITABILITY ESTIMATES IN GROUNDNUT

Character	Range	Mean	Range of environmental variability (CV%)	Genotypic variability (CV%)	Heritability (H^2)
No. of seeds per pod	1.6–2.7	2.3	7.8–10.8	10.4	41.3
100 seed weight (g)	43–58	50.7	10.9–15.1	18.8	70.7
Shelling percentage	68.7–74.3	71.0	5.6–10.2	6.0	31.2
No. of pods	16.5–32.1	24.1	42.2–48.3	45.3	46.0
No. of seeds	39.0–61.0	49.8	44.1–48.9	44.9	46.9
Seed yield (kg/ha)	1792–2201	2093	44.8–51.7	44.1	46.1

characters, particularly those with low environmental variability. Low heritabilities were recorded for those characters with high environmental variability. The environmental variability and heritability were intermediate for the number of nodes.

The heritability values of yield and related characters in groundnut were not as low as those in sesame. The environmental variability of these characters was very similar. In groundnut, the weight of 100 seeds had the highest heritability and the shelling percentage the lowest. Of all the characters with a relatively low environmental variability, only the shelling percentage of groundnut had a low heritability.

3.1.3. Correlations and path coefficients

The genotypic correlations among characters in the 60 cultivars representing the model population in sesame are presented in Table III. All the characters recorded highly significant positive correlations with yield, except the 1000 seed weight and the capsule length, which were negatively related, not only to the yield but also to all the other characters.

Of the characters studied, those that can serve easily as selection criteria in a breeding programme were used in the path coefficient analysis of sesame. The negative correlation of seed size (1000 seed weight) with yield was found to be due to

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TABLE III. GENOTYPIC CORRELATIONS (r_g) AMONG CHARACTERS IN SESAME

	Days to flowering	1000 seed weight	Height to first flowering node	Plant height	No. of nodes	No. of capsules	Seed yield
Capsule length	−0.39*	0.56**	−0.48*	−0.46*	−0.47*	−0.35	−0.39*
Days to flowering	—	−0.32	0.91**	0.96**	0.81**	0.38**	0.86**
1000 seed weight		—	−0.60**	−0.61**	−0.86**	−0.84**	−0.64**
Height to first flowering node			—	0.95**	0.97**	0.68**	0.97**
Plant height				—	0.84**	0.53**	0.90**
No. of nodes					—	0.63**	0.71**
No. of capsules						—	0.60**

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TABLE IV. PATH COEFFICIENTS AND INDIRECT EFFECTS OF YIELD COMPONENTS ON SEED YIELD IN SESAME

Relationship	1000 seed weight	No. of nodes	No. of capsules	Capsule length
Correlation coefficient (r)	−0.636**	0.709**	0.602**	−0.385*
Path coefficient (p)	0.525	0.793	0.504	−0.117
Indirect effects				
1000 seed weight	—	−0.683	−0.422	−0.060
No. of nodes	−0.455	—	0.317	0.054
No. of capsules	−0.442	0.499	—	−0.262
Capsule length	−0.264	+0.100	0.203	—

TABLE V. GENOTYPIC CORRELATIONS AMONG YIELD COMPONENTS IN GROUNDNUT

	No. of seeds per pod	Shelling percentage	100 seed weight	Seed weight per plant
No. of pods per plant	0.121	0.225*	0.444*	0.912**
No. of seeds per pod	—	0.471**	0.092	0.215*
Shelling percentage		—	0.425**	0.416**
100 seed weight			—	0.392**
No. of seeds	0.942**	0.404**	0.305**	0.854**
Pod weight	0.231**	0.400**	0.386**	0.994**

TABLE VI. PATH COEFFICIENTS AND INDIRECT EFFECTS OF YIELD COMPONENTS ON SEED YIELD IN GROUNDNUT

Relationship	No. of pods	No. of seeds per pod	100 seed weight
Correlation coefficient (r)	0.912**	0.215*	0.392**
Path coefficient (p)	0.838	0.076	0.347
Indirect effects			
No. of pods	—	0.107	0.039
No. of seeds per pod	0.009	—	0.007
100 seed weight	0.015	0.032	—

negative indirect effects via the number of nodes and capsules per plant. The direct effect of the seed size was found to be positive. The direct effect of capsule length was negative, but less than its correlation with yield (Table IV).

The number of pods, number of seeds and the pod weight per plant were found to have highly significant genotypic correlations with seed yield in groundnut (Table V). The other characters recorded relatively low values. The number of pods per plant, the number of seeds per pod and the 100 seed weight, which form the seed yield components in groundnut, were used in the path analysis. The indirect effects were negligible and, as a result, unlike sesame, the path coefficients were close to the values of correlation coefficients (Table VI).

3.2. Mutant populations

3.2.1. Variability and heritability in sesame

Figure 3 compares the variability of characters in sesame in the M_2 populations with the parent cultivar MI 2 for some selected gamma ray doses. Doses within the range 300–600 Gy showed consistently higher variability than others. The M_2 populations showed increased variability over the parent for all the characters studied. The relative increase in variability is higher for those characters with less environmental variability, such as the length of the capsule and the plant height at flowering and maturity, than for characters with high environmental variability. Of the characters with high environmental variability, the number of capsules showed a higher relative increase in variability in M_2 than the seed yield and the number of nodes per plant (Fig. 3).

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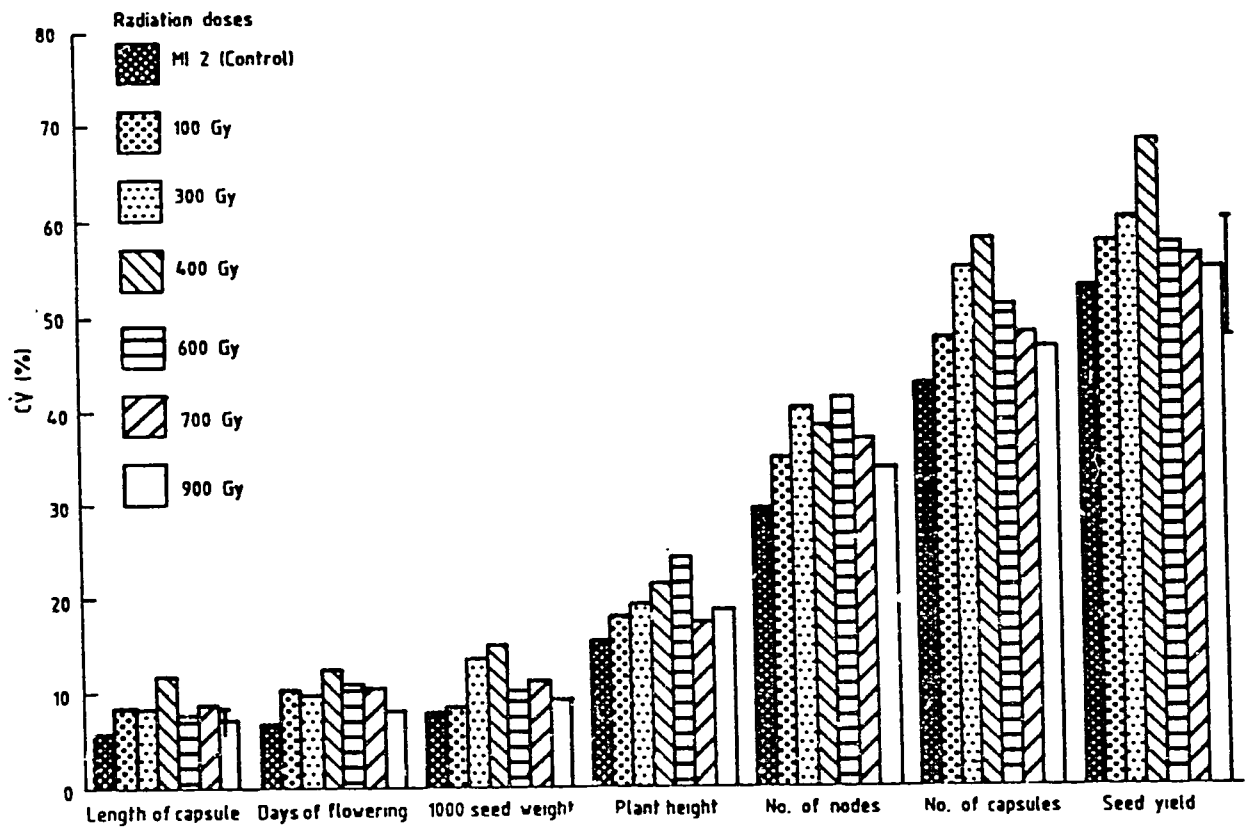

FIG. 3. Variability of characters in the M_2 populations of sesame compared with the parent cultivar MI 2.

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TABLE VII. HERITABILITY IN IRRADIATED POPULATIONS OF SESAME

Population	M_2 parent to M_3 progeny					M_3 parent to M_4 progeny				
Dosage (Gy)	0	100	300	400	700	0	100	300	400	700
Capsule length	3.3	12.4	27.6	29.5	8.4	5.2	18.8	41.4	21.4	10.5
Days to flowering	7.4	21.8	34.6	39.7	20.2	11.2	18.4	38.6	47.6	24.4
1000 seed weight	6.6	8.5	10.4	8.6	11.4	4.2	27.8	54.4	47.8	28.7
Plant height	10.7	13.5	17.9	18.5	10.0	9.5	24.8	39.2	44.4	28.5
No. of nodes	5.3	11.4	12.8	18.4	16.7	3.8	28.[illegible]	32.4	18.7	18.6
No. of capsules	0.7	7.8	9.6	12.7	6.8	4.8	14.8	26.7	18.1	12.0
Seed yield	1.8	3.7	5.7	0.4	8.5	0.2	12.7	26.2	10.5	11.2

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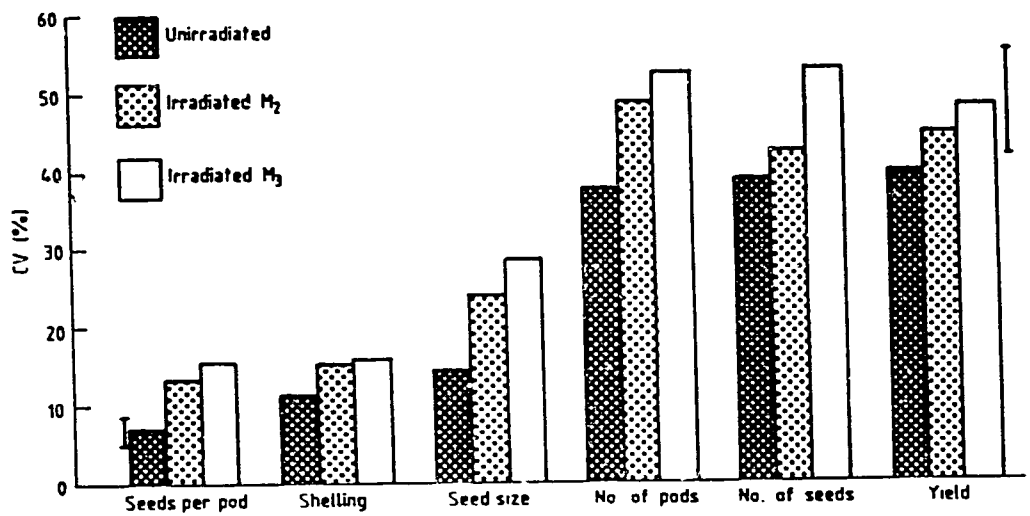

FIG. 4. Induced variability in the 'Vietnam' groundnut.

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The M_2 parent to M_3 progeny and the M_3 parent to M_4 progeny heritabilities in sesame were higher for characters with low environmental variability than for those with higher levels of environmental variability (Table VII). However, in the majority of cases the heritability in irradiated populations was lower than in the model population studied (Tables II and VII). The heritability of characters was higher in the selection experiment carried out in M_3 than in M_2, and also in the dose range 300–400 Gy.

3.2.2. *Variability and heritability in groundnut*

The variability of characters in the M_2 and M_3 populations of the gamma irradiated groundnut variety 'Vietnam' is presented in Fig. 4. The effect of radiation was greater for the 100 seed weight than the number of seeds per pod and the shelling percentage among the characters with low environmental variability. The increase in variability after irradiation was much greater for the number of pods than the seed yield or the pod yield per plant (Fig. 4).

The heritability estimates for the M_2 parent to M_3 progenies and the M_3 parent to M_4 progenies, presented in Table VIII for the two cultivars of groundnut studied, show high values for seed size among the stable characters (characters with low environmental variability) and for the number of pods among the characters with a high degree of environmental variability. The variability was greater in the M_3 than in the M_2 populations for most of the characters. Heritabilities were also higher when selected in M_3 than in M_2 (Table VIII).

TABLE VIII. HERITABILITY IN IRRADIATED POPULATIONS OF GROUNDNUT[a]

Character	M_2 parent to M_3 progeny	Control	M_3 parent to M_4 progeny	Control
No. of branches	0.13	0.08	0.28	0.01
No. of seeds per pod	0.29	0.04	0.34	0.05
Seed size	0.33	0.10	0.49	0.07
Shelling percentage	0.22	0.04	0.28	0.00
No. of pods	0.26	0.07	0.39	0.10
Pod weight per plant	0.24	0.06	0.35	0.02

[a] Pooled data for two varieties and different gamma ray doses.

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4. DISCUSSION

The regularities of environmental variability in the different characters observed in the model populations need consideration in a breeding programme. Thus, selection will be easier for characters with low environmental variability than those with higher values, given that the genotypic variation is the same. However, the data from our experiments show that the genotypic variability of the different characters is not similar.

This dissimilarity is reflected in the differences in heritability of characters with similar environmental variability. Thus, in groundnut, for example, the shelling percentage and the 100 seed weight have low environmental variability. In spite of this, the heritability of shelling percentage is much lower than that of 100 seed weight. The implication is that it is much easier to select for increased seed size than for shelling percentage, although both characters constitute the yield components. Similar results have been reported in studies undertaken with 20 strains of groundnut in Gujarat, India [9]; however, the heritability values were relatively higher than those in our studies.

Although the selection pressure applied on different characters was the same (5%), the resulting high yielding groundnut mutants, which out-yielded the national check in multilocational trials [10], were traced back to selections made for the seed size (mutants 180/21 and 180/22) and the number of pods (mutants 280/20, 980/4 and 1080/2). None of the high yielding mutants resulted from selections for yield per se. When the results of path analysis are considered, it is interesting to note that the number of pods and the 100 seed weight recorded the highest direct effect on yield.

Therefore, it seems that the efficiency of yield breeding could be increased by studying the variability and the relationships among characters in a representative model population grown in the environment for which the breeding programme is planned.

Also, in locations other than ours, there seems to be a potential for improving groundnut yield through selection for increased seed size. Thus, for example, the enhanced pod yield of 'Trombay Groundnut' varieties in India is also attributed to increased kernel size [11-13]. The possibility of improving yield through selection for seed and pod size has been suggested in populations with mean values of the 100 seed weight that are greater than ours [14-16] and in crosses involving large and medium kernel varieties [17-18].

The differences in variability caused by the doses used need to be considered in selecting proper doses for yield breeding using induced mutations. The importance of using larger dose ranges in studying the induced variability for quantitative characters has been stressed [3]. Thus, in our experiments the dose ranges of 300-600 Gy in sesame have recorded more variability in M_2 than in lower or higher doses. This observation is of further importance since sesame shows a high

level of resistance to radiation [4]. When response to selection in different generations is considered, all the characters responded better in the M_3 than in the M_2 generation. The selection response differences in the two generations were higher in groundnut than in sesame. This may be due to the polyploid nature of groundnut, which takes longer to segregate than diploids. The results agree with the recommendations that when seeds are irradiated selection for quantitative characters should begin in generations not earlier than M_3 [2, 3, 19].

In sesame there is a possibility of selecting for improved characters already in M_2, since there is increased variability for most of the characters (Fig. 3) and response to selection (Table VII). As response to selection is better in M_3, it is desirable to start selection in this generation. The response in both generations was similar for the date of flowering, which indicates that early or late flowering mutants may be isolated with success in M_2.

The heritability of characters in the model population of sesame is relatively higher than that in groundnut. This may be due to a diversity in the collection which we received from the Food and Agriculture Organization of the United Nations. The groundnut collection studied represented genotypes better adapted to the country, selected from earlier screening trials. The negative correlation of seed size (1000 seed weight) and capsule length with other characters and yield observed in the sesame collection was suspected to be due to several indehiscent cultivars received from the University of California at Riverside (UCR NS varieties); these have long capsules and few large seeds per capsule, resulting in low yields. The low yield of cultivars that incorporate an indehiscent gene of the *id/id* type has been observed widely [4, 19, 20]. This was confirmed when the data were analysed without the UCR varieties; the correlations were then positive.

The path coefficient analysis further revealed this bias. The direct effect of 1000 seed weight was found to be positive (Table IV) when the effect of the other characters was removed by the partial regression method we employed. The negative effect of capsule length on yield was also less after path analysis.

When the results of the selection experiment in sesame are considered, the high heritability of characters with low environmental variability needs to be emphasized (Table VII). These include capsule length, days to flowering and 1000 seed weight. The plant height, which recorded a medium level of environmental variability, gave a better response to selection than seed yield and the number of capsules, but less response than the more stable characters. The high heritability of capsule length and the medium heritability of plant height have been reported in four crosses of sesame [21].

The number of capsules per plant, 1000 seed weight and plant height in sesame have been considered by many workers as the main contributory factors to yield [19, 22–24]. Probably because of the availability of a great amount of natural variation for quantitative characters [22–24], fewer attempts have been made to improve these characters through mutation breeding. The morphologically changed

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natural (such as the *id/id* genotype for indehiscence) [4, 19, 20, 22] and induced mutants [4, 22, 25] have been used in limited breeding programmes. Therefore, comparison of our data is difficult. The selections made in the present breeding programme are being studied for their performance. The results will be valuable because of the lack of detailed studies on the induced variability of quantitative characters in sesame using mutagenic agents.

ACKNOWLEDGEMENTS

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Appendix 6

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Gamma Ray Induced Sesame Mutants : Their Characteristics and Performance

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Sri Lanka.

ABSTRACT

Mutations were induced with gamma rays in two contrasting sesame cultivars MI2 (Black seeds) and MI3 (White seeds). A range of morphological mutants that can contribute to increased seed yield could be isolated and confirmed. Improvement of one yield component in general led to the decrease in one or more other components. However, some mutants with three capsules per axil, short internodes and long capsules recorded higher yield than the parent variety. Some white seed mutants of MI2 variety and a brittle seed coat mutant which can be easily decorticated recorded higher seed yield in multilocational trials. Brittle seed coat character is being transferred to other cultivars through backcross procedure.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is the major oilseed crop in Sri Lanka. Low and fluctuating yield of this crop has been attributed not only to poor management, low inputs and environmental constraints, but also to the lack of adapted, high yielding cultivars showing good response to fertilizer, pest and disease resistance etc. (Brar and Ahuja, 1979; Weiss, 1983; Anon, 1985, Ashri, 1988). Inspite of the fact that sesame is an ancient crop, the available germplasm for indehiscent capsules, determinate growth habit and resistance to major diseases is very limited and

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very often have undesirable pleiotropic effects or linkages (Weiss, 1983; Ashri, 1988). Therefore, additional variability for these characters were sought by the use of induced mutations. Character improvement that can lead to increased yield, adaptation, improved quality of the crop and enhanced cross pollination to utilize the hybrid vigor that has been observed widely (Goyal and Kumar, 1988; Jadon and Mehrotra, 1988; Osman, 1989; Sasikumar and Sardana, 1990; Sodani and Bhat Nagar, 1990) were aimed in the selection of mutants.

MATERIALS AND METHODS

The cultivars and germplasm entries that have potential for further improvement were identified in the varietal screening and evaluation experiments. The cultivar MI2 which has recorded the highest yield in multilocational trials was used in the mutation breeding experiments with the aim of improving its potential yield and seed quality. This cultivar has a basal branching habit, alternate leaf arrangement, one bicarpellate capsule per leaf axil and black seed coat. The black seeds have a lower market value than the white sesame seed. Therefore improved white sesame lines were also sought in the segregating populations.

The only white sesame cultivar recommended for cultivation in Sri Lanka is MI3. This cultivar has a different plant architecture

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than MI2. It is non branching with three bicarpellate capsules per leaf axil. Leaf arrangement is opposite.

After detailed studies on radiosensitivity, the doses selected for mutation induction in MI2 were 200, 400 and 600 Gy. Twenty grams of seeds per dose were irradiated in MI2 (1000 seed weight = 3.1 g). The MI3 variety was found to be more resistant to irradiation and hence higher dose levels of 600, 750 and 900 Gy were used on 20 g (1000 seed weight 3,1 g) seed samples. Irradiation was carried out with gamma rays from the ^{60}Co source at the Central Agricultural Research Institute, Gannoruwa, Peradeniya, Sri Lanka.

The mutagen treated populations in their first generation were grown in Randomized Complete Block design with two replications and the parent varieties as control. Both single plant and bulk methods were used to sample the M_1 population. Seeds from the first formed five capsules of M_1 plants were harvested. Selection in the M_2 generation was carried out for both morphological and biometrical characters. In the M_3 generation the seed colour and other seed coat characters were observed and selected.

Once confirmed, the mutants with greatest potential were yield evaluated in 15 m^2 plots in Randomized Complete Block design with three replications. Fifteen plants per plot were randomly sampled for estimating the yield components. The most promising mutants from replicated trials were yield tested in three locations in the

Low country Intermediate zone, Mid country Intermediate zone and Low country dry zone. The white seeded MI3 cultivar served as the control for white and cream seeded mutants. The results were statistically analysed using ANOVA and GLM procedures of the SAS package for personal computers (SAS,1987).

RESULTS AND DISCUSSION

The selections for quantitative characters in M_2 and M_3 populations of MI2 variety were evaluated and the selection efficiency in different dose levels and the heritabilities for different characters in mutant populations have been established (Pathirana, 1991). The present paper deals with the performance of individual mutants and the mutants selected for simply inherited characters useful in the breeding programme.

The number of true breeding mutants for different characters isolated from the two recommended varieties is summarized in table 1. The mutants with short internode, branched stem (in MI3), three flowers per leaf axil (in MI2), tetracarpellate ovary and long capsules have the potential to increase the seed yield by influencing yield components and harvest index. Therefore these mutant lines are used widely in the cross breeding programmes.

The mutants with three capsules per leaf axil isolated in MI2 variety were shorter than the parent variety. The branching

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mutants in MI3 variety were also shorter than the non-branching parent variety. Therefore back crossing and crossing with taller, high yielding genotypes is being performed to improve the yield potential of the mutants.

The polypetalous corolla mutant isolated in MI2 variety was highly sterile and only few seeds per plant were produced even in crosses with normal plants. A normal polypetalous corolla mutant has been produced (Murty and Bhatia, 1990) which should be useful in increasing the natural cross pollination for making hybrid seeds.

Some of the characters reported in our study have been induced in Japanese sesame varieties (Kobayashi, 1981), in India (Murty et al. 1990) and Egypt (Ibrahim, 1990). The seed colour in sesame has been found to be inherited by simple genes in many cases and in our programme many mutants with lighter colours could be identified in MI2 which is a black seeded cultivar. Most of the selected types were either brown or cream coloured, but a few white coloured lines could also be identified in M_3 and M_4 generations. Some of the white seeded mutants have similar yield potential as the parent and therefore could be valuable commercially.

The most useful mutant should be the one with brittle seed coat character. The seeds of this mutant can be decorticated by rubbing between hands. The usual procedure for sesame decortication in oil mills or for confectionery purposes is rather

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complicated and leads to some decline in the quality. With the development of proper dehullers, the brittle seedcoat mutant character should solve the problem of decortication at domestic and industrial levels.

Improvement of one yield component can lead to the decline of another due to yield component compensation (Adams, 1967; Adams and Grafius, 1971; Zakri and Grafius, 1978). Therefore, we studied the yield components of the mutants in detail and compared them with the parent variety (table 2). Mutants having short internodes had shorter capsules and lower number of seeds per capsule. However, some lines with shorter internodes have similar or better yield than parent due to higher number of capsules in them. A higher number of seeds per capsule was found in long capsule mutants. Relatively lower number of capsules per plant in many of these mutants would have been the factor that deprived the expected higher yield. Those mutants with three capsules per axil in MI2 variety were the highest yielding among the morphological mutants (Table 2). Using exotic germplasm for the three capsules per axil character, van Rheenen (1988) found that the number of capsules per axil has no influence on the yield in his breeding experiments.

Similar to the germplasm lines with tetracarpellate capsules, the mutants with increased carpel number have shorter capsules resulting in lower number of seeds. Therefore, in sesame, the placental area for seed attachment does not seem to increase proportionate to the increase in the carpel number due to

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shortening of the capsule. Therefore as in most of the recommended cultivars throughout the world, the bicarpellate capsule seem to have the highest yield potential. However, it should be noted that the tetracarpellate capsules have better seed retention at maturity.

The mutants of MI2 variety in their M_4 generation were tested at Girandurukotte in the Low country Intermediate zone where high potential for the expansion of sesame cultivation exists. The results of promising mutants in this experiment conducted in two replications in a Randomized Complete Block design with the parent variety as control is given in table 3. Several mutants which exceeded the parent variety significantly for more than two characters including yield were revealed in this experiment.

As the white sesame seeds have a higher premium price in the market, the mutants with white or cream coloured seeds were tested separately with MI3 as the control. Six such mutants were included in the advanced yield trials conducted in three replications at two locations. The trials were conducted at the experimental field of the Institute of Fundamental Studies at Kengalla in the Mid country Intermediate zone and at Bata atha farm in the Low country Dry zone. The yield, age and the number of capsules per plant of the mutants at these two locations are summarized in table 4. The higher yield of the mutants derived from MI2 variety compared with MI3 variety was not surprising as MI2 variety has a higher yield potential than MI3. The highly branched and tall plants of MI2

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variety produce more capsules than unbranched MI3 variety. The lower yield at Bata atha farm was due to heavy infestation by shoot webber *(Antigastra catalaunalis)* at the early phase of growth which retarded the plants.

These sesame mutants with white and cream seeds were further tested in multi-locational trials during the succeeding seasons in four replications in Randomized Complete Block design. The sites selected for testing were at Kengalla, Mapalana (University farm in the Low country Intermediate zone) and Kurunegala (Low country Dry zone). The results of two seasons of testing are presented in table 5. The results indicated the superiority of MB 29 and MB 33 over the recommended cultivar. During the South west monsoon season MB 1 and MB 13 also recorded higher mean yields than the recommended cultivar. The results of these experiments reveal that the mutant with brittle seed coat character MB 29 can contribute to a great extent in the sesame industry.

Work is underway to transfer the brittle seed coat character to other promising breeding lines which have been developed through cross breeding of cultivars and mutants.

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Table 1 - Useful mutants selected and confirmed in two cultivars of sesame.

Mutant Character	Parent cultivar	Number of True Breeding Mutants
Short Internodes	MI2	5
	MI3	2
Increased Pubescence	MI2	4
	MI3	1
Tetracarpellate Ovary	MI2	6
	MI3	3
Polypetalous Corolla	MI2	1
Changed Corolla colour	MI2	2
	MI3	5
Early maturity	MI2	14
	MI3	2
Late maturity	MI2	3
	MI3	6
Branched stem	MI3	2
Long capsule	MI2	3
	MI3	6
Short capsule	MI2	7
Three flowers per axil	MI2	3
One flower per axil	MI3	8
Changed seed Colour	MI2	12
	MI3	11
Brittle seed coat	MI2	1
Others	MI2	13
	MI3	8

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Table 2 - The variation for some yield components among desirable mutants of MI2 variety.

Mutant character	Capsule length, cm	Number of seeds per capsule	Number of capsules	Yield per plant, g
Short Internode	3.1+0.2 -3.4+0.2	61.3+6.2 -68.4+6.5	50.0+6.9 -52.7+8.2	4.8+0.8 -7.8+1.2
Tetracar--pellate ovary	1.7+0.1 -3.0+0.2	55.8+4.1 -67.8+8.5	36.2+4.5 -41.7+7.1	3.8+0.7 -6.1+0.8
Long Capsule	3.9+0.2 -4.3+0.3	66.4+4.5 -75.2+6.0	45.7+7.2 -48.3+7.5	5.0+0.8 -7.3+1.2
Three flowers per axil	2.8+0.2 -3.4+0.2	52.4+3.8 -67.7+5.2	46.3+6.9 -53.7+8.1	5.7+0.7 -8.2+1.4
Parent (MI2)	3.6+0.2	69.0+5.2	48.8+7.7	6.7+0.9

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Table 3 - Yield and other characters of sesame mutants in M_4 compared with MI2 (Girandurukotte, 1988).

Treatment	Plant height, cm	Number of Capsules per plant	Number of Seeds per Capsule	1000 Seed weight, g	Seed Weight per Plant, g
MB 1	128	54.2	78.0*	2.77	10.2
MB 7	115	68.8*	63.9	3.03	9.1
MB 4	97	91.4*	68.7	3.30	7.2
MB 8	119	74.0*	54.9	3.20	9.1
MB 10	111	44.4	69.2	3.13	8.8
MB 13	129	50.6	79.6*	3.43*	9.1
MB 15	96	47.8	65.6	3.33	7.2
MB 16	114	49.3	62.9	4.20*	12.7*
MB 17	86	31.1	37.0	3.73*	1.3
MB 25	146	61.8	73.5	2.93	7.9
MB 29	108	64.7	59.6	3.57	9.7
MB 30	129	92.9*	58.6	3.40*	13.1*
MB 32	139	80.7*	76.7	3.07	13.8*
MB 33	127	69.9*	59.8	3.33	11.1
MI 2	117	48.1	63.6	3.20	7.2
CV, %	9.0	16.8	13.0	3.5	25.4
pr > F	0.0001	0.0001	0.0003	0.0001	0.0001
LSD	17.8	17.5	14.0	0.20	3.89

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Table 4 - Performance of sesame mutants compared with MI3 variety (1989).

	Age[a]	Number of Capsules [a]	Yield, kg ha^{-1} Pallekele	Bata atha	Mean
MB 1	90	42	721*	495	608
MB 7	86	40	595	601	598
MB 13	87	44	1590**	520	1055
MB 29	88	41	827*	403	615
MB 32	88	40	1090**	540	815
MB 33	85	45	1121**	505	813
MI 3 (control)	80	38	600	524	562
CV%			18.5	37.2	

(a) - mean of two locations

*, ** - yield significantly higher than MI3 at 5% and 1% levels, respectively.

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Table 5 - Performance of sesame mutants in regional trials (means of three locations and two seasons).

Mutant/ variety	Age, days	Plant height, cm	Number of capsules	Mean yield, kg ha^{-1}	
				Maha (a) 1990/91	Yala(b) 1991
MB 1	88	96.8	37.1	492	989
MB 7	85	90.5	40.8	684	725
MB 13	86	99.6	37.5	517	1003
MB 29	86	97.8	40.6	1011	925
MB 32	87	97.0	37.7	620	651
MB 33	86	98.4	40.7	865	1123
MI 3	85	93.7	36.8	824	871

(a) Maha - North east monsoon season

(b) Yala - South west monsoon season

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Appendix 7

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INDUCED MUTATIONS AND ANTHER CULTURE FOR SESAME IMPROVEMENT

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2. Introduction

Sesame (*Sesamum indicum* L.) is the major annual oilseed crop grown in Sri Lanka. The average yields of sesame are rather low with a seasonal fluctuation from 378 kg ha^{-1} to 700 kg ha^{-1} [1]. There is much higher potential for this crop as some sesame varieties have recorded yields over 1450 kg/ha [1,2]. Low yields of sesame have been attributed not only to poor management, low inputs and environmental constraints but also to the lack of suitable cultivars with wide adaptability, high yield potential with tolerance to biotic and abiotic stresses [3 - 6].

Narrow adaptation of cultivars, indeterminate growth habit, shattering losses and pest and disease attack are some of the major constraints to increased yield in sesame [5,6]. Inspite of the fact that sesame is an ancient crop, the available genes for indehiscent capsules, determinate growth habit, resistance to some of the major diseases and pests are very limited and very often have undesirable pleiotropic effects or linkages [3-6]. Therefore character improvement of these genotypes and additional variability in the adapted local varieties through induction of mutations could be rewarding.

It is necessary to decorticate the sesame seeds before processing for oil or use in confectioneries, as the testa carries over 99% of the oxalates, the only toxic constituent in sesame seed. The testa imparts a slightly bitter taste to the meal,

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resulting from chelation of the calcium content by oxalic acid. Decortication also substantially increases palatability, digestibility and protein content of the meal [4,7]. Cultivars whose seeds can be easily decorticated will be useful in developing countries where machinary are not available for this purpose.

Although sesame is considered to be a drought resistant crop, the drought at certain phases of growth can cause severe growth and yield reduction. Water stress at seedling and flowering stages are the most important in this respect. The mutants selected for improved yield and disease resistance in Sri Lanka [8,9] were tested for their resistance to drought by direct and indirect methods and results are reported here.

With the objective of using doubled haploids in the breeding of sesame, attempts are being made to develop a protocol for efficient haploid production from sesame anthers. Optimal stage of the explant and culture conditions for efficient callus induction and sub-culture of anthers of MI 3 variety are described here.

3. Materials and Methods

3.1. Genotypes used for mutation induction

The cultivars that have potential for further improvement were selected for mutagenic treatment. Due to wide variation reported in radio-sensitivity of seeds of sesame cultivars, optimal doses

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for seed irradiation were selected after growth reduction studies with MI 2 and MI 3 cultivars. For MI 2 variety doses in the range of 100 to 1000 Gy were used and for MI 3 variety 100 to 1750 Gy were used for radiation sensitivity studies. The irradiated seeds were grown in three replications in a randomized complete block design and germination percentage, plant height, survival percentage and seed yield were studied.

The cultivar MI 2 which has recorded the highest yield was used to further improve its potential yield and the seed quality. This cultivar has black seeds, basal branching habit and it produces one bicarpellate capsule per leaf axil. The cultivar MI 3 was also used in mutation breeding and is unbranched, has three capsules per leaf axil and white seeds. Both are recommended for cultivation in Sri Lanka.

3.2. Sampling and selection procedures

First formed five capsules of surviving M_1 plants were used to collect seeds for single plant progenies or making bulk populations for selection. Selection for shorter internode, longer capsules, more erect branches (in MI 2) to reduce seed losses at harvest and to accommodate more plants per unit area, narrow leaf, increased pubescence, three capsules per leaf axil (in MI 2) were carried out in the second and third generations after mutagen treatment. Normal looking plats with increased number of capsules were selected for biometrical measurements and the best were carried

foreward. Details of selection procedures for quantitative characters have been reported [10].

In the third and subsequent generations, selected plants as well as plants grown in bulks from unselected material were screened for seed colour and other desirable characters.

3.3. Yield evaluation

After preliminary screening in breeding nurseries, the promising mutants were tested in larger plots with other promising breeding lines and with MI 3 variety as the control. The multi-locational experiments were conducted at the Research Farm of the Faculty of Agriculture at Mapalana in the low country of southwestern Sri Lanka; at the Field Station of the Institute of Fundamental Studies at Kengalla in the mid country intermediate zone, at Kurunegala in the low country dry zone and at the Government Farm, Batatha, in the low country dry zone of southern Sri Lanka. The varietal screening methodology has been described in detail [8]. Some mutants were back crossed to the parent variety or crossed with other material where further improvement was desired.

Seeds of UCR 82-203 NS, a cultivar with indehiscent capsules but with other undesirable side effects [4,6,11] were irradiated with gamma rays, treated with Ethyl methane sulphonate and diethyl sulphate, with the objective of breaking any linkages as it has not

been possible to separate the indehiscence from the undesirable traits so far [11].

3.5 Anther culture

For establishing callus from sesame anthers, Murashige and Skoog (MS) medium [12] supplemented with different concentrations of 2,4 dichlorophenoxy acetic acid (2,4-D), 3-indole acetic acid (IAA) and 6-benzylaminepurine (BA) were used, as earlier studies have indicated the suitability of this medium [13]. Different concentrations of hormones and light regimes used have been described [14]. Anthers of white seeded recommended cultivar MI 3 were used.

4. Results

4.1. Varietal differences in radiation sensitivity

The growth reduction studies conducted with two recommended cultivars MI 2 and MI 3 revealed that MI 3 is more resistant to gamma radiation than MI 2 and that 50% growth reduction (GR 50) for plant height was in the range of 750-1000 Gy for MI 3 and 500-600 Gy for MI 2 (Fig. 1). The germination percentage also showed similar trends (Fig. 1).

4.2. Selection of mutants and variability created

Selections in irradiated populations were carried out for obvious morphological changes which could contribute to increased yields as well as for quantitative characters. For this purpose bulk populations sampled from first formed five capsules of the M_1 plants as well as single plant progenies were used. Results of selection, heritability and correlations among characters in different generations have been reported elsewhere [10]. The variation observed in selections in preliminary screening of mutants in M_4 and M_5 generations is presented in table 1. At the preliminary screening stage the mutants recorded higher mean yield, plant height and number of capsules. The mean number of seeds per capsule was less in mutants selected from MI 2 variety than in the parent as only few selections could be made directly for this character. The mean number of seeds per capsule has also recorded an increase in the mutants of MI 3 variety although direct selection for this character was attempted only in few cases. This variety generally records lower number of seeds per capsule (Table 1).

4.3. performance of mutants in regional trials

The high yielding mutants were included in the major yield trials of the breeding programme and were tested in different locations along with the other higher yielding breeding lines selected from the segregating populations and germplasm collection.

The mean performance of the mutants compared with the control MI 3 variety at two or three locations during each growing season is given in table 2.

The mutants MB 29 and MB 33 have consistently outyielded the recommended variety, throughout the period of testing. Out of the six seasons of testing MB 15 recorded lower yield than MI 3 only during the northeast monsoon season of 1990/91. Due to relatively poor performance of MB 7 and MB 32, they were discontinued from the testing programme in 1992.

4.4. Drought resistance of mutants

Different criteria were used to study the degree of drought resistance of the mutants as against the parent cultivars.

4.4.1. Seed germination under increased osmotic pressure

Moisture stress can be simulated by increasing the osmotic pressure of water. Germination in solutions of high osmotic pressure is one way of estimating the tolerance of genotypes to moisture stress at seedling stage. Germination percentages of mutants in manitol solutions upto 8 atm and polyethylene glycol upto 4 atm are given in table 3. Seed germination in manitol was highest in MI 3 variety. Many mutants derived from MI 2 variety had greater capacity to germinate than the parent. Particularly noteworthy in this respect are MB-29w and MB 33.

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There was a relationships between germination percentage in manitol and polyethylene glycol. The mutants MB 29w and MB 33 recorded higher germination percentage in polyethylene glycol solutions as in the case of manitol.

4.4.2. Stomatal frequency and behavior

Stomatal frequency and behavior of stomata under water stress could give further indications of drought resistance of genotypes. Some of the mutants of MI 2 variety, including MB 33 had less number of stomata per unit area than the parent cultivar (table 4). Faster reaction of stomatal apparatus to water stress induction could be another indication of drought resistance. This was studied in several mutants. Leaves at different stages of growth were studied in separate experiments but the reaction of stomata to simulated water stress was similar in leaves of different age in the same genotype. Therefore mean values of percentage decrease in width of stomatal aperture in some of the mutants studied is given in Fig. 2. Mutants MB 1-1 and MB 30 recorded more efficient closure of the stomatal apperatus to induced water stress, compared to the parent variety MI 2. The rate of closure of stomata of MB-29w was much slower.

4.4.3. Other characters of mutants

The morphological and agronomic characters of the most promising mutants of MI 2 variety selected from the preliminary

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screening nurseries were studied in detail. Some of the characters which could contribute to increased drought resistance are presented in table 4. The total leaf area was least in C 37 and greatest in C 10. Other mutants had a leaf area comparable to the parent variety MI 2. Although the stomatal frequency of mutants was not much different from the parent in general, MB 30 recorded a relatively higher number of stomata. The MI 3 variety which was used in later experiments on mutation breeding had less than half the number of stomata per unit area than MI 2 variety. It had a lower shoot/root ratio as well. Most of the mutants also recorded less shoot/root ratio than MI 2 variety. The mutants MB 29w, MB-1 and MB 1-1 had the most prolific root system among the mutants.

Drought during the flowering period can severely affect seed yield of sesame [3,4]. The shortest period of flowering was recorded in MB 29w and C-8. The leaf pubescence, although did not exhibit much variation, was higher in MB-1 and MB 1-1.

4.5. Results of seed irradiation of UCR 82-203 NS variety

In a population of more than 150000 M_2 plants of UCR 82-203 NS variety, representing about 40000 M_1 parent plants, two mutants were recovered which have lost the undesirable effects associated with the indehiscent character. At maturity it was found that these mutants had lost the indehiscent character as well and were like a normal shattering variety. In subsequent trials they recorded 2-3 times higher yield than UCR 82-203 NS [15].

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4.6. Isolation, confirmation and improvement of morphological mutants

The number of mutants of MI 2 and MI 3 varieties with changed morphological characters which could be effectively used in a breeding programme are given in table 5. These mutants have been confirmed in subsequent generations after selection. Details of these mutants have been described earlier [8].

A mutant isolated in the M_3 generation of MI 2 variety treated with Ethyl methene sulphonate was found to have a brittle testa which can be easily removed by rubbing the dry seeds. This mutant was back-crossed to parent variety and was also crossed with white seeded cultivars for selecting improved, brittle seed coat genotypes. A line MB -29w selected from a cross of MB 29w with local variety Sudu Thala has shown promise.

The yield of MB 29 and MB 29w is similar to MI 2 and is higher than that of MI 3 variety, the only white sesame cultivar released in Sri Lanka.

The polypetalous corolla mutant (table 5) was found to be sterile and could not be maintained over several generations.

4.7. Sesame anther culture

Flower buds of 5-7 mm length and 36-48 h prior to anthesis incubated at 8°C for 24 h gave the highest rate of callus induction. Continuous darkness after plating was better than a photoperiod of 12 h. The MS 5 medium recorded the highest rate of callus induction (Table 6) as well as callus formation after reculture (Table 7). MS 5 medium contained MS salts supplemented with 10 mg l^{-1} 2,4-D, 2 mg l^{-1} of both IAA and BA. Other media used in the studies and details of culture conditions for successful callus induction have been discussed in detail [14].

5. Discussion

5.1. Raidosensitivity

High level of resistance of sesame seeds to irradiation when formation is considered [16] and the differences in the radiation sensitivity of different sesame genotypes observed by other workers [16-20] are substantiated by our experiments with two morphologically different sesame cultivars. It is clear from the results that the MI 3 variety tolerates much higher doses of gamma rays than MI 2 variety considering germination after irradiation as well as growth reduction studies. There seems to be a very high variation in growth reduction in sesame genotypes after irradiation and therefore different genotypes to be used in mutation breeding should be tested for radiosensitivity before deciding on doses.

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5.2. Variability created through mutation induction

Easily identifiable, morphological mutants or those with resistance to diseases have been induced in sesame using mutation breeding methodology. Induced variability in quantitative characters has been dercribed recently [18] and seems to be promising from the results presented here. When selected for one yield component, a change in the other components generally occurs due to compensatory effects [19]. Nevertheless, yield increase can be achieved in individual lines as well as in the mean yield of all selected lines if the population is large enough and selection pressure is high (Table 1). Thus, except for number of seeds per capsule in MI 2 variety, all the other yield components have higher mean values in the selected mutant lines even compared with the parent. The range of variability is also quite high, offering many lines for advancing to yield trials.

The mutants selected purely for quantitative characters and those with changes morphological characters can be treated as exceptionally valuable material for further improvement of sesame because there mutants were induced in locally adapted, recommended and high yielding cultivars . These mutants have been included in the ongoing hybridization programme. The mutants MB 29w (white seeded brittle seed coat genotype) and MB 13 will be handed over to the Department of Agriculture for testing in the National Coordinated Trials.

5.3. Drought resistance

The water requirements of different genotypes can differ at various growth phases of the plant. A genotype may be more tolerant to water stress at an early growth phase while a second may be more tolerant at a later stage of growth. Seeds of MB 29w and MB 33 have shown better capability of germinating at high asmotic potentials than other mutants. Low shoot-root ratio of MB 29w, MB 1 and MB 1-1 should be considered as advangeous when growing them in drought prove environments. Short period of flowering in MB 29w is also advantageous as drought at flowering stage can substantially reduce yields of sesame [20]. Shorter flowering period reduces the risk of drought and also facilitates synclrronous maturity minimizing shattering losses.

5.4. Indehiscence in sesame

The two mutants which have lost the undesirable effects of *id id* genotype were later found to have lost the indehiscent character as well. These were recovered from a population of about 150000 M_2 plants. The indehiscent character is governed by a single recessive gene [21] and our objective was to break the linkage of indehiscent character with other undesirable effects. The lose of undesirable characters along with the indehiscent character implies that the undesirable effects are a pleiotropic effect rather than a liked character as speculated by some anthers [22]. Therefore,

search for genotypes should be made to improve the seed retention of sesame at maturity.

5.5. Callus induction from anthers

According to the only published results of anther culture in sesame, the Korean cultivars used have recorded 5501% callus induction when 25 mg l^{-1} of 2,4-D and 1 mg l^{-1} of BA were included in the MS medium [23]. In the present experiment, the MS medium containing 10 mg l^{-1} of 2,4-d with 1 mg l^{-1} of BA recorded a higher rate of callus induction than MS 2 medium with 15 mg l^{-1} 2,4-D. These results are consistent with the findings that the different genotypes may require different media for callus induction from anther of the same species [24].

Shortening of the breeding cycle using doubled haploid technology can be achieved only if different stages take short periods [25]. All the media which were capable of producing callus were efficient in this context as they induced callus within 2-4 weeks. Nevertheless, MS 5 was the most successful medium where callus induction took place within 2 weeks and a greater proportion of anthers produced callus.

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Table 1 - Means and ranges of performance of mutant lines compared with parent cultivars.

Materials	Age, days	Plant height, cm	Capsules per plant	Seeds per capsules	1000 seed weight, g	Seed yield of plant^{-1}
Mutants of	93	119	54	59	3.3	8.4
'MI2'43	(78-99)	(81-146)	(31-93)	(37-80)	(2.8-4.2)	(1.3-13.8)
lines in M_4^0						
MI2	89	117	48	64	3.2	7.2
33 lines	90	123	56	63	3.1	8.7
in M_5^0 MI2	(83-96)	(90-137)	(28-69)	(44-82)	(2.7-3.5)	(3.8-10.2)
MI2	90	131	53	69	2.9	8.4
Mutants of	91	98	41	63	3.2	7.7
'MI3' 78	(74-96)	(83-126)	(29-54)	(41-81)	(2.7-3.9)	(3.5-12.7)
lines in M_4^0						
MI3	85	94	35	58	3.1	5.8
35 lines in	88	95	42	57	3.3	7.4
M_5^0	(75-92)	(78-119)	(30-62)	(38-74)	(2.8-3.7)	(3.811.4)
MI3	84	89	37	52	3.2	5.2

The range for mutants are given in paranthesis 40 high yielding mutants selected visually.

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Table 2 - Performance of sesame mutants in regional trials

Treatment	Age	Yield, kg/ha					
		Maha [a] 1989/90	Yala [b] 1990	Maha 1990/91	Yale 1991	Maha 1991/92	Yala 1992
MB 1	88	-	628	492	989	508	968
MB 7	85	-	721	684	725	339	-
MB 13	86	1055	742	517	1007	461	793
MB 29	86	-	-	1011	925	555	655
MB 32	87	815	718	620	651	387	-
MB 33	86	-	-	865	1123	475	804
MI 3(control)	85	561	684	824	871	440	728
Number of locations		3	2	2	3	2	2

a - Maha - Northeast monsoon season.
b - Yala - Southwest monsoon season.

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Table 3 - Percentage germination in Manitol and Polyethylene Glycol solutions.

Treatments	Water	Manitol solution			Polyethylene glycol		
		4 atm	6 atm	8 atm	3 atm	3.5 atm	4 atm
MB 1	100	42	4	0	87	66	42
MB 1-1	100	39	7	0	85	68	53
MB 2	98	44	8	3	81	71	54
MB 7	100	58	14	6	91	51	45
MB 13	99	39	8	2	76	49	35
MB 29w	100	67	28	13	92	79	81
MB 30	100	48	10	4	80	67	45
MB 32	100	59	11	3	83	59	40
MB 33	99	63	19	8	86	76	70
C 8	98	57	10	4	78	62	54
C 10	100	38	6	0	79	63	52
C 37	100	22	4	0	86	78	56
MI 2	98	53	7	0	79	54	37
MI 3	99	94	74	30	91	69	53

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Table 4 - Some characters related to drought resistance in sesame mutants.

Treatment	leaf area, cm^2	Stomata per mm^2	Shoot: root ratio by weight	Days to flower	Duration of flowering, days	Leaf pubescence[a]
MB 1	443	50	5.6:1	40	35	3.0
MB 1-1	430	43	5.4:1	41	35	2.7
MB 2	367	52	13.5:1	35	31	2.0
MB 29w	411	56	5.3:1	42	26	2.0
MB 30	510	73	7.6:1	36	31	2.0
MB 33	449	42	8.7:1	36	34	3.5
C 8	660	46	7.0:1	42	27	2.0
C 10	995	46	9.0:1	41	25	2.3
MI 2	438	56	12.6:1	36	33	2.1
MI 3	340	24	6.8:1	35	36	2.3

a - Glabrons - 0; Moderate pubescence - 5;
Extemely high pubescence - 10

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Appendix 8

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OPTIMIZATION OF MEDIA AND CONDITIONS FOR CALLUS INDUCTION FROM ANTHERS OF SESAME CULTIVAR MI 3

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Abstract : Murashige and Skoog (MS) medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), 3-indoleacetic acid (IAA) and 6-benzylaminepurine (BAP) were used to induce callus from anthers of sesame (*Sesamum indicum* L.) cultivar MI 3 as an initial step towards developing a protocol for the haploid production for use in the breeding Anthers from flower buds 36-48 h before anthesis incubated at 8^0 C for 24 h in the dark gave the best results. Out of the media studied, MS_5 medium containing 10 mg/l of 2,4-D and 2 mg/l each of IAA and BAP gave the highest rate of callus induction (46%) within 2-3 weeks of plating. For sub-culturing, both MS_5 and MS_4 (5 mg/l of 3-indoleacetic acid and 3 mg/l of benzylaminepurine) media gave satisfactory results, with MS_5 recording 78% and MS_4 60% callusing Calli produced in these two media were soft and yellow-coloured. In MS_5 medium they exceeded 2 mm in diameter. These calli have been transferred to regeneration media for optimising conditions for haploid production.

Key words: Anther culture, Sesame, Gingelly, *Sesamum indicum* L., haploids, callus induction

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an important oilseed crop with a large number of land races and cultivars adapted to a wide range of climatic conditions.[1-3] It is economically the most important annual oilseed crop in Sri Lanka. For all practical purposes it is treated as a self-pollinated species although varying degrees of cross-pollination have been reported.[1,3-7] Doubled haploids (DH) offer an efficient and rapid method of improving self-pollinated crop species if suitable protocols for haploid production, diploidization and regeneration are available.[8-10] The DH method has already been used to improve a range of agronomically important characters in several crops such as rice,[11,12] barley,[13,14] wheat[15,16] and *Brassica sp.*[17-19] Modified Murashige and Skoog (MS) medium has been used successfully to induce callus from sesame anthers of cultivars of exotic origin.[20,21] However, under the same culture conditions significant differences among genotypes have been observed.[10,15,22-25] Therefore, efficient haploid production from locally adapted high yielding cultivars is a prerequisite for using this methodology in crop improvement.

Optimal stage of the explant and culture conditions for efficient callus induction and sub-culture of anthers of MI 3 variety, the only recommended white seeded sesame cultivar in Sri Lanka are reported here.

MATERIALS AND METHODS

The cultivar MI 3 used in this study is the only recommended variety in Sri Lanka having white seeds. It has a non-branching stem, opposite leaf arrangement with three bicarpellate capsules per leaf axil. Flower buds in three stages of development were studied for callus formation from anthers. These were the small (48-60 h before anthesis), medium (36-48 h before anthesis) and large buds (24-36 h before anthesis). Flower buds were surface-sterilized with 70% ethanol for 5s and then rinsed in autoclaved distilled water prior to extracting anthers under aseptic conditions for plating. Callus formation from anthers treated at 8^0 C for 24 h and 48 h was compared with untreated ones which were directly plated without cold treatment.

Murashige and Skoog (MS) medium[26] has been the most effective one for callus induction in sesame.[20,21] Five MS based media with different levels of hormones were used in the present studies (Table 1). The pH value of media was adjusted to 5.8 ± 0.1 with 0.1 N NaOH or 0.1 N HCl. The effect of the presence or absence of light on the callus induction of plated anthers was also investigated. The effects of the total absence of light and a photoperiod of 12 h per day from fluorescent tubes at 2000 lux were compared. The plated anthers and subcultured calli were maintained at a temperature of 25 ± 1^0 C.

Table 1: Hormone composition of MS media used in the experiments (mg/l)

	Medium				
Hormones	MS_1	MS_2	MS_3	MS_4	MS_5
2,4-D	10	15	20	-	10
IAA	-	-	-	5	2
BAP	1	1	-	3	2

RESULTS

Flowers at the 'medium stage' having a length of 5-7 mm and 36-48 h prior to anthesis gave the highest rate of callus induction. The flower buds of this stage were therefore used in subsequent experiments. Out of a total of 1077 anthers which produced calli, 947 (88%) remained green until longitudinal splitting. Of the 6123 anthers which turned brown within a few days of plating, only 613 (10%) produced calli. Thus, 90% of the anthers which turned brown failed to produce callus.

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In the preliminary studies with different temperatures of pretreatment of anthers, the highest number of calli (199 out of 1200 plated anthers) were produced when the anthers were incubated at 8^0 C for 24 h. Incubation at 8^0 C for 48 h resulted in the callus production from 154 out of 1200 anthers. Direct plating without a cold treatment resulted in only 71 anthers producing callus out of 1200 anthers. This experiment was conducted only with MS_1 medium and further experiments were carried out using anthers from flower buds of 'medium stage' of development incubated at 8^0 C for 24 h.

Among the media used MS_1, MS_2, MS_4 and MS_5 were capable of inducing callus from anthers of MI 3 variety (Table 2). All four media had one or two of auxins 2-4 dichlorophenoxyacetic acid (2,4-D) and 3-indoleacetic acid (IAA) and a cytokinin 6-benzylaminepurine (BAP), whereas MS_3 medium which failed to induce callus had only 2,4-D, but in greater concentration than the rest (Table 1). Callus induction in MS_5 medium occured within 2 weeks of plating compared to 3-4 weeks in other media. A relatively greater proportion of anthers plated on this medium produced callus (Table 2). The first response of most anthers that gave callus was the longitudinal splitting within a week after plating. Continuous darkness was better than a photoperiod of 12 h per day on MS_2 and MS_4 media for callus formation (Table 2). Experiments on callus induction with MS_5 medium were therefore continued in the absence of light.

Table 2: Callus induction capacity of media from anthers of medium size (5-7 mm) flower buds of sesame

Medium	Treatment	Number of anthers producing callus*	Percentage of callus induction
MS_1	Light	199	17
MS_2	Light	80	7
	Dark	111	9
MS_3	Light	0	0
	Dark	0	0
MS_4	Light	60	5
	Dark	80	7
MS_5	Dark	547	46

* 1200 anthers were plated for each treatment.

Calli derived from anthers were cultured in four different MS based media viz. MS_2, MS_3, MS_4 and MS_5 (Table 1). Response to reculture was found to be different

in the four media (Table 3). The MS_5 medium containing three hormones proved to be the best among those tested. Calli formed on the MS_2 medium were relatively hard and brownish. They were globule like structures less than 1 mm in diameter. When these calli were transferred again to MS_4 or MS_5 media, they formed callus tissues similar to those usually grown in the latter two media, being yellowish and soft. However, calli subcultured from MS_4 and MS_5 media were more capable of callusing than those from MS_2.

Table 3: Callus formation on different media after reculture

Medium	Response of plated callus		Characteristics
	Percentage of callus formation	Size of callus formed*	
MS_2	45.5 ± 0.7	+	brown, globular and hard calli
MS_3	0	--	--
MS_4	60.0 ± 1.7	+	yellowish soft calli
MS_5	77.8 ± 1.8	+ + +	yellowish soft calli

* + - All calli less than 1 mm in diameter
+ + - More than 80% of calli of 1-2 mm diameter
+ + + - More than 80% of calli greater than 2 mm in diameter

DISCUSSION

Shortening of the breeding cycle using DH technology can be achieved only if different stages take short periods.[8,13,17,18] All the media which were capable of producing callus were efficient in this context as they induced callus within 2-4 weeks. Nevertheless, MS_5 was the most successful medium as callus induction in it took place within 2 weeks compared to 3-4 weeks in other media. Moreover, a greater proportion of plated anthers produced callus in this medium.

The position of anthers on the medium at the time of plating also seems to have an influence on the callus induction. This aspect needs further investigations and should contribute to increasing the callus yield.

According to the only published results of anther culture in sesame, the Korean cultivars used have given 55.1% callus induction when 25 mg /l of 2,4-D and 1 mg/l of BAP were included in the MS medium.[20] Our experiments indicate that

concentration of 15 mg/l of 2,4-D with 1 mg/l of BAP (MS_2) does not give a satisfactory rate of callus induction. The MS_1 medium containing 10 mg/l of 2,4-D with 1 mg/l of BAP recorded a higher rate of callus induction than MS_2 medium with 15 mg/l 2,4-D. These results are consistent with the findings that the different genotypes require different media for callus induction from anthers of the same species.[10,15,22-25]

Anthers from flower buds 36-48 h before anthesis, incubated for 24 h at 8^0 C when plated on MS_5 medium and kept in total darkness produced the highest rate of callus induction in the present experiment. With the objective of producing haploid plants, calli obtained from anthers of MI 3 variety have been transferred to regeneration media with different ratios of hormones. Besides, different media with natural sources are being tested for anther culture of several promising sesame genotypes including MI 3.

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