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Rhizosphere inorganic carbon enhancement of crop production

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אוניברסיטת בן-גוריון בנגב
Ben-Gurion University of the Negev
The University celebrates its 30th Anniversary

Our ref: 88107
2 March 2000

Mr. Bill Crane
Environment Science and Technology Counselor
U.S. Embassy
71 Hayarkon Street
Tel Aviv 63903

Dear Mr. Crane,

Re: US-Israel AID-CDR Program
Grant No: TA-MOU-97-C16-100
"Rhizosphere Inorganic Carbon Enhancement of Crop Production"
Prof. H. Lips, Dr. M. Sagi - Israel
Dr. F. Faustino, Dr. E. M. T. Mendoza - Philippines

Enclosed please find one (1) copy of the, 'Annual Report', covering the period of March 1, 1998 to February 29, 2000, submitted by Prof. Lips and Dr. Sagi of Ben-Gurion University of the Negev for the above-mentioned project.

Thank you for your continuing support of this project.

Yours sincerely,

Mrs. Simone Herishanu
Acting Head, Research Liaison Section

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Executive Summary

During preliminary experiments in Ramat Negev (Israel) using local brackish water (± 7 dS m^{-1}) or good quality water salinized with NaCl to the same electroconductivity, it was observed that plants grew better with well brackish water. It was later observed that this was due mostly to the presence of considerable amounts of HCO_3^- ions. Further experimental work pointed out to the fact that HCO_3^- was used by the roots and fixed by the enzyme PEPc (phosphoenol pyruvate carboxylase) into OAA (oxaloacetic acid). This was very important to the plant under saline conditions because oxo-glutarate required for the assimilation of ammonium became very scarce and OAA could easily replace it as a carbon skeleton to bind ammonium ions yielding aspartate and asparagine.

The practical application of HCO_3^- fertilization to crops may especially effective for crops irrigated with brackish water and for legumes fixing N_2, two types of crops requiring the assimilation of relatively large amounts of ammonium in their roots. A fringe benefit of HCO_3^- fertilization is higher harvest index (biomass of fruits+seeds/total biomass of the plant) and a higher fruit quality as observed in Israel with tomatoes, melons, peppers and cucumbers. In the present project the Philippino investigators will study the response of relevant local crops to HCO_3^- fertilization while the Israeli group will continue their efforts to optimize this process and establish which are the crops and environmental conditions under which the response is more possitive. Both groups will also study the biochemical changes in nitrogen assimilation enhanced by HCO_3^-.

During the last year work has been carried out along these lines and some of the preliminary results are described bellow. Roberta N. Garcia has been studying the levels of PEPc (the enzyme incorporating HCO_3^- into OAA) in Sede Boqer in cooperation with local researchers. Her work is now being prepared for publication.

The collaboration between the groups has permitted an integrated approach to study basic and applied aspect of the effect of bicarbonate fertilization on crops under salinity and especially on the productivity of leguminous crops.

Section I

A) Research objectives

To investigate the influence of increased concentrations of inorganic carbon applied to the root medium on the growth and fruit quality of tomato plants with a view to improving production (quality and quantity), while minimizing environmental pollution due to unused fertilizers and maximizing the marketing and nutritional value of the produce.

a) Investigation of the physiological and metabolic mechanisms through which rhizosphere dissolved inorganic carbon (DIC = CO_2 + HCO_3^-) affects carbon and nitrogen assimilation. To understand the relationships between increased DIC supply and; (1) anaplerotic carbon provision through PEPc activity, (2) NO_3^- and NH_4^+ uptake, (3) N metabolism and (4) respiration, (5) regulation of assimilate allocation to growth sinks.

b) To study the enhancement of salt-tolerance through the enrichment of rhizosphere DIC. To optimize additions of dissolved inorganic carbon to fertigation under saline conditions caused by either saline soils or water. To understand the mechanisms through which enriched rhizosphere DIC ameliorates salinity stress.

c) To test the potential of enriched rhizosphere DIC to overcome the natural limitation of plants to use NH_4^+ at levels which could support vigorous growth of crops. Plants grown with NH_4^+ nutrition have high rates of DIC utilization and may thus benefit from enriched rhizosphere DIC concentrations. Improved NH_4^+ utilization by crop plants would be beneficial due to decreased NO_3^- accumulation in agricultural produce and reduced leaching of NO_3^-fertilizers into water resources.

d) Investigation of the utilization of DIC derived from natural sources. Manure releases CO_2 and NH_4^+. Rhizosphere DIC enrichment under these conditions and increased DIC in the soil resulting from plastic mulching may enhance an environmentally safer (low NO_3^- applications) crop production in rural areas. These techniques may be used to enhance salinity tolerance of plants grown without drip irrigation.

B) Research accomplishments:

I. Melon nitrate reductase (NR), fruit production and quality as affected by salinity and $NaHCO_3$

Chen Guoxiong and Moshe Sagi and Herman S. Lips

Melon (*Cucumis Melo* L. C-8) plants were grown in pots in a greenhouse in a mixture of vermiculite, perlite and fine sand (1/1/2, v/v/v). The effects of NaCl (100mM), $NaHCO_3$ (10mM) and NaCl (100mM)+$NaHCO_3$ (10mM) on melon leaf NRA, fruit yield and quality were studied. The treatments were applied to 28 days old melon seedlings. Leaf NR was slightly increased by $NaHCO_3$ and decreased by NaCl as compared to controls in 46 days old plants, which started to bear fruit . The plants exposed to NaCl were smaller than controls and similar to control with $NaHCO_3$. $NaHCO_3$ additions to the medium did not affect melon yield significantly, while NaCl and NaCl+$NaHCO_3$ treatments decreased the yield by about 33% of the control, and increased the dry weight to fresh weight ratio. $NaHCO_3$ treated plants exhibited a false 'mature signal'. NaCl and NaCl+$NaHCO_3$ treatments **improved fruit quality** through the increased in total soluble solid and total soluble sugar.

Materials and Methods

The experiment was carried out in the a greenhouse in Sede Boqer campus from May to September in 1999. The greenhouse transmits 1100 to 1700 μE of PAR radiation. Temperatures in the greenhouse ranged from 20.5°C to 30.7°C during the day and 16°C and 24°C during the night. The greenhouse was cooled by an automatic water cooling/ventilating system.

The growth substrate consisted of a mixture of perlite, vermiculite and dune sand at a ratio of 1:1:2 in volume. Thirty six black plastic pots each with a volume of 15 liter were filled with the solid substrate up to 7 cm from the top. The filled pots were extensively washed with tap water in order to eliminate excess alkalinity and salt.

Melon seeds were sown in the media at a depth of 2 cm, 10 seeds per pot, on May 15. The seedlings appeared on May 19. The seedlings were thinned to 4 plants per pot at the 2 leaf stage and to 2 plants per pot at the 4 leaf stage. The seedlings were selected for uniformity during thinning. A frame was set up when the plants had 5 leaves to support the elongating plants until 2 m length. Shoot lateral branches were cut during growth.

Plants were irrigated with 1/4 strength Long Ashton nutrient solution until the 5 leaf stage (May 19-June14). Nitrogen concentration was 4 mM NH_4NO_3 and were irrigated once a week with 6 L per pot. The strength of the solution was increased to 1/2 strength Long Ashton nutrient solution during June 15 to 29. Salinity and bicarbonate treatments were applied at the same time by addition of 50 mM NaCl or 10 mM $NaHCO_3$ or both to the irrigation solution. Control plants were irrigated with the original solution. The nutrient solution was adjusted to pH 6.5 using 1 N HCl. After June 29, the NaCl concentration was increased to 100 mM and the irrigation frequency was increased to once every 4 days and 8 L per pot.

Manual pollination was conducted at 7:30-8:30 A.M. every day from July 2 to August 3. Fruits were harvested on August 16 and 20 and September 1 and 12. A total number of 64 fruits (one from each plant) were harvested on August 16 for quality analysis.

In vitro nitrate reductase (NADH-NR, EC 1.6.6.1) of the leaves was determined shortly after the application of NaCl and $NaHCO_3$ and on July 4 when the plants started fruiting. The extraction medium consisted of 200 mM Tris buffer (pH7.5), 2 mM EDTA, 3 mM dithiothreitol, 2% (w/v) casein and 10% (v/v) glycerol with the addition of 0.1g polyvinylpyrrolidone to each extraction. The homogenates were centrifuged at 30000g in a Sorvall RC-5 refrigerated centrifuge at 4° C for 10 min. The supernatant was used to measure NR *in vitro*. The reaction mixture consisted of 100 mM phosphate buffer (pH7.5) with 1 M KNO3 and 0.4 mM NADH. The reaction was initiated with addition of 100 µl enzyme extract to 300 µl reaction mixture and incubated at 30° C for 10 min. The reaction was terminated by adding with vigorously mixing a 0.1 ml mixture of 1.3 mM phenazine methosulfate and 1 M Zn acetate (1/1, v/v) to remove residual NADH. The solution was reacted with 1 ml of a 1:1 (v/v) mixture of 1% (w/v) sulphanilamide in 3.0 M HCl and 0.02%(w/v) N-naphthyl-(1)-dihyduochloride. absorbance was measured at 540 nm after 20 min.

The plants were separated into root, stem, petiole and leaf constitutes and fresh and dry weights were measured in the end of the experiment. The plant materials were dried in a ventilated oven at 80°C for 96 hours.

Results

NRA in leaves

Salinity and bicarbonate were applied when the plants were 28 days old and started to produce flowers. Leaf NR of NaCl treatment was the highest, as high as 300% times the levels in controls, 250% times that of $NaHCO_3$ treated plants and 200% of NaCl+$NaHCO_3$ treated plants, one day after application of the treatments (Fig 1). Leaf NR of salinity and bicarbonate treated plants decreased with time while that of the control plants remained quite constant with time. Leaf NR in vitro of melon plants was stimulated by NaCl and $NaHCO_3$ treatments initially -and inhibited afterwards.

Salinity significantly inhibited leaf NR while the effect of bicarbonate was not significant in the 46 days old plants, which started to produce fruit (Table1). $NaHCO_3$ did not affect leaf NR but NaCl had a significantly inhibitory effect in fruit-producing plants.

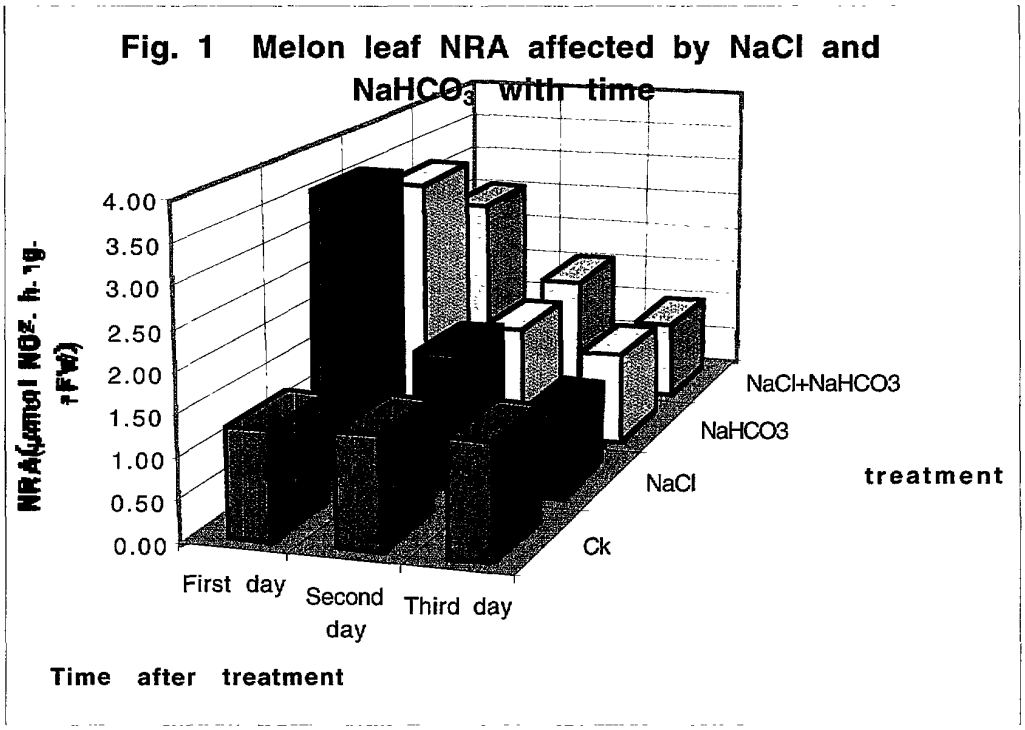


Table 1: Melon leaf NR as affected by NaCl and $NaHCO_3^-$.

Treatment	Control	NaCl	$NaHCO_3$	$NaCl+NaHCO_3$
NR (μmol NO_2 h^{-1} g^{-1}FW)	1.17	0.85	1.22	0.60

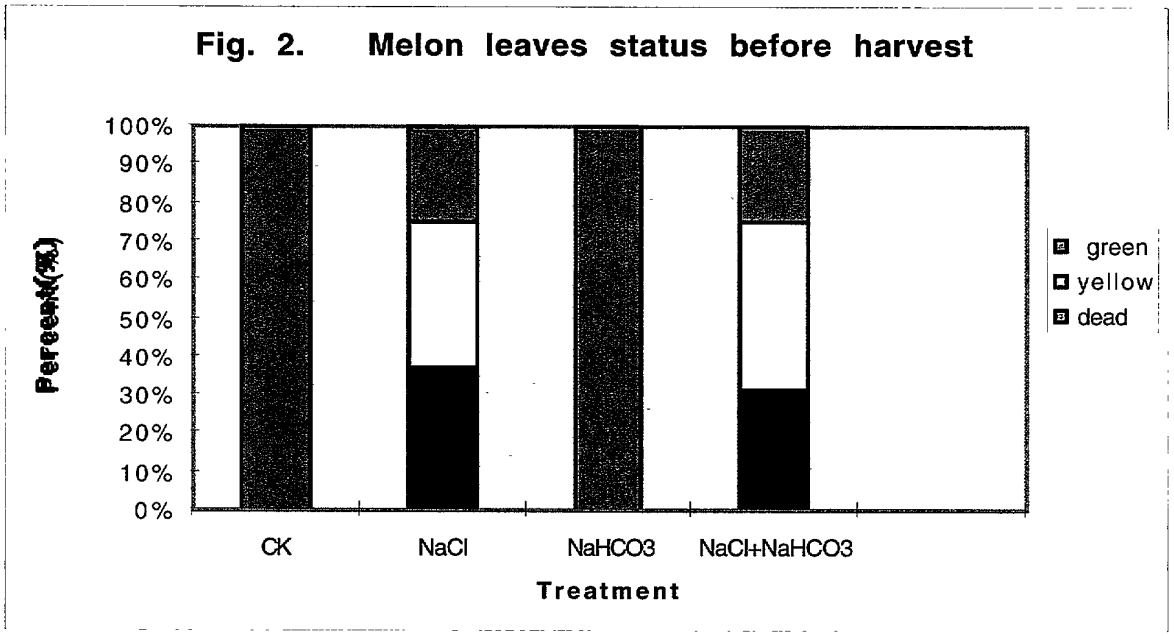
Plant growth

Salinity and bicarbonate decreased the size of plants (Table 2). The effect of $NaHCO_3$ was limited. Salinity substantially decreased plant height, leaf number per plant and size of leaf, petiole and stem.

Table 2. Growth of melon plants as affected by NaCl and $NaHCO_3^-$

Treatment	Control	NaCl	$NaHCO_3$	$NaCl+NaHCO_3$
Leaves	72.0	51.3	69.3	49.5
Dead leaves	3.0	6.0	3.0	10.0
Live leaves	69.0	45.3	66.3	39.5
Leaves weight(g)	284.0	148.5	266.9	123.3
Gram per leaf	4.1	3.3	4.0	3.1
Petiole weight(g)	95.5	56.4	87.4	45.4
Gram per petiole	1.4	1.2	1.3	1.1
Stem length(cm)	349.3	184.5	321.3	190.0
Stem weight(g)	164.3	78.6	146.4	81.1
Gram per cm stem	0.5	0.4	0.5	0.4
Root weight(g)	64.7	28.6	40.1	18.6

Salinity enhanced senescence of the plants while the effect of $NaHCO_3$ treatment was not evident (Fig 2). The percentage of yellow leaves in the plants receiving NaCl and $NaCl+NaHCO_3$ was 37.5% and 43.8%, respectively. The dead leaf percentage of the plants treated with NaCl and $NaCl+NaHCO_3$ was 37.5% and 31.5%, respectively.



Salinity and bicarbonate treatments affected plant biomass (Table 2 and 3). The four treatments could be divided into two groups. The first group was Control and $NaHCO_3$, the second was NaCl and NaCl+$NaHCO_3$. There was not significant difference within each group, but a significant difference existed among groups. The first group weight was higher than that of plants in the second group. Therefore $NaHCO_3$ affected melon vegetative growth slightly, NaCl and NaCl+$NaHCO_3$ inhibited melon vegetative growth severely.

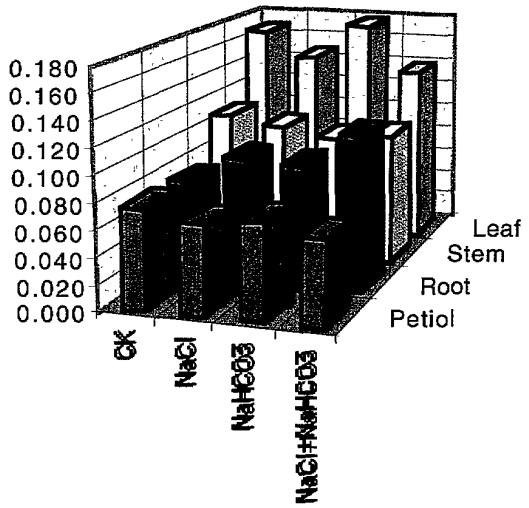
Dry matter distribution in melon plant was different according to the treatment. Compared to controls, the $NaHCO_3$ grown plants had less dry matter in root, petiole and stem and increased biomass of leaf. NaCl+$NaHCO_3$ decreased biomass of leaf and increased that of root, petiole and stem. NaCl plants were similar to NaCl+$NaHCO_3$ plants except for the slightly decreased in stem.

Table 3. Dry waight of melon plants as affected by NaCl and NaHCO

Treatment	Control	NaCl	$NaHCO_3$	NaCl+$NaHCO_3$
Root	4.8	2.7	2.9	2.2
Petiole	7.2	3.8	6.4	2.9
Stem	18.2	8.3	14.3	8.4
Leaves	47.9	22.1	47.2	17.0

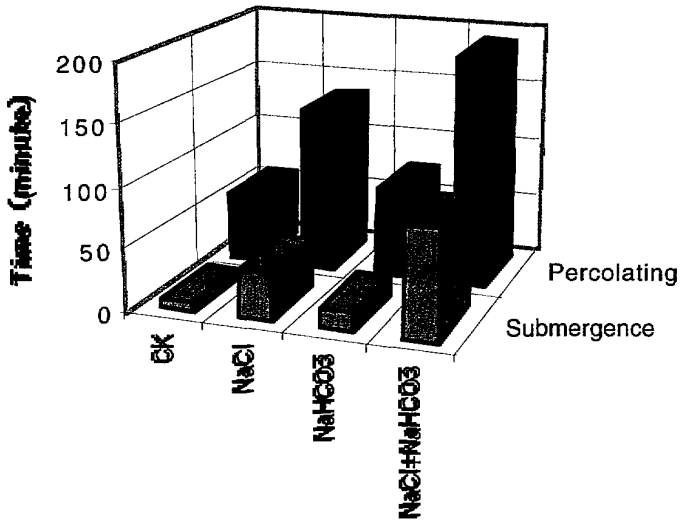
Except for the roots almost all parts of the melon plants subjected to the three treatments dropped the ratio of dry weight to fresh weight as compared to controls (Fig3). The ratio of the plants in the three treatment should be higher than in controls. Why were the results reversed? The reason may have been the moisture in the growth substrate. The fresh weight was measured 3 days after irrigation, when the moisture in NaCl, NaCl+$NaHCO_3$ was much better than in CK and $NaHCO_3$ because the latter had bigger plant which transpired more, and because NaCl decreased the permeability of the growth substrate storing more moisture in the NaCl containing matrix (Fig 4). So the plant in CK and $NaHCO_3$ contained less water which resulted in a lower fresh weight, thus had higher ratio of dry weight to fresh weight.

Fig. 3. Ratio of dry weight to fresh weight



The root, removed from the matrix and washed, had a similar water content. The ratio of dry weight to fresh weight of root may express the affect of the treatments accurately: all the three treatment increase the ratio in the order of $NaHCO_3$, NaCl and NaCl+$NaHCO_3$.

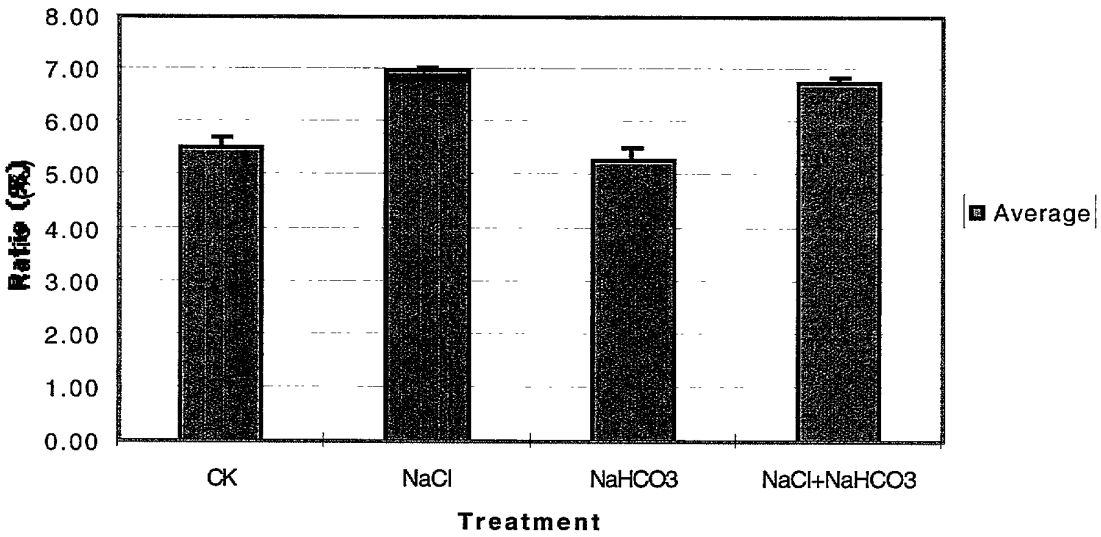
Fig. 4. Waterlogged time of matrix



Fruit yield and quality affected by NaCl and $NaHCO_3$

Fruit yields per plant from CK, NaCl, $NaHCO_3$ and NaCl+$NaHCO_3$ treatments were 1847, 731, 1795 and 613 g, respectively. $NaHCO_3$ slightly decreased fruit yield, but NaCl and NaCl+$NaHCO_3$ significantly decreased fruit yield. $NaHCO_3$ exerted little effect on fruit size, NaCl and NaCl+$NaHCO_3$

Fig. 5. The weight ratio of dry to fresh of melon fruit



caused a reduction of fruit size. Average weight per fruit of controls, NaCl, $NaHCO_3$ and NaCl+$NaHCO_3$ plants were 950, 571, 804 and 496 g/fruit respectively.

The specific weights of fruits from different treatments had almost the same value of 1 g/cm^3. But there was significant difference in the weight ratios of dry to fresh weight of the melon fruits between treatments (Fig 5). The ratios of fruits of NaCl and NaCl+$NaHCO_3$ grown plants were about the same, but obviously larger than the ratios in controls and $NaHCO_3$ treated plants. $NaHCO_3$ treatment had no significant effect on this ratio.

Dry matter distribution between vegetative and reproductive organs differed among treatments (Fig 6). In NaCl and NaCl+$NaHCO_3$ a decrease in dry matter allocation to fruit was observed, while $NaHCO_3$ increased slightly dry matter allocation to fruits.

The effect of NaCl and $NaHCO_3$ on fruit quality was shown in table 4. NaCl and NaCl+$NaHCO_3$ increased concentration of total soluble solids to the same degree, but both of them did not affect electrical conductivity. **Sucrose concentration of melon fruits was significantly improved by NaCl and $NaHCO_3$**, while NaCl treated plants had a slightly lower glucose concentration and $NaHCO_3$ plants slightly higher than controls. Total soluble sugar concentration was highest in NaCl+$NaHCO_3$ grown plants, followed by NaCl treated plants and lowest in controls. No significant difference of titratable acid existed between control, NaCl, and NaCl+$NaHCO_3$ treatments. TA (total acidity) in $NaHCO_3$ treatment was too high, resulting in additional results (TSS, EC, SUC and GLUC) which need verification. NaCl and NaCl+$NaHCO_3$ reduced melon netting quality, the appearance of melons harvested from NaCl and NaCl+$NaHCO_3$ treatments was not ideal.

Fig. 6. Dry matter distribution between vegetative and reproductive organs

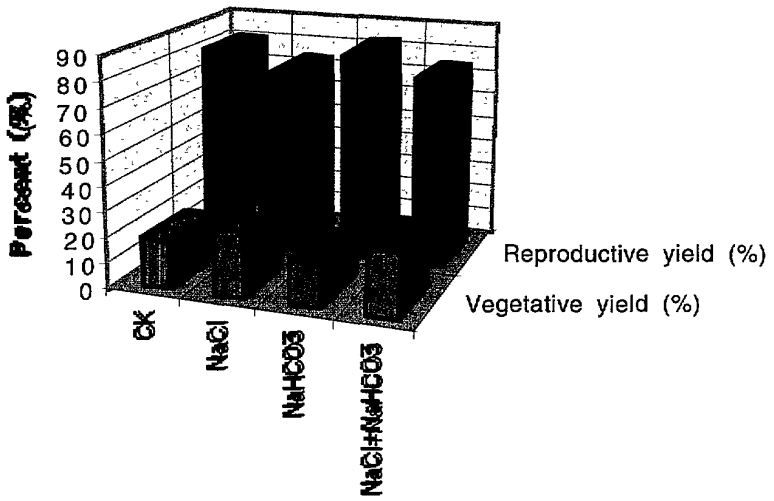


Table 4. Melon quality as affected by NaCl and $NaHCO_3^-$.

Treatment	TSS (%)	EC (ds/m)	*SUC* (mg/gfw)	GLUC (mg/gfw)	TA (meq/gfw)
Control	9.7	9.8	24	27	0.021
NaCl	10.4	8.7	36	23	0.018
$NaCHCO_3$	8.2	7	33	21	0.049
$NaCl+NaHCO_3$	**10.4**	**9.8**	**35**	**29**	0.021

Discussion

Low concentration of NaCl may be benefitial to plant growth. NaCl 21.8 mM stimulated tomato growth (Adams P., 1988), 2-4g/l NaCl increased Poa Pratensis and Lolium Perenne seedling height (Chen G. et al., 1996). In carrot cells the initial exposure to NaCl increased malate concentration threefold in the cytosol and is concomitant with an equivalent increase in cytosolic Na^+, and when the cells have adjusted to the external salinity, malate concentration in the cytosol returned to the non-saline level (Reuveni et al., 1991). This suggested that initial exposure to low levels of NaCl stimulated NR activity in cells.

Low NaCl concentration may stimulate NR activity in plants. NR activity increased in pigeon pea at low levels of NaCl (Sagar A., 1993). NR activity was found to increase in the presence of NaCl over that of the control and increasing NaCl concentration caused a further increase in NR activity in a concentration dependent manner (Misra N., 1990). The NR activity doubled within 24 hours by the addition of Na+ (Ohta D., 1989).

The Na^+ concentration in melon leaf cells increased after melon root exposed to NaCl or $NaHCO_3$, causing a NR activity rise due to the stimulation by low levels of Na^+ (Fig.2). Leaf NR increased with Na^+ accumulation in leaf cells, then decreased to the level of controls with increasing Na^+ concentration.

The melon plants exposed to NaCl and $NaHCO_3$ had smaller leaves, stems, roots and fruits. This result held identical views from others (Silberbush, 1991; Mendlinger S., 1992b; Ulery A. L., 1997; Mirza J. I., 1993; Parasher A., 1992). Reproductive yield was more affected by NaCl and $NaHCO_3$ than vegetative yield, the amounts of dry matter allocated to reproductive organs was reduced by NaCl and $NaHCO_3$ as compared to controls.

NaCl increasing TSS and total sugar content (Table 5). $NaHCO_3$ effect on fruit quality (Table 5) was contrary to the results reported by Gao in which $NaHCO_3$ improved tomato fruit quality. According Mendlinger S.(1992a), NaCl did not affect electrical conductivity and pH of the fruit. But the titratable acid of $NaHCO_3$ grown plants was higher than in plants of the other three treatment, the EC and TSS was lower (Table 5). For acid content, there was in general a decline pattern with the maturity but a significant reduction at the final stage of maturity was observed (Quadir M. A., 1990; Hubbard N. L., 1989; Kenneth A. C. 1988). TSS increased with maturation of (Yoshida, 1990; Nojima H., 1993; Chachin K., 1988). All melons tested were harvested on the same day. The basic part connecting to the stem turned yellow, which was considered as premature signal. So it may be concluded that $NaHCO_3$ treatment showed premature signal earlier than it should by the state of maturity of its fruits.

II. Growth, N metabolism and root HCO_3^- fixation by tomato plants irrigated with saline water as affected by bicarbonate fertilization.

Gao Zhifang, M. Sagi and S. H. Lips

Tomato plants grown under saline conditions reduce and assimilate a large proportion of nitrate taken up in the roots, contrary to plants under non-stress conditions which transport most (up to 98%) of the root nitrate to the shoots for its assimilation into organic nitrogen compound. Bicarbonate enrichment of the rhizosphere increased the anaplerotic provision of carbon skeletons for ammonium assimilation in the roots. Salinity treated plants produced more dry mass and organic N when their roots were supplied with CO_2-enriched air.

While salinity treated plants the products of $DI^{14}C$ (dissolved inorganic CO_2) were diverted into amino acid synthesis while non-stressed plants diverted most to organic acids. Enriched rhizosphere DIC increased the anaplerotic supply of carbon skeletons for amino acid synthesis in roots, contributing to the sustained production of biomass under saline conditions.

Effect of NaCl on the concentration of malate, K^+, NO_3^- and reduced N (mM) in the xylem sap of plants of detopped tomato plants, 11 d after transfer into hydroponics.

Xylem content	NaCl mM		
	0	100	200
Malate	1.72±0.34b	0.57±0.14a	0.12±0.07a
K	30±2a	31±4a	30±2a
Nitrate	9.79±1.61b	4.44±1.39a	1.83±0.19a
Reduced-N	2.92±0.07a	6.72±1.21b	8.45±1.16b
Nitrate:reduced N	3.40±0.63b	0.63±0.09a	**0.89±0.12a**

Nitrate and K^+ uptake and rates of HCO_3^- fixation (nmole g^{-1} root DW) in tomato plants 14 d after transfer into hydroponic culture with 0 or 100 mM NaCl and aeration with either low (360 µM) CO_2. Different letters following the means±SE indicate that salinity had a significant (P>0.05), Fisher's protected LSD) influence (n=4).

Root CO2 level	Salinity (mM)	
	0	100
	Nitrate uptake	
Low	31.3±3.2b	17.0±2.7a
High	34.1±4.4b	26.5±1.3b
	K uptake	
Low	5.9±0.4b	0.0±0.5a
High	7.0±0.7b	1.2±0.2a
	Bicarbonate fixation	
Low	2.9±0.2a	1.0±0.1a
High	26.4±4c	11.0±0.7a

High bicarbonate affected nitrate uptake mainly under saline conditions. This reflects the fact that salinity inhibited transport of nitrate to the shoot but stimulated root uptake, reduction and assimilation of nitrate into amino acids. Under these conditions the supply of organic N to the shoot was improved, allowing enhanced production of biomass – which is one of the characteristics of stress tolerance.

III. Carbon and nitrogen metabolism in salinity-treated tomato plants as affected by rhizosphere dissolved inorganic carbon.

M.D. Cramer, M. D., Z.F Gao, S.H. Lips

The influence of variation in the concentration of dissolved inorganic carbon (DIC) in the form of CO_2 and HCO_3^- in the root media on the C and N metabolism of *Lycopersicon esculentum* (L.) Mill. cv F144 was investigated under both saline and non-saline conditions. Tomato seedlings were grown in hydroponic culture (pH 6.5) with and without NaCl and the root solution was aerated with either ambient CO_2 concentration (360 µmol mol^{-1}) or CO_2 enriched air (5 000 µmol mol^{-1}). Nitrate uptake and root tissue NO_3^- concentrations were slightly increased by elevated rhizosphere DIC concentrations in both control and salinity-treated plants. This was associated with 46% higher nitrate reductase activity in the roots of control plants supplied with elevated DIC than in those supplied with ambient DIC. The *in vitro* activity of phosphoenolpyruvate carboxylase (PEPc) in control and salinity-treated plants was unaffected by the supply of elevated rhizosphere DIC concentrations. However, *in vitro* PEPc activity was considerably higher than the rates of *in vivo* PEPc activity reported previously, indicating that PEPc activity *per sé* was not a limitation on the provision of anaplerotic C. Thus, elevated rhizosphere DIC concentration stimulated the uptake of NO_3^- and provided alternative C skeletons for the assimilation of the NH_4^+ resulting from NO_3^- reduction into amino acids within the roots. Salinity stimulated root glutamine synthetase (GS) activity up to 2-fold over GS activity in control plants. Furthermore, elevated DIC caused an increase in leaf and root GS activity of control plants while inhibiting GS activity in the roots of salinity-treated plants. Glutamine:2-oxoglutarate aminotransferase (GOGAT) activity of salinity-treated plants was increased 2-fold by elevated rhizosphere DIC concentrations. These changes in GS and GOGAT activity must reflect changes in amino acid synthesis. Under saline conditions the xylem transport of NO_3^- is partially blocked and a

larger root assimilation develops requiring not only transamination of 2-oxoglutarate to glutamate, but also oxaloacetate to aspartate and transamidation of aspartate to asparagine.

INTRODUCTION

The biological components of soil (plant roots and microflora) produce CO_2 through respiration, which accumulates at concentrations between 2 000 and 5 000 μmol mol^{-1} (Norstadt & Porter, 1984) or even higher. In artificial culture systems aerated with ambient air, the concentration of rhizosphere dissolved inorganic carbon (DIC) in the form of CO_2 and HCO_3^- (CO_3^{2-} < 0.1% of DIC if pH < 7.5) is often less than 1 000 μmol mol^{-1}. Elevated rhizosphere DIC concentrations (5 000 μmol mol^{-1}) have been shown to increase the growth of plants (Vapaavuori & Pelkonen 1985; Bialczyk, Lechowski & Libik, 1994; Bialczyk, Lechowski & Libik, 1996), especially those exposed to salinity (Cramer & Lips, 1995) or grown under high light intensity (Cramer & Richards, 1999). This may be related to increased root assimilation of DIC (Cramer & Lips, 1995), influences of DIC on NO_3^- uptake (Cramer, Savidov & Lips, 1996), decreases in root respiration measured as CO_2 flux (Qi, Marshall & Mattson, 1994; van der Westhuizen & Cramer, 1998) or alteration in the partitioning of C and N to organic acid and amino acid synthesis. Assimilation of DIC from nutrient solutions by plant roots has been widely observed (Splittstoesser, 1965; Vapaavuori & Pelkonen, 1985; Vuorinen *et al.*, 1992; Cramer & Lewis, 1993).

Phosphoenolpyruvate carboxylase (PEPc), catalysing the synthesis of oxaloacetate from PEP and HCO_3^-, is an ubiquitous enzyme in plants which may function in the anaplerotic replenishment of TCA cycle intermediates, NAD(P)H generation, re-fixation of respiratory CO_2 (Vuorinen & Kaiser, 1997), as a pH-stat or for the provision of carboxylates to maintain ionic balance in the xylem sap (Schweizer & Erismann, 1985; Arnozis, Nelemans & Findenegg, 1988). Spontaneous pH-dependant inter-conversion of CO_2 and HCO_3^- is slow and carbonic anhydrase is required to catalyse the reaction (Hatch & Burnell, 1990; Raven & Newman, 1994). In tomato plants the assimilation of DIC into acid stable products was increased 10-fold by 14-fold higher rhizosphere DIC concentrations (Cramer & Lips, 1995). Although this increased assimilation of DIC could be expected to result in increased activity of PEPc, this was not found to be the case in barley (Cramer *et al.*, 1996).

Ammonium nutrition and salinity result in diversion of the products of $DI^{14}C$ assimilation into amino acid synthesis, whereas NO_3^- nutrition diverts $DI^{14}C$-derived ^{14}C into organic acids (Cramer & Lewis, 1993; Cramer *et al.*, 1995). In salinity-treated plants, assimilation of DIC into amino acids was increased by elevated rhizosphere DIC concentrations (Cramer & Lips, 1995). Since elevated rhizosphere DIC concentrations promoted growth of salinity-treated plants, it was concluded that elevated DIC could ameliorate salinity stress by supplying anaplerotic C for assimilation of NH_4^+, the reduction product of NO_3^-, into amino acids.

Uptake of NO_3^- has been shown to be increased by elevated rhizosphere DIC concentrations (Cramer, Lewis & Lips, 1993; Cramer *et al.*, 1996). DIC fixation may increase the assimilation of NH_4^+ into amino acids in the roots as a consequence of the improved supply of anaplerotic C. This was likely to be more significant in plants which reduce and assimilate NO_3^- into amino acids in the roots which is the case in salinity-treated plants (Siddiqi *et al.*, 1990). Nitrate uptake is inhibited by salinity through a restriction of the activity of the NO_3^- transporter, which is more sensitive to Cl^- than to Na^+ (Aslam, Huffaker & Rains, 1984). Increased uptake of NO_3^- by salinity-treated plants supplied with elevated rhizosphere DIC (Cramer & Lips, 1995) may be attributed to greater utilisation of N for the synthesis

of amino acids and translocation of these to the shoot. Translocation of these amino acids to the shoot may remove feedback inhibition of NO_3^- uptake. In barley, however, the effect of DIC on NO_3^- uptake was independent of the activity of nitrate reductase (Cramer *et al.*, 1996). The effect of DIC on NO_3^- uptake may thus be through electrochemical changes across the root plasmalemma due to cytosolic CO_2 and HCO_3^- inter-conversion or exchange of HCO_3^- for NO_3^-. Whatever the mechanism, increased uptake of NO_3^- is likely to place additional demands for the supply of anaplerotic C within the root.

Although assimilation of DIC through PEPc activity in the root is responsible for only a small contribution to the C budget of the whole plant, the rate of assimilation is a significant proportion of the C budget of the root. DIC assimilation could occur at rates equivalent to 30% of the rate of respiration in plant roots exposed to 5 000 μmol mol^{-1} CO_2 (Cramer & Lips, 1995). DIC has been shown to influence respiration rate (Qi *et al.*, 1994) which will have significant consequences for the physiology of the roots.

We investigated the physiological changes associated with enhanced uptake of NO_3^- in plants supplied with elevated CO_2 together with salinity stress. The benefit of enriched DIC supply for the uptake of NO_3^- could be more likely to occur when the external NO_3^- concentration is low (< 0.5 mM) and when energy is required for the uptake of NO_3^-. We measured the uptake of NO_3^-, accumulation of NO_3^- in the tissue, accumulation of total and reduced-N in the tissue and the activities of nitrate reductase (NR), PEPc, glutamine synthetase (GS) and glutamate-oxoglutarate amino transferase (GOGAT) in an attempt to further our understanding of the mechanisms through which elevated rhizosphere DIC concentrations may benefit plant growth.

Abbreviations - DIC, dissolved inorganic carbon (HCO_3^- + CO_2); IRGA, infra red gas analyser; PEPc, phosphoenolpyruvate carboxylase; NR(A), nitrate reductase (activity); GS, glutamine synthetase; GOGAT, Glutamine:2-oxoglutarate aminotransferase.

MATERIALS AND METHODS

PLANT MATERIAL

Twenty-day-old seedlings of *Lycopersicon esculentum* (L.) Mill. cv F144 grown on vermiculite were transferred into nutrient solutions after carefully rinsing the roots with deionised water. The hypocotyls of the plants were wrapped with foam rubber and the plants inserted through holes in the lids of tanks (12 plants per tank) containing 130 l half-strength Long Ashton nutrient medium (Hewitt, 1966), which was modified to contain 0.5 mM $NaNO_3$ and 60 mg l^{-1} sodium ferric ethylenediamine di-(o-hydroxyphenylacetate), known under the trade-name 'Fe-sequestrene 138' (Ciba-Geigy, Petah Tikva, Israel), as the iron source. After transfer of the plants into nutrient solutions, the concentration of NaCl was increased at a rate of 50 mM d^{-1} to reach a final concentration of 100 mM. Controls had no NaCl added. The plants were utilised 7 to 10 days after transfer into hydroponics.

Carbon dioxide was supplied to the roots at elevated levels (5 000 μmol mol^{-1}) by enriching ambient air with pure CO_2 through a needle valve. Control plants received aeration with ambient air. The CO_2 concentration was monitored continuously with an APPA-3 infrared gas analyser (Anarad Inc., Santa Barbara, CA, USA). The excess CO_2 was flushed from below the lids of the containers with a high flow rate of ambient air and a fan was used to maintain air movement over the plant shoots. The air sampled routinely from above the lids of the hydroponic containers with an ADC LCA2 infrared gas

analyser (Analytical Development Corporation, Hoddesdon, UK) was found not to differ from ambient CO_2 concentrations.

NITRATE UPTAKE

Five plants of each treatment were transferred into 200 ml fresh half-strength Long Ashton nutrient solution containing 5 mM MES (pH 6.5) and 1 mM $NaNO_3$. The solutions were aerated with either 360 or 5 000 μmol mol^{-1} CO_2 at a flow rate sufficient to ensure thorough mixing of the nutrient solutions. The pH of the solutions did not vary over the uptake period.

The plants were equilibrated for a period of 6 h prior to the beginning of the experiment at 28 ± 2°C with an irradiance of about 450 μmol m^{-2} s^{-1}. Sub-samples of 1 ml were taken at 30 min intervals for 6 hours from the nutrient solution. The volume of the solution was determined gravimetrically. At the end of the uptake experiments, the plants were removed from the cups, and the volume of the remaining solution determined. The plant roots were rinsed in distilled water, blotted dry and weighed.

The concentration of NO_3^- in the nutrient solutions was measured using the dissimilatory NR enzymatic method of McNamara *et al.* (1971) modified to use 0.6 M Na-formate as a source of reductant. The concentration of K^+ in the nutrient solutions was determined using a Corning 410 Flame Photometer (Halstead, UK). The changes in the concentrations of NO_3^- and K^+ were corrected for the decrease in volume of the nutrient solution over the uptake period, which was assumed to be a linear function of time.

Tissue concentrations of NO_3^-, total-N and reduced-N

The oven-dried plant material of each treatment was milled in a Wiley mill (A. H. Thomas, Philadelphia, PA, USA) using a 60 mesh screen. Samples of 50 mg material were digested in 35-cm long tubes with 4 ml 3.4% (w/v) salicylic acid in 13.5 M sulphuric acid with 0.2 g selenium. The samples were digested at room temperature for 2 h, 200°C for 1 h, 270°C for 1 h and 370°C for 1 h. The digest was diluted and assayed for NH_4^+ (Solorzano, 1969).

Tissue NO_3^- concentrations were determined by killing a homogeneous sample of about 0.3 g (FW) of tissue in liquid N_2. The sample was suspended in 10 ml distilled water and extracted in a water bath at 80°C for 2 h. Each extract was mixed and sub-samples of 1 ml centrifuged at 1 300 g for 5 min; the NO_3^- concentration of the supernatant was determined using the modified dissimilatory NR enzymatic method of McNamara *et al.* (1971). Reduced-N concentration was calculated by subtracting the NO_3^- concentration from the total-N concentration.

IN VITRO NR, PEPc, GS AND GOGAT ASSAYS

Five plants of each treatment were harvested between 10.00h and 12.00h and the roots washed in deionised water. The leaf and root components were sliced into 1 to 2 mm sections and the samples were snap-frozen in liquid N_2 prior to storage at -80°C. Leaf and root soluble proteins were extracted by homogenising the frozen material in a pre-cooled mortar in a ratio of 0.5 g fresh weight to 4 ml ice-cold extraction buffer. All extracts were centrifuged at 12 000g in a Sorvall RC-5 refrigerated centrifuge at 4°C for 10 min. Enzyme assays were conducted using the supernatant.

The extraction medium for NR (NADH-nitrate reductase, E.C. 1.6.6.1) contained 200 mM Tris-HCl (pH 7.8), 2 mM EDTA, 3 mM dithiothreitol, 2% (w/v) casein, 10% glycerol (v/v) and 0.1 g polyvinylpyrrolidone per sample. The reaction mixture for NR contained 30 mM phosphate buffer, 50 mM Tris-HCl (pH 7.5), 25 mM KNO_3 and 0.4 mM NADH in 200 μl. The reaction was initiated by the addition of 100 μl enzyme extract and incubated at 30°C for 10 min. The reaction was stopped by

addition of the reagents for NO_2^- determination (Snell & Snell, 1949). The samples were centrifuged at 4 000 g for 5 min and the absorbance measured. NR activity was calculated from the amount of NO_2^- accumulated.

The extraction buffer for GS (EC 6.3.1.2), PEPc (EC 4.1.1.31) and GOGAT (EC 2.6.1.53) contained 100 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 3 mM dithiothreitol, 1 mM reduced glutathione, 10% (v/v) glycerol, 1% (w/v) of bovine serum albumin and 0.2 g polyvinylpyrrolidone per sample. GS activity was measured through the accumulation of glutamyl-hydroxamate and root GOGAT activity was measured by following NADH oxidation at 340 nm according to the methods of Lea (1985). PEPc activity was measured at 340 nm by coupling the reactions to NADH-oxidation (Cramer *et al.*, 1996).

Statistical analysis

Results were subjected to analysis of variance (ANOVA) to determine the significance of differences between the responses to the treatments. Fisher's protected least significant difference (LSD) tests (95%) were conducted to determine the differences between the individual treatments using Statgraphics Ver. 7.0 (1993). For each set of data the different plant components (leaf, stem and root) were tested separately. Different letters on the graphs indicate significantly different results, but do not indicate differences between plant components. Two-way ANOVA was followed by calculation of significance of interaction between rhizosphere DIC concentration and the level of salinity.

RESULTS

There was a significant decrease in the water contents of the leaves ($P<0.001$) and stems ($P<0.001$) of the salinity-treated plants in comparison to the control plants (Figure 1). In two-way ANOVA there was a significant effect of the rhizosphere DIC on both stem ($P=0.040$) and root ($P=0.017$) water contents. Increased water contents have been noted previously in tomato plants supplied with elevated DIC and grown at 4 mM NO_3^- (Cramer & Richards, 1999). The stems of the salinity-treated plants had considerably lower water contents than the controls. However, since in this paper no stem tissue was measured, no misinterpretation of the data took place by expression of enzyme activities on the basis of fresh weight.

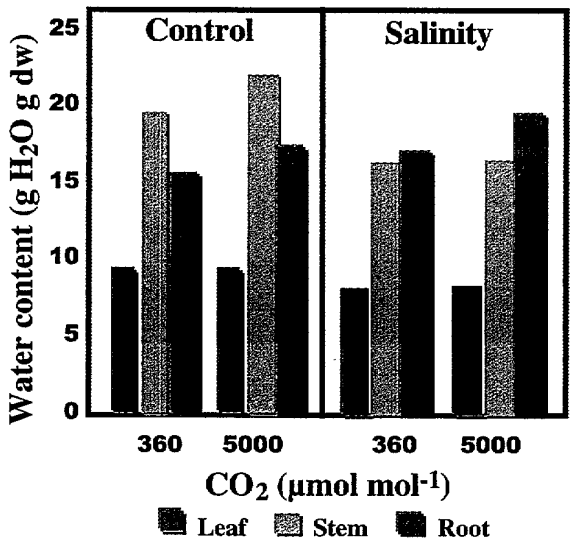


Figure 1 Typical water contents (g H_2O g^{-1} FW) of the leaf, stem and root tissues of control and salinity-treated plants grown in hydroponic culture aerated with either 360 or 5 000 µmol mol^{-1} CO_2. Different letters indicate significant ($P>0.05$, $n=5$) differences between means determined from ANOVA followed by Fisher's protected LSD tests (leaf, stem and root components were tested separately). There was no significant interaction between CO_2 concentration and salinity in two-way ANOVA for the leaf ($P=0.993$), stem ($P=0.080$) or root ($P=0.698$) components.

In control plants, elevated DIC resulted in a 19% increase in NO_3^- uptake from nutrient solutions initially containing 1 mM NO_3^- in comparison to plants supplied with ambient DIC concentrations (**Figure** 2).

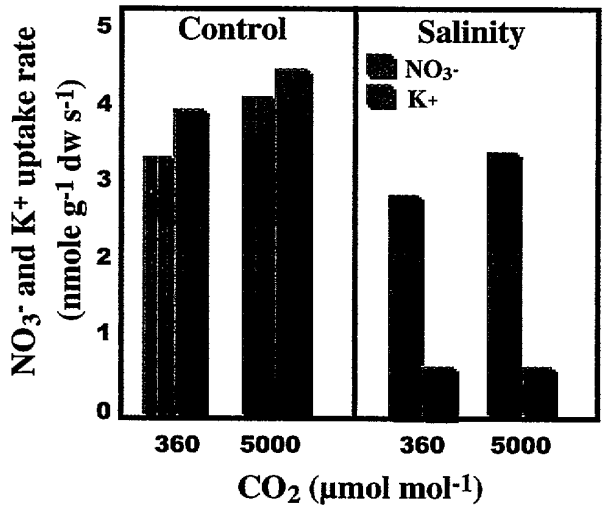


Figure 2 The rate of NO_3^- and K^+ uptake (nmol g^{-1} root FW s^{-1}) from the nutrient medium and the ratio of NO_3^-:K^+ uptake (values above bars) of control and salinity-treated plants aerated with either 360 or 5 000 µmol mol^{-1} CO_2. Different letters indicate significant (P>0.05, n=5) differences between means determined from ANOVA followed by Fisher's protected LSD tests (NO_3^- and K^+ uptake rates tested separately). There was no significant interaction between CO_2 concentration and salinity in two-way ANOVA for the either the NO_3^- (P=0.897) or K^+ (P=0.233) uptake rates or for the ratio of NO_3^-:K^+ uptake (P=0.804).

The effect of DIC on NO_3^- uptake in salinity-treated plants was not statistically significant. However, two-way ANOVA showed that both rhizosphere DIC (P=0.015) and salinity (P=0.039) had a significant effect on NO_3^- uptake with no significant interaction between these two factors. Uptake of K^+ by control plants was increased by elevated DIC but salinity reduced K^+ uptake by about 91%. The concentration of NO_3^- in the leaf, stem and root tissues of the control plants were greater than in the salinity-treated plants (

Figure 3). The tissue concentration of NO_3^- was between 4- and 6-fold greater in the roots than the leaves. Higher concentrations of NO_3^- were observed in the roots of both control and salinity-treated plants supplied with elevated DIC in comparison to plants supplied with ambient DIC.

Figure 3 The concentration of NO_3^- (µmol g^{-1} root FW) of the leaf, stem and root tissue of control and salinity-treated plants grown in hydroponic culture and aerated with either 360 or 5 000 µmol mol^{-1} CO_2. Different letters indicate significant (P>0.05, n=5) differences between means determined from ANOVA followed by Fisher's protected LSD tests (leaf, stem and root components were tested separately). There was no significant interaction between CO_2 concentration and salinity in two-way ANOVA for the leaf (P=0.566), stem (P=0.809) or root (P=0.956) components.

The concentrations of total-N and reduced-N in the roots of control plants supplied with elevated DIC concentrations were higher than in plants supplied with ambient DIC. These differences were not apparent in plants grown with 100 mM NaCl. The concentrations of total-N and reduced-N of the stems were increased in salinity-treated plants in comparison to the control plants. There were significant interactions between rhizosphere DIC and the level of salinity for both total and reduced-N concentrations, indicating that salinity eliminated the effect of rhizosphere DIC on total and reduced-N.

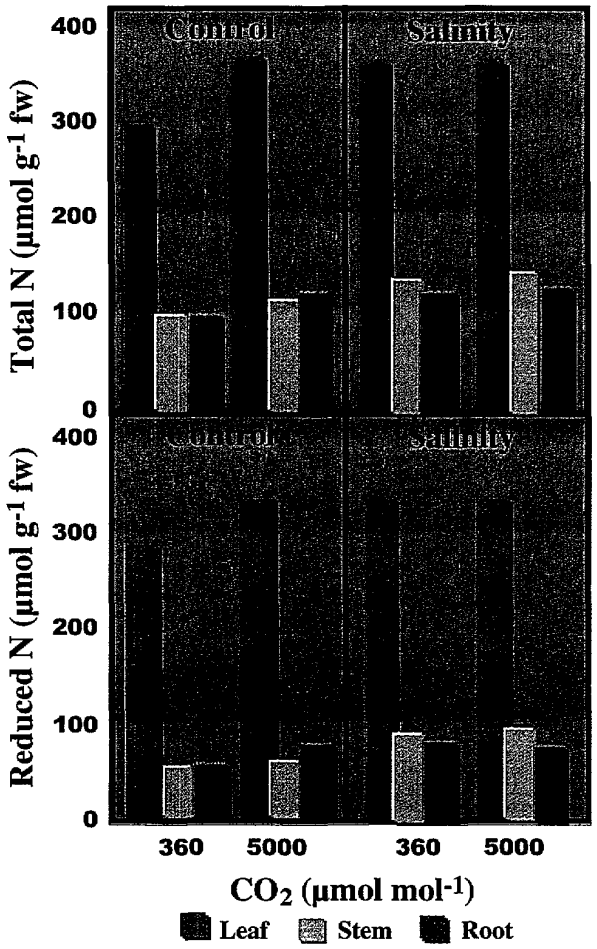


Figure 4 The concentration of (A) total-N and (B) reduced-N (µmol N g^{-1} root FW) of the leaf, stem and root tissue of control and salinity-treated plants grown in hydroponic culture and aerated with either 360 or 5 000 µmol mol^{-1} CO_2. Different letters indicate significant (P>0.05, n=5) differences between means determined from ANOVA followed by Fisher's protected LSD tests (leaf, stem and root components were tested separately). There was significant interaction between CO_2 concentration and salinity in two-way ANOVA for the root (total-N, P=0.020; reduced-N, P=0.020), but, not for the leaf (total-N, P=0.323; reduced-N, P=0.316) or stem components (total-N, P=0.862; reduced-N, P=0.893).

Leaf NRA in both control and salinity-treated plants was unaffected by the concentration of rhizosphere DIC. In contrast, root NRA of control plants supplied with enriched DIC was 46% higher than that in plants supplied with ambient DIC. This response was, however, not observed with plants grown with salinity. In two-way ANOVA salinity had a significant effect on leaf ($P<0.001$) but not root ($P=0.223$) NRA and there was significant interaction between rhizosphere DIC and the level of salinity for roots. The lower stem NO_3^- concentrations (Figure 3), higher stem reduced-N concentrations (Figure 4) and lower ratio of leaf:root NRA of plants exposed to salinity in comparison to control plants may indicate that salinity restricted NO_3^- transport to the shoot. Furthermore, in control plants elevated rhizosphere DIC triggered a partial shift in the site of NRA towards the root in comparison to plants supplied with ambient CO_2.

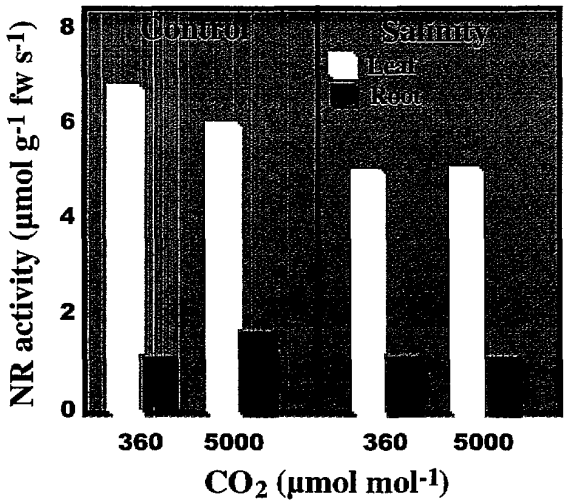


Figure 5 Nitrate reductase activity measured *in vitro* as the accumulation of NO_2^- (nmol g^{-1} FW s^{-1}) in the leaf and root components of control and salinity-treated tomato plants grown in hydroponic culture aerated with either 360 or 5 000 µmol mol^{-1} CO_2. The leaf:root ratio of NRA is shown above the bars. Different letters indicate significant ($P>0.05$, $n=5$) differences between means determined from ANOVA followed by Fisher's protected LSD tests (leaf and root components were tested separately). There was significant interaction between CO_2 concentration and salinity in two-way ANOVA for the root ($P=0.028$) and leaf:root ratios ($P=0.005$), but not for the leaf tissue NRA ($P=0.691$).

There was no effect of DIC concentration or salinity on leaf PEPc activity in either control plants or salinity-treated plants. Root PEPc activity was not affected by DIC concentration, although, root PEPc activity was higher in salinity-treated plants than in control plants. There was, however, a significantly greater leaf:root ratio of PEPc activity in the control plants supplied with ambient DIC relative to plants supplied with enriched DIC.

Root GS activity was considerably lower than leaf GS activity. Leaf GS activity in control plants was 23% higher in plants supplied with enriched DIC than in plants receiving ambient DIC. The activity of GS in the roots of the control plants was also increased by elevated DIC. GS activity in the roots of salinity-treated plants was considerably greater than that of the corresponding control plants. The GS activity in the roots of salinity-treated plants supplied with elevated DIC concentrations were about 20% lower than in the plants supplied with ambient DIC concentrations. The leaf:root ratio of GS activity was significantly lower for salinity-treated plants than for control plants. Furthermore, DIC concentration had a significant effect on the leaf:root ratio of GS activity in salinity-treated plants. Two-way ANOVA revealed significant interactions between DIC and salinity treatments for both leaf and root GS activities indicating that salinity modified the response of Gs to elevated DIC.

Rhizosphere DIC concentration had no effect on the GOGAT activity in the roots of control plants. In salinity-treated plants the activity of GOGAT in the roots of plants supplied with ambient DIC

concentrations was 47% lower than that of the salinity-treated plants supplied with elevated rhizosphere DIC concentrations and 43% lower than the activity in control plants.

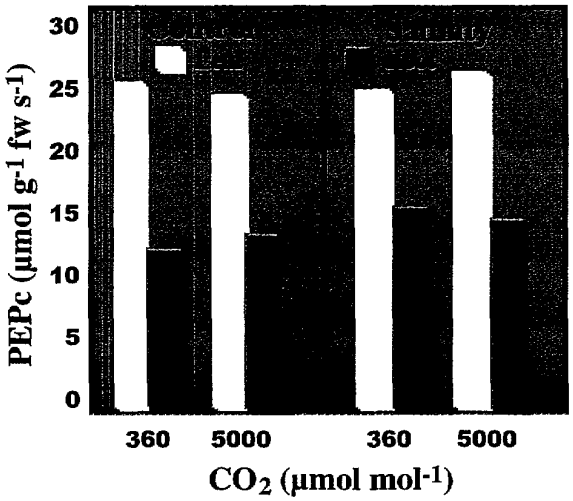


Figure 6 Phosphoenolpyruvate carboxylase activity (nmol g^{-1} FW s^{-1}) measured *in vitro* in the leaf and root components of control and salinity-treated tomato plants grown in hydroponic culture and aerated with either 360 or 5 000 µmol mol^{-1} CO_2. The ratio of leaf:root PEPc activity is shown above the bars. Different letters indicate significant (P>0.05, n=5) differences between means determined from ANOVA followed by Fisher's protected LSD tests (leaf and root components were tested separately). There was significant interaction between CO_2 concentration and salinity in two-way ANOVA for the leaf:root ratios (P=0.033), but not for the leaf (P=0.060) or root (P=0.120) PEPc activities.

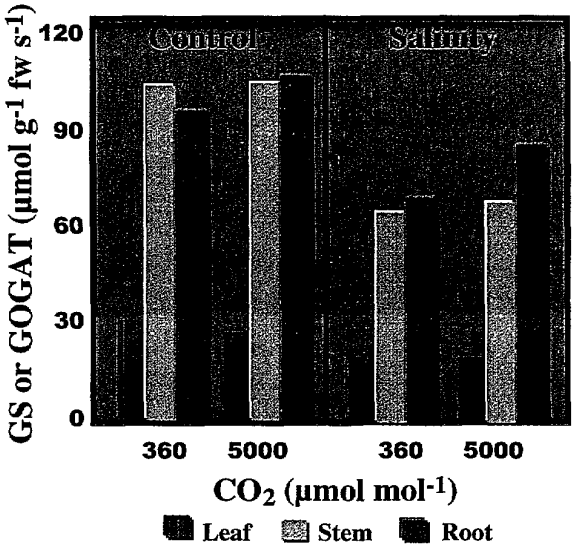


Figure 7 *In vitro* leaf and root GS and root GOGAT activity (nmol g^{-1} FW s^{-1}) of control and salinity-treated tomato plants grown in hydroponic culture and aerated with either 360 or 5 000 µmol mol^{-1} CO_2. The ratio of leaf:root GS activity is shown above the bars. Different letters indicate significant (P>0.05, n=5) differences between means determined from ANOVA followed by Fisher's protected LSD tests (leaf and root components were tested separately). For GS activity there was significant interaction between CO_2 concentration and salinity in two-way ANOVA for the leaf (P=0.032) and root (P=0.0003) GS activities, but not for the leaf:root ratios of GS activity (P=0.110). For GOGAT activity there was no significant interaction (P=0.058) between CO_2 concentration and salinity in two-way ANOVA.

DISCUSSION

Assimilation of DIC by roots influences both C and N metabolism within the root. We can thus expect a cascade of metabolic and enzymatic responses to changes in rhizosphere DIC concentration depending on factors such as N and DIC concentration and form, salinity and other environmental factors which influence C metabolism. Some of the physiological changes observed in this study confirm trends observed previously for tomato plants (Bialczyk *et al.*, 1994; Cramer *et al.*, 1995). However, in this investigation the concentration of NO_3^- in the hydroponic solutions was considerably lower than that used previously, starting at 0.5 mM and declining to about 0.1 mM over a 7 d period (data not shown) prior to replacement of the nutrient solutions. Under these conditions NO_3^- uptake is likely to be growth limiting and require energy expenditure.

Uptake of NO_3^- was slightly stimulated by elevated rhizosphere DIC concentrations (**Figure** 2), confirming previous results. DIC assimilation may enhance incorporation of N into amino acids in the roots as a consequence of a greater supply of anaplerotic C (Cramer *et al.*, 1993). The increased uptake of NO_3^- and DIC assimilation into amino and organic acids may have contributed to the significant increase in stem and root water contents in plants supplied with elevated DIC due to increased tissue osmotic potentials. In previous work the stimulation of NO_3^- uptake by DIC was greater in salinity-treated plants than in control plants (Cramer & Lips, 1995). That this did not occur in this investigation may be ascribed to the low concentration of NO_3^- supplied to the plants or the fact that the uptake measurements were performed under relatively low-irradiance (450 μmol m^{-2} s^{-1}) controlled temperature conditions and also at a higher NO_3^- concentration than that used for growth, in contrast to the experimental conditions used by Cramer & Lips (1995). However, elevated DIC has been shown to elicit a greater increase in NO_3^- uptake in non-salinised plants supplied with 0.5 mM NO_3^- than at either 2 or 4 mM NO_3^- (van der Merwe & Cramer, unpublished). The positive effect of elevated DIC on NO_3^- uptake in barley grown with NH_4^+ nutrition was independent of NRA and thus of anaplerotic C provision for N assimilation (Cramer *et al.*, 1996). This led to the hypothesis that elevated DIC caused changes in the electrochemical conditions across the root plasmalemma resulting in increased anion and decreased cation uptake. The fact that K^+ uptake was increased by DIC concentration does not support this hypothesis (**Figure** 2). It was previously found that K^+ uptake rates were not significantly influenced by the concentration of rhizosphere DIC, although, there was a significant influence of DIC on the concentration of K^+ in the tissue and in the xylem sap (Cramer & Lips, 1995). Vuorinen (1997) has reported no effect of elevated DIC on K^+ uptake in birch but significant stimulation of K^+ uptake in willow. The effect of DIC on NO_3^- and K^+ uptake is thus probably mediated by mechanisms other than changes in the electrochemistry across the root plasmalemma. The increased uptake and translocation of K^+ may be associated with uptake and translocation of NO_3^- as proposed by Ben-Zioni, Vaadia & Lips (1971). The large decrease in the rate of K^+ uptake in the salinity-treated plants is probably due to the competitive displacement of K^+ by Na^+.

Increased uptake of NO_3^- in plants supplied with elevated DIC was associated with higher concentrations of NO_3^- in the root tissue (

Figure 3). Lower tissue NO_3^- concentrations in the salinity-treated plants compared to the control plants could result from the interference of salinity with NO_3^- uptake (Aslam *et al.*, 1984) or greater root NRA. In control plants, higher total and reduced-N concentrations were found in the roots of control plants supplied with enriched DIC than in plants supplied with ambient DIC (Cramer & Lips, 1995). This was probably due to both the increased uptake of NO_3^- and the increased supply of anaplerotic C from DIC assimilation for N assimilation. However, changes in NO_3^-, total and reduced-N were relatively small, reflecting the limited supply of NO_3^- available from the nutrient medium. Salinity-treatment resulted in higher concentrations of reduced-N in the roots and stems compared to control plants. As in previous work (Siddiqi *et al.*, 1990; Cramer *et al.*, 1995), salinity resulted in a partial shift of the site of NO_3^- reduction from the being mainly shoot-based to greater root participation. The evidence for this is derived from lower concentrations of NO_3^- in the stems and reduced leaf:root ratios of NRA. The larger proportion of reduced-N in the stem tissue may reflect greater xylem transport of

reduced-N as found previously (Cramer & Lips, 1995), although stem reduced-N concentrations may be largely associated with phloem sap (Jeschke & Pate, 1992). It was previously found that root *in vivo* NRA was greater in plants treated with 200 mM NaCl in comparison to either 100 mM NaCl-treated or control plants, which had similar NRA (Cramer *et al.*, 1995). *In vivo* NR measurements for roots are of dubious value due to the lack of any interference with NO_2^- reduction in this system. *In vitro* NRA, as measured in this investigation, indicates potential NRA, but not the actual *in situ* NRA which is limited by substrate availability and under regulation through phosphorylation (Glaab & Kaiser 1995). Activation of NR through dephosphorylation of the NR enzyme is stimulated by cytoplasmic acidification (Kaiser & Brendle-Behnisch, 1995). Elevated DIC may cause partial acidification of the root cytoplasm through CO_2 hydration to HCO_3^-, thus stimulating NRA (Mengel, Robin & Salsac, 1983).

Despite changes in N metabolism with elevated DIC, there was no effect of DIC concentration on *in vitro* PEPc activity with altered rhizosphere DIC concentrations, although the distribution of PEPc activity between the leaf and root was significantly altered. The *in vivo* activity of PEPc measured by the incorporation of ^{14}C into acid-stable products is decreased by salinity and increased by elevated DIC concentrations (Cramer & Lips, 1995). Vuorinen & Kaiser (1997) have shown that the "potential" *in vivo* activity of PEPc measured at high DIC concentrations does not change greatly with elevated DIC in comparison to ambient DIC. These authors did, however, show a more than 2-fold increase in "potential" *in vivo* PEPc activity in plants grown with 0.74 mM $KHCO_3$ in comparison to plants grown with ambient DIC concentrations. Furthermore, the *in vitro* activity of PEPc (Figure 6) was at least an order of magnitude greater than the *in vivo* activity of this enzyme in willow, barley (Vuorinen & Kaiser, 1997) and in tomato (Cramer & Lips, 1995) indicating that the level of PEPc is not a limiting factor for the assimilation of DIC from the root medium. The activity of this enzyme is either regulated or limited by other processes *in vivo* (Turpin, Weger & Huppe, 1997).

The roots of plants grown under saline conditions had significantly higher *in vitro* PEPc activities than control plants, although, previous work has shown a reduction of $DI^{14}C$ assimilation with salinity (Cramer *et al.*, 1995). Similar enhancement of *in vitro* root PEPc activity by salinity was observed with plants supplied with 4 mM NO_3^- (data not shown). The difference between the results obtained with the *in vitro* and *in vivo* estimates of PEPc activity may arise from stimulation by salinity of the respiratory release (Cramer *et al.*, 1995) of $^{14}CO_2$ during the *in vivo* assay. Increased root N assimilation in salinity-treated plants (Cramer *et al.*, 1995) and in plants supplied with elevated DIC would require an increased provision of C skeletons for assimilation of NH_4^+ into amino acids and higher anaplerotic PEPc activity. For every 2-oxoglutarate transaminated by GS/GOGAT to glutamate, one oxaloacetate produced via PEP carboxylation and one acetyl-CoA must enter the TCA cycle. Thus two additional molecules of PEP are required to enter the TCA cycle for the synthesis of glutamate (Turpin *et al.*, 1997). Salinity induces the accumulation of proline up to 200-fold that in control plants (Stewart & Larher, 1980, Berteli *et al.*, 1995). Synthesis of proline from glutamate (Ireland, 1997) may depend on the anaplerotic provision of C by PEPc. Furthermore, oxaloacetate derived from carboxylation of PEP may be directly transaminated and transamidated to yield aspartate and asparagine, respectively (Cramer *et al.*, 1993).

Elevated DIC concentration stimulated GS activity in the roots and leaves of control plants, but had no effect on root GOGAT activity. The increased GS activity probably reflects the increased uptake and assimilation of NO_3^- with elevated DIC. The lack of correlation between the response of GS and GOGAT activities to elevated DIC indicates that the GS and GOGAT activities are not tightly coupled. This is consistent with the fact that the main amino acids synthesised from root $DI^{14}C$ assimilation are glutamate, glutamine, aspartate and asparagine (Cramer *et al.*, 1993). The roots of salinity-treated plants had higher GS activity, although, elevated DIC concentrations caused a significant decrease in root GS activity of these plants. Very similar results for GS activities were obtained with a set of plants grown on 4 mM NO_3^- (data not shown). The lower leaf:root ratios of GS in salinity-treated than in control plants provides further evidence for the shift of N reduction and assimilation towards the roots. In contrast to GS, the activity of GOGAT was significantly higher in the roots of salinity-treated plants supplied with elevated DIC as compared to plants with ambient

DIC. Bourgeais-Chaillou, Perez-Alfocea & Guerrier (1992) found similar magnitude changes in root GOGAT activity in soyabean in response to salinity. Other authors have also shown a lack of correlation between the responses of GS and GOGAT activity to salinity. In detached tomato leaves, Berteli *et al.* (1995) found a reduction in GS activity and an increase in GOGAT activity in response to salinity.

Changes in GS and GOGAT activity observed in this investigation may be related to the requirement for C to meet the additional demands resulting from enhanced root-based reduction and assimilation of NO_3^-, the transamination of oxaloacetate and transamidation of aspartate to asparagine which may accumulate under salinity stress (Venekamp, 1989), or possibly for the synthesis of proline. With salinity the plants may experience a shortage of 2-oxoglutarate in the root due to high rates of TCA cycle activity, as shown by high rates of respiratory CO_2 loss. This may limit GOGAT activity while the root has to meet the challenge of NH_4^+ derived from NO_3^- reduction, which tends to maintain high GS activity. High GS relative to GOGAT activity may also result in enrichment of xylem sap with glutamine rather than glutamate, which is typical of plants supplied with NH_4^+ rather than NO_3^- nutrition (Cramer *et al.*, 1993). Further studies are needed to elucidate the interactions between salinity, DIC and amino acid synthesis.

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IV. Physiological and biochemical basis of salt tolerance in maize

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Abstract

The ability of crops to tolerate saline environment has gained much attention due to the imperative demands for higher crop productivity and optimal land utilization. Understanding the underlying mechanism for salt tolerance is a key step for breeding/engineering crops suitable for brackish areas. The present study elucidates the mechanism of salt tolerance in corn, focusing on the dry matter production, ion uptake and activity profile of nitrate reductase (NR) and phosphoenolpyruvate carboxylase (PEPC).

Screening for salt tolerance at the seedling and reproductive stages of growth identified 12 tolerant, 2 moderately tolerant and 6 sensitive entries. Comparison between a salt tolerant Pi 31 and a salt-sensitive (Pi 11) revealed significant differences in root growth, Cl^- and Na^+ uptake and NR and PEPC activities between the two inbreds. Extending the analysis to 4 inbreds (2 tolerant and 2 sensitive) generated differential response to NaCl-salinity between the two sensitive inbreds while the tolerant inbreds consistently exhibited low Na^+ uptake combined with high K^+ uptake. Better growth performance of tolerant inbreds indicated their ability to withstand higher salt concentration than the sensitive inbreds. Significantly high root NR activity was observed in Pi 31 while Pi 21 gave the highest leaf NR activity. High K^+ in the cell was suggested to counteract he ionic imbalance due to excess Na^+ and Cl^- ions while elevated activities of NR and PEPC were postulated to provide a mechanism for the enhance assimilation of nitrogen and production of organic acids in the roots of plants growing under saline conditions.

Keywords: corn, salt tolerance, ion uptake, nitrate reductase, phosphoenolpyruvate carboxylase

Introduction

Salinity is a widespread phenomenon and it was reported that 344 million ha of land are affected by salinity in the world. Of this area, 230 million ha are not extremely saline and are potentially available for crop production of salt-tolerant species. The inhibition of growth under saline conditions may be due to water and osmotic stresses, specific ion toxicity and or ionic imbalance acting on biophysical and metabolic processes of growth (Greenway and Munns, 1980). Na^+ or Cl^- uptake is often associated with the salt-induced reduction in plant growth.

As salinity increases to higher levels most metabolic functions of the plants are affected. Nitrate assimilation is a major anabolic process in photosynthetic metabolism and has considerable influence on plant growth and protein content. Nitrate reductase [NR; EC 1.6.6.1], the first enzyme of the nitrate assimilation pathway, was found to be inhibited by chloride ions (Leidi et al., 1991). Phosphoenolpyruvate carboxylase [PEPC; EC 4.1.1.31] catalyzes the B- carboxylation of PEP using HCO_3^- as a substrate in a reaction, which yields oxaloacetate and Pi. The connection of PEPC to nitrogen metabolism and its contribution to N assimilation has been well established by previous studies (Sugiharto and Sugiyama, 1992; Suzuki et al., 1994). Feeding corn leaves with $NH4^+$ or NO_3^- increased the levels of C_4 PEPC protein and mRNA via cytokinin-dependent stimulated transcription of C_4 PEPC gene. NR and PEPC has been implicated major components of salt tolerance in some species (Piepenbrock and Schmitt, 1991; Cushman et al., 1990; Sagi et al., 1998; Omarov et al., 1998).

The ability of crops to tolerate saline environment has gained much attention due to the imperative demands for higher crop productivity and optimal land utilization. Much effort has been done toward understanding the mechanisms of plant tolerance with the eventual goal of improving the performance of crop plants in saline soils (Binzel and Reuveni, 1994). These efforts continue to yield valuable knowledge about plant osmotic stress responses, cellular ion homeostasis and expression of genes associated with salt tolerance (Hurkman, 1992; Niu et al., 1993; Bohnert et al., 1995; Niu et al., 1995; Zhu et al., 1997).

The present study elucidates the mechanism of salt tolerance in corn, focusing on the dry matter production, ion uptake and activity profile of NR and PEPC.

Materials and methods

Screening for Salt Tolerance

Seedling stage. Seeds of 31 yellow corn inbred lines were obtained from the Cereals Division of the Institute of Plant Breeding, UPLB and screened for salt tolerance in three batches. The seeds were surface sterilized with commercial chlorox preparation containing 3% sodium hypochlorite for 10 minutes, rinsed thoroughly with sterile distilled water and germinated in moistened filter paper at room temperature. After four days, uniform seedlings were selected and transferred in plastic trays with 10L half-strength Long Ashton nutrient solution aerated for 6-8 hours/day. The nutrient solution was kept at pH 5.5 with H2SO4 or KOH as needed. The volume of the nutrient was maintained at 10L by the addition of distilled water. The seedlings were grown for five days in half-strength nutrient solution. Thereafter, the nutrient solution was replaced with full strength every three days until the end of the experiment.

Salinity treatment was imposed after three days. Sodium chloride (NaCl) at 25 mM was added in nutrient solution for four successive days up to a final concentration of 100mM (EC=13 dS/m) for experiment 1 and 3, and 150 mM (EC=16dS/m) for experiment 2. Seedlings were evaluated based on vigor, degree of leaf damage and overall stand two weeks after transplanting. The rating scale for salt tolerance used at seedling stage is described in Table 1.

Table 1. Tolerance rating scale used at seedling stage.

Numerical rating	Tolerance rating	Description
1	Tolerant	No visible damage, healthy plants with no stunting or wilting
2	Relatively tolerant	Leaf rolling observed in older leaves
3	Moderately tolerant	Necrosis and/or chlorosis observed on tips and margins of old leaves
4	Relatively sensitive	Necrosis and/or chlorosis observed in old leaves or premature yellowing of old leaves
5	Sensitive	Stunted growth, chlorosis and/or necrosis of both young and old leaves and in some cases death of seedlings

Reproductive stage. Twenty inbreds were selected and furrher screened for salt tolerance at the reproductive stage. Seedlinaó fro} ti... nutrient culturm solution were transplanted in #12!ãlay pots containin approximately 10-12 kg of {oil mixture. The seedlings were allowed to recoöer from stress after which, they were subjected to 100mM NaCì (EC=13dS/m) until the silking(#tage. Visual evaluation was conducted 45 daøs after transplanting. Table 2 indicates the visual scoring used at the reproductive stage.

Table 2. Tolerance rating scale used at the reproductive stage.

Numerical rating	Tolerance rating	Description
1	Tolerant	Plants able to produce tassel, silk and ears; leaves remained green
2	Relatively tolerant	Plants able to produce tassel, silk and ears; leaves become necrotic
3	Moderately tolerant	Plants produce tassel and silk but not at the same time
4	Relatively sensitive	Plants die after producing tassel and/or silk
5	Sensitive	Plants die early

Physiological and Biochemical Studies

Two experiments were set up to study physiological and biochemical traits related to salt tolerance. In Experiment 1, there were two entries, namely Pi 11 (sensitive) and Pi 31 (tolerant). In Experiment 2, the number of entries was increased to four with the addition of SMCE 21-28 (sensitive) and P1 21 (tolerant).

Seeds were surface sterilized with commercial chlorox preparation containing 3% sodium hypochlorite for 10-15 min, then rinsed several times with sterile distilled water. Pre-germinated seeds were transplanted and grown in 10 L (Experiment 1) or 20 L (Experiment 2) plastic trays with half strength Long Ashton solution for 5 days. Full strength nutrient solution was used starting on the 5th day; henceforth, changes were done every three days. Salt treatments of 0, 25, 50, 75 and 100mm NaCl were added to the medium at 25 mM increment starting from day 3. Fe+++ was added everyday, pH was adjusted to 5.5-6.0 and the volume of the solution was maintained between changes by adding distilled water. Seedlings were grown inside the greenhouse for two weeks prior to sampling.

Growth parameter measurements

Plant materials were dried at 70°C for 48 hours and the dry matter was determined for the shoot and roots. Plant height and shoot:ratio were also measured.

Ion Analysis

K^+ and Na^+ were analyzed using flame photometry, Cl^- by Mohr method. Total N was determined by Kjeldahl method.

Enzyme Assays

Nitrate reductase. Ground leaf samples were manually extracted using a glass homogenizer with 50 mM MOPS buffer, pH 7.5 containing 1 mM EDTA, 1 mM DTT and 10 uM FAD at 1.0g sample per 1.8 mL of buffer. Extraction of sample was done at 4°C. The homogenate was centrifuged at 10,000 rpm for 30 min at 4°C after which, the supernate was collected and placed over ice until assay. The reaction mixture was composed of 600 uL 50 mM MOPS, pH 7.8, 100uL 20 mM KNO3, 100 uL 0.4 mM NADH and 200 uL sample to a total volume of 1.0 mL. The reaction mixture was incubated at 27C for 10 min after which, 1 ml of 1% sulfanilic acid in 3N HCl and 1 mL of 0.2% naphthyl-diamine ethylene diHCl were added. The solution was allowed to stand for 15 min. Absorbance was read at 540 nm. NR activity was expressed as nmol/min/g sample.

Phosphoenolpyruvate carboxylase. Ground root sample was extracted manually with 50 mM Tris-Cl, pH 8.0 containing 5 mM MtgSO4, 5 mM NaHCO3 and 1 mM DTT at 1.0 g sample/1.8 mL buffer solution using a glass homogenizer. The resultant homogenate was centrifuged at 10,000 rpm at 4C and then the supernatant was collected and kept over an ice bath until assay. PEPC activity was determined by monitoring the decrease in absorbance due to NADH at 340 nm. The reaction mixture consisted of 250 uL 50 mM Tris-Cl pH 8.0, 100 uL 50 mM NaHCO3, 100 uL 50 mM MgCl2, 50 uL 4 mM NADH, 50 uL 96 units MDH, 250 uL 10 mM PEP and 200 uL enzyme extract. The last 4 components were kept at 4C while the enzyme assay was conducted at 30C. PEPC activity was expressed as nmol/min/g sample.

Data Analysis

Data were analyzed with analysis of variance for factorial design with 3 replications using SAS statistical software (SAS Institute, Cary, NC). Means were compared using DMRT.

RESULTS AND DISCUSSION

Screening for Salt Tolerance

The inbreds were grouped into three categories for ease of classification namely: tolerant (visual score of 1), moderately tolerant (visual score of 2 and 3) and sensitive (visual score of 4 and 5). The inbreds gave varying degrees of tolerance to salinity. Of the 31 entries screened at seedling stage, 14 were rated tolerant, 8 were moderately tolerant and 9 were sensitive (Table 3). From among the 20 inbreds screened at the reproductive stage, 12 were classified as tolerant, 2 as moderately tolerant and 6 were sensitive (Table 3). However, 5 inbreds which exhibited tolerance to salt stress during the seedling stage succumbed to the stress at the reproductive stage. Selected for the physiological and biochemical studies described below were four inbreds, Pi 11 and SMCE 21-28, both sensitive at seedling stage and Pi 21 and Pi 31, rated tolerant at both seedling and reproductive stage.

Table 4. Growth performance of four yellow corn inbreds under five salt levels. Plants were grown for two weeks in nutrient solution supplemented with NaCl at 0 , 25, 50,75 and 100 mM. Plant materials were dried at 70°C for 48 hr.

Inbred	mM NaCl	Plant height (cm)	Plant dry weight (g)	Shoot dry weight (g)	Root dry weight (g)
Pi 11	0	60.45 a	0.618 a	0.051 a	0.113 a
	25	50.15 b	0.446 b	0.375 b	0.071 b
	50	35.10 c	0.304 c	0.261 c	0.042 c
	75	31.75 cd	0.310 c	0.264 c	0.046 c
	100	27.45 d	0.260 c	0.228 c	0.032 c
SMCE 21-28	0	52.10 a	0.726 a	0.629 a	0.098 a
	25	42.30 b	0.556 b	0.470 b	0.086 ab
	50	32.35 c	0.414 c	0.344 c	0.064 bc
	75	26.95 c	0.312 c	0.259 c	0.053 c
	100	25.85 c	0.317 c	0.269 c	0.047 c
Pi 21	0	49.71 a	0.516 a	0.430 a	0.086 a
	25	48.65 a	0.484 a	0.400 a	0.084 a
	50	45.30 ab	0.420 ab	0.349 ab	0.071 ab
	75	39.75 b	0.355 b	0.291 bc	0.064 ab
	100	32.90 c	0.307 b	0.254 c	0.053 b
Pi31	0	50.00 a	0.664 a	0.574 a	0.090 a
	25	48.50 a	0.549 b	0.520 a	0.079 ab
	50	39.75 b	0.391 c	0.355 b	0.055 c
	75	35.40 b	0.354 c	0.302 b	0.052 c
	100	33.55 b	0.369 c	0.312 b	0.058 bc

Genetic variability in response to salt stress has been reported in corn (Lessani and Marschner, 1978; Hajibagheri et al., 1987; Cramer et al., 1990; Cramer, 1992; Nordquist et al., 1992; Alberico and Cramer, 1993; Maiti et al., 1996). In the present, study, variation in response to salinity was observed among 31 yellow corn inbreds at the seedling stage based on visual scores (i.e. seedling vigor). However, some inbreds which were tolerant or moderately tolerant during the seedling stage showed sensitivity to salinity at the reproductive stage. This differential response demonstrates the complexity of plant adaptation to salt stress. Although visual screening at the seedling stage provides benchmark information, identification of additional selection criteria criteria is of utmost importance.

Noble and Rogers (1992) suggested that selection and breeding to increase salt tolerance might be more successful is selection is based directly in the physiological mechanism or characters conferring salt tolerance. On the other hand, Hurkman (1992) stressed that identification of genes whose expression enables plants to adapt to or tolerate salt stress is essential for salt tolerance.

Physiological and Biochemical Studies

Two salt-sensitive inbreds, Pi 11 and SMCE 21-28-2-22-1-2-2-2 (SMCE 21-28) and two salt-tolerant inbreds, Pi 21 and Pi 31 were used in the following studies. The four inbreds were ranked based on vigor and leaf damage as follows: Pi 31>Pi 21>SMCE 21-28>Pi11. The results obtained in Experiment 1 were confirmed in Experiment 2. Thus, only results from Experiment 2 and those from Experiment 1 which were not covered in Experiment 2 will be presented and discussed.

Effect of increasing saline conditions on growth of developing corn

The tolerant Pi 21 and Pi 31 showed greater capacity to accumulate dry matter at higher NaCl concentrations than the sensitive Pi 11 and SMCE 21-28 (Table 4). While the tolerant lines maintained their growth terms of plant height, plant, shoot and root dry weight at 25 mM (both for Pi 21 and Pi 31) and even at 50 mM (for Pi 21), the sensitive lines started decreasing in these aspects at 25 mM. Under the extreme conditions of 100 mM NaCl, the salt sensitive lines suffered 40 to 70% decrease in plant height, plant, shoot and root weight. On the other hand, the tolerant lines exhibited from 33% to 45% reduction in plant height, plant, shoot and root weight.

In rice, the salt sensitive variety decreased by 30% in plant dry weight while the tolerant variety decreased by 25% (Faustino et al., 1996). In corn, increasing concentrations of NaCl inhibited root elongation and leaf elongation rate with the sensitive variety more severely affected by salinity than the tolerant variety (Cramer et al., 1990; Cramer, 1992). In sugar beet, growth and leaf area expansion were reduced even at very low salinity levels (Terry and Waldron, 19) while restriction of leaf expansion was (Gao et al., 1998) seen in tomato.

Table 4. Growth performance of four yellow corn inbreds under five salt levels. Plants were grown for two weeks in nutrient solution supplemented with NaCl at 0 , 25, 50,75 and 100 mM. Plant materials were dried at 70°C for 48 hr.

Inbred	NaCl mM	Plant height (cm)	Plant dry weight (g)	Shoot dry weight (g)	Root dry weight (g)
Pi 11	0	60.45 a	0.618 a	0.051 a	0.113 a
	25	50.15 b	0.446 b	0.375 b	0.071 b
	50	35.10 c	0.304 c	0.261 c	0.042 c
	75	31.75 cd	0.310 c	0.264 c	0.046 c
	100	27.45 d	0.260 c	0.228 c	0.032 c
SMCE 21-28	0	52.10 a	0.726 a	0.629 a	0.098 a
	25	42.30 b	0.556 b	0.470 b	0.086 ab
	50	32.35 c	0.414 c	0.344 c	0.064 bc
	75	26.95 c	0.312 c	0.259 c	0.053 c
	100	25.85 c	0.317 c	0.269 c	0.047 c
Pi 21	0	49.71 a	0.516 a	0.430 a	0.086 a
	25	48.65 a	0.484 a	0.400 a	0.084 a
	50	45.30 ab	0.420 ab	0.349 ab	0.071 ab
	75	39.75 b	0.355 b	0.291 bc	0.064 ab
	100	32.90 c	0.307 b	0.254 c	0.053 b

In rice, the salt sensitive variety decreased by 30% in plant dry weight while the tolerant variety decreased by 25% (Faustino et al., 1996). In corn, increasing concentrations of NaCl inhibited root elongation and leaf elongation rate with the sensitive variety more severely affected by salinity than the tolerant variety (Cramer et al., 1990; Cramer, 1992). In sugar beet, growth and leaf area expansion were reduced even at very low salinity levels (Terry and Waldron, 19) while restriction of leaf expansion was (Gao et al., 1998) seen in tomato.

The observed reduction in plant growth was attributed to diminished surface area available for photosynthesis, changes in plant water status and Na toxicity effects on leaf elongation.

Ion uptake

A clear increase in Cl- absorption at the whole plant level was seen at 25 mM NaCl compared to control in the four inbreds (Table 5). Gradual increase in the Cl- content with increasing salinization was observed in Pi 11, Pi 21 and Pi 31 while maximal Cl- content was already attained at 50 mM NaCl in SMCE 21-28. Cl- distribution followed the order: leaf blade>leaf sheath>roots. Comparable amounts of leaf blade Cl- were found among SMCE 21-28 (4.51%), Pi 21 (3.99%) and Pi 31 (4.34%) (Table 6). Cl- content in the leaf blade of the two tolerant inbreds was statistically similar to Pi 11 which showed the lowest value of 3.86%. Pi 11 had higher leaf sheath Cl- than the other inbreds. Root Cl- content did not vary among the 4 inbreds.

Table 5. Chloride content (%) at different salt levels of four yellow corn inbreds on the whole plant basis. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C for 48 h and analyzed for chloride content using the Mohr method.

mM NaCl	Inbred				
	Pi 11	SMCE 21-28	Pi 21	P1 31	S-mean
0	0.95 d	0.99 c	1.12 d	1.12 d	1.05 e
25	1.95 c	2.61 b	2.25 c	2.30 c	2.28 d
50	3.92 b	3.80 a	2.91 c	3.59 b	3.56 c
75	4.31 b	3.97 a	3.94 b	4.11 ab	4.08 b
100	5.23 a	4.29 a	4.92 a	4.38 a	4.71 a
V-mean	3.27	3.13	3.03	3.10	3.13

In a column, means followed by a common letter are not significantly different at 5% level of significance.

The two sensitive genotypes accumulated Cl^- in different parts of the plant – Pi 11 in the leaf sheath while SMCE 21-28 in the leaf blade (Table 6). The tolerant ones seemed to exclude toxic amounts of the ion.

Table 6. Chloride content (%) in the different plant parts of four yellow corn inbreds as affected by NaCl salinization. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C for 48 h and analyzed for chloride content using the Mohr method.

Inbred	Plant part			V-mean
	Leaf blade	Leaf sheath	Root	
Pi 11	3.86 b	3.45 a	2.51 a	3.27 a
SMCE-21-28	4.51 a	2.73 b	2.16 a	3.13 a
Pi 21	3.99 ab	2.82 b	2.28 a	3.03 a
P1 31	4.34 ab	2.65 b	2.31 a	3.10 a
Mean	4.17	2.91	2.32	3.13

In a column, means followed by a common letter are not significantly different at 1% level of significance.

Absorption of Na+ continually increased as NaCl was supplied to the growth medium (Table 7).

Lower Na+ content was found in Pi 21 (4.31%) and Pi 31 (4.25%) compared to Pi 11 (5.05%) (Table 8). SMCE 21-28 had similar Na+ content (4.59%) with the other 3 inbreds. The four inbreds differed significantly in Na+ in the leaf sheath and roots but not in the leaf blade (Table 8). Pi 21 and Pi 31 had the least amount of Na^+ in the roots (3.24%) and leaf sheath (3.65%), respectively. Pi 11 had the highest Na^+ content (4.84%) in the roots.

Table 7. Sodium content (%) at different salt levels of four yellow corn inbreds on the whole plant basis. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C and analyzed for sodium content using the flame photometry.

mM NaCl	Inbred				
	Pi 11	SMCE-21-28	Pi 21	P1 31	S-mean
0	0.659 d	0.396 d	0.463 c	0.499 e	0.579 e
25	4.322 c	2.868 c	3.589 c	3.308 d	3.522 d
50	6.044 b	4.842 b	5.737 b	4.698 c	5.330 c
75	6.678 b	6.228 a	6.614 a	5.721 b	6.310 b
100	7.555 a	6.608 a	6.554 ab	7.319 a	7.009 a
V-mean	5.052	4.592	4.309	4.248	

In a column, means followed by a common letter are not significantly different at 5% level of significance.

Table 8. Sodium content (%) in the different plant parts of four yellow corn inbreds as affected by NaCl salinization. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C and analyzed for sodium content using flame photometry.

Inbred	Plant part			V-mean
	Leaf blade	Leaf sheath	Root	
Pi 11	5.30 a	5.01 a	4.84 a	5.05 a
SMCE-21-28	5.43 a	4.51 a	4.03 b	4.59 ab
Pi 21	5.17 a	4.52 a	3.24 c	4.31 b
P1 31	4.92 a	3.62 b	4.17 b	4.25 b

In a column, means followed by a common letter are not significantly different at 1% level of significance.

Uptake of K^+ was apparently reduced by NaCl stress, as K^+ uptake decreased as the NaCl concentration in the medium increased (Table 9). Among the four inbreds, Pi 31 showed the least lowering of K^+ content. On the other hand, Pi 21 was more efficient in extracting K+ from the culture medium most of which was found in the roots (2.67%) (Table 10). K+ content of Pi 21, Pi 31 and SMCE 21-28 were identical. Pi 31 showed the highest leaf blade K+, whereas, Pi 11 gave the highest leaf sheath K+ which was comparable to that of Pi 21.

Table 9. Potassium content (%) at different salt levels of four yellow corn inbreds on the whole plant basis. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C and analyzed for K+ content using flame photometry.

mM NaCl	Inbred				
	Pi 11	SMCE-21-28	Pi 21	P1 31	S-mean
0	2.96 a	2.64 a	2.84 a	2.99 a	2.87
25	2.11 a	2.33 a	2.14 b	2.39 b	2.24
50	1.70 c	1.78 b	1.61 c	2.01 c	1.77
75	1.45 cd	1.37 c	1.27 d	1.57 d	1.41
100	1.36 d	1.39 c	1.13 d	1.53 d	1.35
V-mean	1.91	1.80	2.10	1.93	1.93

In a column, means followed by a common letter are not significantly different at 5% level of significance.

Table 10. Potassium content (%) in the different plant parts of four yellow corn inbreds as affected by NaCl salinization. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C and analyzed for K+ content using flame photometry.

Inbred	Plant part			V-mean
	Leaf blade	Leaf sheath	Root	
Pi 11	2.13 c	1.54 a	2.08 b	1.91 b
SMCE-21-28	2.40 b	1.09 c	1.90 b	1.80 b
Pi 21	2.25 bc	1.37 ab	2.67 a	2.10 a
P1 31	2.69 a	1.22 bc	1.79 c	1.90 b

In a column, means followed by a common letter are not significantly different at 1% level of significance.

Increasing the salt concentration in the root medium of both tolerant and sensitive inbreds increased the Na+/K+ ratio (Table 11). SMCE 21-28, a sensitive inbred had the highest Na+/K+ ration (4.28), significantly higher than that of the tolerant inbreds Pi 21 (2.99) and Pi 31 (2.93). The two tolerant inbreds had comparable Na+/K+ ratio to SMCE 21-28.

Table 11. Na+/K+ ratio at different salt levels of four yellow corn inbreds.

mM NaCl	Inbred				
	Pi 11	SMCE-21-28	Pi 21	P1 31	S-mean
0	0.226 c	0.158 d	0.161 c	0.263 b	0.202 e
25	2.159 bc	1.829 cd	1.514 c	1.328 b	1.708 d
50	3.682 ab	3.783 bc	2.516 bc	2.862 ab	3.211 c
75	5.037 a	6.177 b	4.749 ab	4.885 a	5.212 b
100	5.602 a	9.445 a	6.025 a	5.293 a	6.591 a
V-mean	3.341	4.278	2.993	2.926	3.385

In a column, means followed by a common letter are not significantly different.

Concentrations of Cl- increased rapidly in response to salt shock of 100 mM NaCl in the compared with the unsalinized roots in maize seedlings (Rodriguez et al., 1997). In contrast, Na+ in the 0-3mm root zone failed to increase at all during exposure to NaCl. This might be explained by Na+ translocation to the apical zone from the caryopsis. These authors also observed that K+ and Na+ strongly accumulated in the same zone.

Increase in K+, and Cl- were consistently higher at 120 mM NaCl that at 0 mM NaCl along the leaf axis from the leaf base in wheat (Hu et al., 1998). Sodium and Cl- concentrations were statistically significantly increased by 120 mM NaCl. Zhu et al. (1998) reported that their results indicated that the level of salt tolerance as measured by root growth correlate closely with K+ content. Because of the critical role of K+ homeostasis, relatively small variation in cellular K+ content are expected to cause large differences in plant growth. They further explained that mechanistically K+ nutrition is critical for Na+ tolerance because Na+ and K+ are chemically very similar. High concentrations of Na+

inhibit K+ absorption by plant roots. K+ plays vital roles in various aspects of plant cell growth and metabolism and is needed in large quantities. Hajibagheri et al. (1989) reported salt tolerant varieties of maize whose resistance has been related to Higher K+ fluxes and cytoplasmic concentrations and lower Na+ and Cl- fluxes and cytoplasmic concentrations while Cerda et al. (1995) reported maize cultivars which had greater capacity to exclude Na+ and Cl- from the leaves and to maintenance of higher K+/Na+ ratio.

Effects on nitrogen content of the plant

Total nitrogen content of the salt tolerant inbreds was not affected by increasing salt in the substrate while that of the salt sensitive inbreds decreased at 50mM for SMCE 21-28 and at 100mM for Pi 11 (Table 12). Variation in total N content in the leaf blade and roots of four inbreds was observed. The sensitive Pi 11 had the least amount of total N in the leaf blade and roots (Table 13).

Table 12. Total nitrogen content (%) at different salt levels of four yellow corn inbredson whole plant basis. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C and analyzed for total N content using Kjeldahl method.

mM NaCl	Inbred				
	Pi 11	SMCE-21-28	Pi 21	P1 31	S-mean
0	3.09 ab	3.65 a	3.31 a	3.20 a	3.32 ab
25	3.24 a	3.43 ab	3.51 a	3.29 a	3.37 a
50	3.04 ab	3.36 b	3.30 a	3.27 a	3.24 abc
75	3.07 ab	3.31 b	3.32 a	3.18 a	3.22 bc
100	2.91 b	3.19 b	3.25 a	3.13 a	3.12 c
V-mean	3.07	3.39	3.34	3.21	3.25

In a column, means followed by a common letter are not significantly different at the 5% level of significance.

Table 13. Total nitrogen content (%) in the different plant parts of four yellow corn inbreds as affected by NaCl salinization. Seedlings were grown in nutrient solution supplemented with 0, 25,50, 75 and 100 mM NaCl for two weeks, dried at 70°C and analyzed for total N content using Kjeldahl method.

Inbred	Plant part			V-mean
	Leaf blade	Leaf sheath	Root	
Pi 11	3.51 b	2.90 a	2.80 b	3.07 c
SMCE-21-28	3.78 a	3.03 a	3.86 a	3.39 a
Pi 21	3.87 a	2.97 a	3.17 a	3.34 a
P1 31	3.97 a	2.83 a	2.84 b	3.21 b

In a column, means followed by a common letter are not significantly different at the 1% level of significance.

Increased in total N concentration due to salinity was reported in wheat (Hu et al., 1998) and rice (Faustino et al., 1996). In rice, the sensitive and tolerant varieties showed comparable total N concentrations in the leaf blade, leaf sheath and roots. The increase in total N might be due to the enhancing effect of NaCl on the synthesis of N-containing compounds in the blade. Whereas, results in wheat showed reduction in NO3- concentration at high 120 mM NaCl. The lower supply in of NO3- to growing leaves may be responsible for the inhibition of growth under saline conditions. Cram (1973) showed that net NO3- influx was reduced by Cl- concentration in the root tissue such that if the abundance of the latter increased the concentration of the former decreased.

Effects on nitrate reductase (NR) and PEP carboxylase (PEPC) activities

Leaf NR and root PEPC activities in both salt and sensitive inbreds were significantly reduced by 25mM NaCl (Fig 1a and Fig 1c). Leaf NR of the salt-tolerant Pi 31 showed higher activities than those observed from the sensitive inbred Pi 11 in most of the salt levels. A similar response was observed for root PEPC. The salinity threshold level observed for both enzymes was 25 mM NaCl wherein they showed an abrupt decrease in their activities.

Root NR of Pi 31 showed decreased activities at 25-100mM NaCl, while root NR of Pi 11 decreased at 25-75mM NaCl but markedly increased at higher salt concentrations (Fig 1c). On the other hand, leaf PEPC activities of Pi 31 and Pi 11 increased at 25 mM NaCl and then decreased at increasing salt levels until they reached stability at 50 mM and 125 mM NaCl, respectively (Fig 1d).

Results indicate that the tolerant Pi 31 can withstand a wider range of salinity compared to the susceptible Pi 11.

Root NR of resistant Pi 31 showed the highest activity over resistant Pi 21 and sensitive Pi 11 and SMCE 21-28. Pi 21 gave lower values which were comparable to the sensitive inbreds (Fig. 2). In contrast, leaf NR of Pi 21 was highest among the 4 inbreds at 25 to 75 mM NaCl. However, increasing salt concentrations induced the activity of Pi 31 leaf NR (Fig. 2).

NaCl-salinity markedly inhibited *in situ* and *in vitro* NR activity in rice seedlings (Richharia et al., 1997). Salinity causes decreased uptake and assimilation of NO3- and leads to a decreased level of *in situ* NR activity in many species (Dubey and Pessarakli, 1995). Decreased NR activity might be due to decreased synthesis of NR protein or brought by direct inhibitory effects of salt ions on NR activity. On the other hand, Sagi et al. (1998) reported that NR and PEPC activities increased with salinity and N level in ryegrass. They noted that PEPC activity correlated positively with organic acid concentrations in both shoots and roots, irrespective of salinity level. While PEPC activity was higher in roots than in shoots, the concentration of organic acids was higher in shoots than in roots of ryegrass. It was inferred that significant amounts of organic acids produced in the roots were used as carbon skeletons for transamination reactions.

DISCUSSION

Comparison of dry matter accumulation between salt-sensitive inbreds and salt-tolerant inbreds of corn suggested adaptive mechanism of the two types during response to salt stress. During period of water

deficit at low NaCl levels, dry matter content of the tolerant and sensitive inbreds was reduced within acceptable levels. Whereas, during period of salt toxicity corresponding to exposures to high salt levels, dry matter accumulation was markedly reduced even resulting to death of the sensitive plants. In contrast, the tolerant inbreds were able to sustain root growth at higher levels of NaCl.

The severe reduction in growth may be attributed to high Na^+ and Cl^- content in the sensitive inbreds. Hajibagheri et al (1989) also reported higher Cl^- content of a salt sensitive variety of maize. Cerda et al. (1995) accounted the large growth reduction of a sensitive corn cultivar to a higher Na^+ and Cl^- concentration in the leaves compared to a tolerant cultivar. Izzo et al. (1991) reported that the ability of maize to resist the effect of salinity rest on its ability to restrict or prevent the entry of Na+ into the shoots.

The remedial effect of high K^+ content in the tissue of the tolerant inbreds on salt-induced growth reduction was proposed to be due to its action on counteracting the excess Cl^- ions (Lopez and Satti, 1996). A direct consequence of such neutralization of excess negative charge is high organic acid levels. K+ is known to play a major role in osmotic adjustment thereby increasing the driving force for water uptake. In leaves, a great deficiency in photosynthetic efficiency was observed with decreasing supply of K+ in the roots (Chow et al., 1990). Hajibagheri et al. (1989) reported that salt tolerance appears to be related to higher K^+ fluxes and high cytoplasmic concentrations. Na^+/K^+ ratio was known to regulate biochemical events and developmental processes (Greenway and Munns, 1980). Brady et al. (1984) observed that excess Na^+ in the root media may result in a passive accumulation of this ion in the root and shoot which result to a high Na^+/K^+ ratio which lead to metabolic disorders such as reduction in protein synthesis and enzymatic activities. The lower Na^+/K^+ ratio of tolerant corn inbreds could presumably moderate root and shoot biomass.

Salt stress modulates the levels of a number of gene products such as thaumatin-related polypeptides, germin-related polypeptides and mRNA transcripts associated to salt-stress. PEP carboxylase has also been implicated in salt tolerance (Piepenbrock and Schmitt, 1991; Cushman et al., 1990). Results of the biochemical study showed higher NR and PEPC activities in the tolerant corn inbred Pi 31 compared to sensitive Pi 11. The increased leaf NR activities in Pi 31 might be due to increased nitrate transport to the shoot for reduction and assimilation. This in turn resulted in vigorous growth of Pi 31 compared to the death of the salt sensitive of Pi 11. Nitrate transport is one of the earliest processes affected by salinity (Silverbush et al., 1989). NR activity is coordinated with the rate of photosynthesis and the availability of C skeletons. The latter could be supplied by the enhanced activity of PEPC. High levels of NaCl were found to reduce NR activity. As NR is a substrate inducible enzyme, its decreased activity under salinization was attributed to reduced NO3- uptake by the plants under salt stress (Lacuesta et al., 1990).

Increased root PEPC activities as a response to salt stress was reported by Cushman et al. (1989) in ice plant. They demonstrated that salt stress increased the transcription of PEPC isogene Ppc1 with additional regulation occurring at the post-translational level. The role of post-transcriptional regulation in Ppc1 expression in salt stress was further strengthened by the identification of specific DNA-binding factor interactions with this promoter that were modified by salt-stress (Cushman and Bohnert, 1992).

Another interesting observation was the difference in ion preference between the two sensitive inbreds, Pi 11 and SMCE 21-28. Pi 11 accumulated higher amounts of Na+ while SMCE 21-28 absorbed more of the Cl- ions and transported to the leaf blade.

Based on the present results, salt tolerance may be attributed to high K+ in the cell which counteract the ionic imbalance sue to excess Na+ and Cl- ions and enhanced activities of NR and PEPC which provide a mechanism for the enhanced assimilation and production of organic acids in the roots of plants growing under saline conditions.

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V. Preliminary assessment on the effect of bicarbonate on the growth of a salt-sensitive peanut variety Pn-10
Flordeliza Faustino

The objectives were to determine the response of peanut grown in bicarbonate-augmented saline medium; and to find a suitable and practical method for incorporation of bicarbonate into the growing medium.

A salt-sensitive Peanut 10 variety (isolated in AID/CDR C12-157) was used to initially assess the suitability of the growing media and the plant material for the present studies. The peanut seedlings were grown in two media, namely, sand and solution cultures.

Under normal conditions, UPL PN-10 matures at about 90-95 days after sowing. Its pod yield is about 1.8-2.5 tons per hectare; seed yield is 1.7 tons per hectare. The pod contains 2 large plump pink seeds. It is resistant to *Aspergillus flavus*. It is also highly resistant to defoliators.

Sand culture. Pre-germinated seeds were sown in plastic pots filled with acid-washed sand that was previously drained with rain water to wash off residual acid solution. At the early stage of growth, the seedlings were maintained with half-strength NO_3^- - type Long Ashton nutrient solution (pH 5.9) and alternatively irrigated with distilled water. Two-week old plants were then treated with 60 mM NaCl. A day after the addition of salt, the plants (bicarbonate-treated pots) were irrigated with 22.72 mM $KHCO_3$ which gives 0.1% CO_2 (Bialczyk and Lechowski, 1992). Incorporation of the dissolved bicarbonate was facilitated by dripping the solution from an inverted dextrose bottle with the rubber tube imbedded into the bottom part of the pot. Initial electrical condutivities (EC's) of the treatment solutions were recorded and similarly, EC's of the soil-solution samples were monitored. Number of compound leaves, flowers and pegs were counted and recorded. Harvesting was done 87 days after sowing. Plant height, number of mature and immature pods. Fresh and dry weights of roots, stem, leaves and mature/immature pods were also obtained.

Table 1. The effect of dissolved inorganic carbon on the growth of a relatively salt-sensitive peanut cultivar UPL Pn-10 grown in sand culture.

Parameter	0 mM NaCl 0% CO_2	0 mM NaCl 0.1% CO_2	60 mM NaCl 0% CO_2	60 mM NaCl 0.1% CO_2
Fresh weights, g				
Shoot	21.05	31.20	17.20	25.45
Stem	7.20	17.25	7.80	16.20
Leaves	13.85	13.95	9.40	9.25
Roots	6.95	10.90	3.95	9.80
Mature pods	6.70	9.55	9.65	8.30
Immature pods	1.25	0.60	1.40	0.70
Total biomass	35.95	53.05	31.40	44.25
Plant height, cm	39.00	31.50	26.75	33.00
Number of flowers	31	46.5	49.5	46
Number of compound leaves	37.5	41.5	37	37.5
Number of pegs	17.5	21.5	20.5	24
Number of mature pods	**4.5**	**8.5**	5.5	5.5
Number of immature pods	6.5	10	5	6.5

Hydroponic (solution) culture. Pre-germinated UPL Pn 10 seeds were transferred in pots filled with 11 liters of NO_3^- - Long Ashton nutrient solution (pH 5.9). Seedlings were allowed to establish for 9 days before commencement of the salt treatment (50 mM NaCl). 22.72 mM $KHCO_3$ was incorporated into the nutrient-salt combined solution giving 0.1% CO_2 treatment (Table 1). Plants were harvested after 23 DAS. Leaf area, fresh/dry weights of roots and shoots were measured.

A. Screening of mungbean varieties for salt tolerance

Since the laboratory has no preliminary data on the salt sensitivity of mungbean, screening was carried out to identify adequate experimental material for the project.

Hydroponic culture. Four recommended mungbean varieties, namely, Pag-asa 3, 7, 9 and 11 were evaluated for their response to different levels of NaCl-salinity: control (0 mM), mild salinity (60 mM) and high salinity (140 mM). Pre-germinated seeds were allowed to establish in trays filled with 10 liters of half strength NO_3^- - type Long Ashton nutrient solution. Salt solution was added during the first true leaf stage (14 DAS). The experiment was terminated 8 days after the first true leaf stage. The plants appeared lanky and did not adapt well to the hydroponic culture. Only visual scores of the four cultivars were taken at end of experiment.

Sand culture. The four cultivars used in the above screening were also used to determine the combined effects of salinity and bicarbonate employing sand culture as in the previous experiment with peanut. Mungbean seeds were directly seeded in the trays with sand. The salinity levels were 0, 50 and 100 mM NaCl and the bicarbonate levels were 0 and 22.72 mM $KHCO_3$ (0.1% CO_2). The seedlings were initially irrigated with 1/4 strength strength NO_3^- - type Long Ashton nutrient solution and salt treatment was applied 14 DAS in 20 mM NaCl-increment. The bicarbonate solution was incorporated a day after the last salt increment. EC and pH of the soil solutions were monitored through drippings collected; salt and/or nutrient solutions were added as deemed necessary. Visual ratings were done whenever differences in response to treatments were observed. Plant were harvested 38 DAS. Fresh and dry weight of roots and shoots were measured

Table 2. Preliminary evaluation on the effect of dissolved inorganic carbon on biomass accumulation in four mungbean varieties grown in salt using sand culture (unreplicated trial).

Treatments	Fresh Weight, g			Dry Weight, g		
	Shoot	Root	Plant	Shoot	Root	Plant
50 mM NaCl No added HCO_2^-						
Pag-asa 3	5.13	0.93	6.06	0.67	0.12	0.79
Pag-asa 7	**3.08**	**0.53**	**3.61**	**0.45**	**0.07**	**0.52**
Pag-asa 9	5.31	1.10	6.41	0.79	0.14	0.93
Pag-asa 11	2.38	0.40	2.78	0.33	0.06	0.39
50 mM NaCl + 0.1% HCO_2^-						
Pag-asa 3	4.57	2.50	7.07	0.56	0.35	0.91
Pag-asa 7	**4.76**	**0.85**	**5.61**	**0.70**	**0.12**	**0.82**
Pag-asa 9	2.53	0.43	2.96	0.37	0.07	0.44
Pag-asa 11	5.58	0.75	6.33	0.81	0.10	0.91
100 mM NaCl No added HCO_2^-						
Pag-asa 3	4.78	1.03	5.81	0.69	0.14	0.83
Pag-asa 7	**1.75**	**0.48**	**2.23**	**0.35**	**0.08**	**0.43**
Pag-asa 9	4.48	0.90	5.38	0.64	0.12	0.76
Pag-asa 11	4.40	1.23	5.63	0.81	0.15	0.96
100 mM NaCl + 0.1% HCO_2^-						
Pag-asa 3	4.61	0.73	5.34	0.58	0.10	0.68
Pag-asa 7	**3.41**	**0.68**	**4.09**	**0.52**	**0.10**	**0.62**
Pag-asa 9	3.40	0.58	3.98	0.46	0.08	0.54
Pag-asa 11	1.56	0.30	1.86	0.22	0.04	0.26

B. Influence of potassium bicarbonate on growth of salt-grown mungbean varieties

Based on the above screening, Pag-asa 7 and Pag-asa 9 were used in the experiment. *Pag-asa 7* matures at 60-67 days after sowing. It yields 1.68 tons per hectare. It is a non-shattering type and produces large, glossy, green seeds. It possesses relative tolerance to drought. It is moderately resistant *to Cercospora* leafspot. *Pag-asa 9* matures at 60-62 days after sowing. It yields a. 2.2 tons grains per hectare. Plant height is about 45 cm during the dry season and 64 cm during the wet season. It has glossy green seeds.

Sand culture. Pag-asa 7 was considered to be salt-sensitive and more responsive to incorporated inorganic carbon while Pag-asa 9 appeared moderately tolerant to salinity. The salt treatments were 0, 60 and 100 mm NaCl and the CO_2 treatments were 0, 5.68 mM $KHCO_3$ (0.025% CO_2) and 22.72 mM $KHCO_3$ (0.1% CO_2) following the nutrient formulation by Lechowski (1992, 1995). The experiment consisted of 9 treatment combinations, replicated thrice. Each pail was punched with a hole at the bottom and then plugged with rubber stopper. The hole was designed for draining whenever nutrient solution will be flushed or sand was washed. The pail was first lined with pebbles (about 6 cm from the bottom) to allow aeration. An inch- diameter pipe was put vertically at the center of the pail before adding 12 kg sand. Ten seeds of each variety were sown in each pail which were then trimmed to two plants per variety. The seedlings were established with strength of the nutrient solution. Succeeding

additions of water or solutions were done through the pipe. The initial height of water in the pipe was noted and maintained to that level by addition of nutrient solution whenever needed. Incorporation of treatment solutions (three weeks after sowing) were done in controlled manner through the plastic tubes inserted into the pipe up to the level of the pebbles. Soil solutions were collected from the pail by unplugging the rubber stopper at the bottom from to time to determine the EC and pH. Plants were harvested 36 DAS. Plant height, fresh and dry weights of roots, stem and leaves were measured. A plant per variety per treatment was set aside for enzyme assay.

Table 3. Effect of potassium bicarbonate on fresh biomass of salt-grown mungbean varieties.

Plant part	0% CO_2		0.025% CO_2		0.1% CO_2	
	Pag-asa 7	Pag-asa 9	Pag-asa 7	Pag-asa 9	Pag-asa 7	Pag-asa 9
0 mM NaCl						
Stem	0.60	0.67	0.97	0.95	0.67	0.80
Root	6.70	5.50	7.28	8.10	6.95	9.93
Plant	7.30	6.17	8.25	9.05	7.62	10.70
60 mM NaCl						
Shoot	0.70	0.85	0.70	0.68	0.73	0.70
Root	7.30	8.73	6.66	7.65	7.78	6.38
Plant	8.00	9.53	7.37	8.32	8.52	7.08
100 mM NaCl						
Shoot	0.47	0.62	0.76	0.83	0.72	0.73
Root	3.98	6.33	6.05	7.90	6.73	6.42
Plant	4.45	6.95	6.81	8.73	7.45	7.15

Table 4. Effect of potassium bicarbonate on dry matter accumulation (g dry weight) of salt-grown mungbean varieties.

% HCO_3^- ->	0% HCO_3^-		0.025% HCO_3^-		0.1% HCO_3^-	
	Pag-asa 7	Pag-asa 9	Pag-asa 7	Pag-asa 9	Pag-asa 7	Pag-asa 9
Plant parts			**0 mM NaCl**			
Shoot	0.807	0.559	0.714	0.782	0.738	1.061
Leaf	0.453	0.353	0.403	0.467	0.427	0.687
Stem	0.354	0.206	0.311	0.315	0.311	0.374
Root	0.077	0.053	0.101	0.090	0.089	0.086
Plant	0.884	0.612	0.814	0.871	0.827	1.148
Plant parts			**60 mM NaCl**			
Shoot	0.688	0.715	0.825	0.763	0.592	0.607
Leaf	0.407	0.440	0.480	0.440	0.340	0.357
Stem	0.281	0.275	0.345	0.323	0.252	0.250
Root	0.087	0.087	0.094	0.084	0.062	0.076
Plant	0.775	0.802	0.919	0.847	0.653	0.683
Plant parts			**100 mM NaCl**			
Shoot	0.437	0.785	0.600	0.997	0.727	0.698
Leaf	0.253	0.480	0.360	0.597	0.413	0.407
Stem	0.187	0.305	0.237	0.400	0.314	0.291
Root	0.071	0.097	0.080	0.111	0.093	0.083
Plant	0.508	0.882	0.677	1.108	0.821	0.781

C. Effects of rhizosphere inorganic carbon enhancement on key enzymes in mungbean grown under saline conditions

Two mungbean varieties, Pag-asa 7 and Pag-asa 9 were used. Pag-asa 7 is salt-sensitive while Pag-asa 9 is salt-tolerant. Pag-asa 7 and Pag-asa 9 were grown under three levels of salinity (0, 60 and 100 mM NaCl) and three levels of CO_2 (0, 0.025 and 0.1%). NR activity was assayed as previously described (C12-157).

VI. Influence of dissolved inorganic carbon on flowering and fruiting habit, fruit quality and yield of Garcinia mangostana (Mangosteen)

Flordeliza Faustino

Garcinia mangostana, commonly known as mangosteen, is a relatively slow-growing fruit crop grown in major islands of the Philippines. It is considered a high-value crop because of its big economic potential. The quality of its fruit commands high price in the market. It has a delicious aril, sweet but with a certain subtle "acid or sour kick" which imparts a unique taste on the fruit. The rind of the fruit is thick which probably accounts for its resistance to fruit borer. It has two fruiting seasons a year, with the heavy crop occurring from September to November while the next smaller crop follows some months later. Initial flowering was observed to occur after 7 to 20 years from sowing. Fruit yield ranges from five to six kilos per tree during the first year of fruiting. Sixteen year old trees were reported to yield 16 tons of fruit from 900 trees, although there were trees reported to yield 200 to 300 kilos per tree. In order for the tree to give good fruit yield, growers usually fertilize the tree with 4 kilos of complete fertilizer (14-14-14) and another 4 kilos of organic fertilizer after harvest. During fruit set, the trees are fertilized with 4 kilos of a combination of 18-40-0 (1 bag) and 0-0-60 (2 bags). Muriate of potash is applied to the tree 100 days after fruit set. Mangosteen grows best from sea level to 600 meters above sea level and in a place with an annual rainfall of 1270 mm that is well-distributed throughout the year.

Acceleration of the vegetative and reproductive phases of growth of *Garcinia mangostana*

Soil culture in big drums. Under normal conditions, mangosteen trees exhibit a prolonged juvenile phase prior to first flowering. The crop has a very slow rate of vegetative growth during the first three years from seed germination. The present study aims to hasten vegetative growth of mangosteen seedlings from seed germination and then to initiate early flowering and fruit set. Fourteen-month old mangosteen seedlings were obtained from a commercial nursery. They will be grown in big plastic drums by soil culture. Due to limited availability of seedlings, a few treatments are being done as an initial work. The experiment has just begun. A follow-up set up is being ready as soon as the seed materials collected from Mindanao become available.

Conclusions of the work with mungbean, peanut and maize

a. The recommended IPB mungbean, peanut and maize inbreds showed genetic variability in their response to salinity and supplemental rhizosphere inorganic carbon. **These results are very significant since they present avenues for varietal improvement of mungbean, peanut and maize for salt tolerance** and offers the farmers new strategy or technology to enhance crop production under normal and saline environment.

b. Rhizosphere inorganic carbon enhancement of mungbean growth seems to have a greater potential for **actual field applications both in normal and saline conditions.**

c. The salt-sensitive mungbean variety Pag-asa 7 showed better response to supplemental rhizosphere inorganic carbon than that of the relatively tolerant Pag-asa 9 particularly under salt stress, 100 mM NaCl.

d. The enhancing effect of added bicarbonate was evident at the early stage of growth of mungbean in both normal and saline environment. Under normal conditions, supplemental CO_2 increased plant growth, but this effect diminished with increasing concentrations of bicarbonate. In fact, those plants grown at 0.5% and 1% CO_2 levels died within a week of treatment. Growth increases over that of the treatment without added DIC were manifested on the following parameters, based on the average values of 2 varieties at 0.05% CO_2 root DW by 20%, leaf DW by 21% and total plant DW by 10%. Under salt stress, 0.05% CO_2 improved growth of the two varieties of mungbean by 7%, 24% and 46% in root DW, leaf DW and total plant DW, respectively. (Remarks: The above values need to be further confirmed since the observations were based on 18-day old plants which were subjected to CO_2-treatment for only 3 days, yet with remarkable response. There were 3 replications but only one plant was harvested per replication).

e. The positive response of the salt-sensitive Pag-asa 7 to rhizosphere DIC under salt relative to control (without CO_2) was largely exhibited in the total number of flowers initiated (10% higher), percentage of pod set (16% more), total number of pods developed (50% more), pod yield (4% higher) and total leaf FW (23% higher).

g. Our experiment on defining the CO_2 levels for optimum growth response of salt-sensitive Pag-asa 7 and relatively salt-tolerant Pag-asa 9 under normal and saline cionditions showed that future studies should be done in the range less than 0.5% CO_2, using potassium bicarbonate as the source.

VII Differential gene expression in corn induced by inorganic carbon enhancement and salinity.

Roberta Garcia and Mae Mendoza

Pi 11 and Pi 31, salt-sensitive and -tolerant yellow corn inbreds, respectively were used in the study based on the results of the previous project. Plants were grown for 2 weeks in sand culture with 100 mM NaCl supplemented with 0 and 0.1% CO_2 as sodium bicarbonate.

Total RNA from roots was extracted with Rneasy Plant Kit from QIAGEN. The Delta™ Differential Display Kit from CLONTECH was used for differential gene expression study.

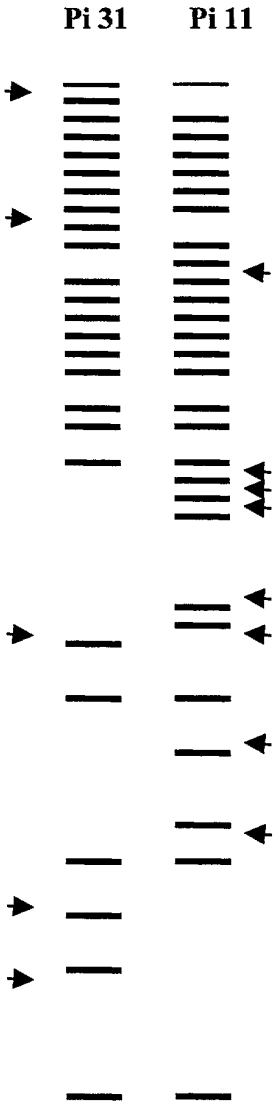

Fig.2. Differential display of Pi 31 and Pi 11 grown under 100 mM NaCl + 0.1% CO_2. RNA isolation was done using Rneasy Plant Kit from Qiagen. 2ug of RNA was used for first strand cDNA synthesis. The arbitrary primer used for differential display was 5'-ATTAACCCTCACTAAATG GAGCTGG-3' and the oligo(dT) primer was 5'-CATTATGCTGAGTGATAT CTTTTTTTTTCG-3'. The Delta™ Differential Display Kit from Clontech was used for the analysis.

Conclusions

Salt-tolerant and sensitive maize seedlings grown in sand culture supplemented with 100 mM NaCl and 0.1% CO2 showed differentially expressed DNA fragments in the roots. The salt-tolerant inbred

has 5 DNA fragments that were not expressed in the sensitive inbred, while, the sensitive inbred showed 8 DNA fragments not seen in the tolerant inbred.

While these results are very interesting, it is still too early to derive definitive conclusions. Present efforts include the analysis of seedlings not exposed to salt and CO_2 treatments to determine intrinsically expressed DNA fragments and those induced by the treatments.

The relationship of the salt expressed bands to several enzymes whose expression increases under stress conditions (see work in Sede Boqer) will be studied shortly.

C) Scientific impact of collaboration: The initial research on the effect of CO_2 enrichment of the root zone of plants was carried out in Israel with tomato and ryegrass. This work is being now extended by our colleagues in Los Baños to leguminous crops and fruit trees typical of the Philippines. The rational behind the work with leguminous plants is that, since N_2-fixation requires an intensive supply of carbon skeletons to bind the NH_4^+ generated by nitrogenase, the synthesis of oxaloacetate from PEP + CO_2 could greatly relieve the demand for oxoglutarate which is also required to generate the ATP required by the fixation process. There is no doubt now that bicarbonate fertilization is specially effective under saline and other stress conditions, leading to the amelioration of yield loss caused by the stress and increasing assimilate allocation to reproductive growth at the expense of vegetative growth

D) Description of Project Impact: Bicarbonate fertilization elicited a lot of interest for several reasons:

B. CO_2 fixation by roots improves stress tolerance to salinity and drought by enhancing ammonium assimilation into organic N compounds in roots, thus sustaining the provision of these essential growth elements to the shoot.
C. The reduction of the NO_3^-/organic N ratio in the xylem sap increases the harvest index of crops, through the enhanced allocation of assimilates to fruits and seeds instead of diverting the fresh assimilates to vegetative sinks.
D. The enhanced allocation of assimilates to reproductive organs in response to the lowered NO_3^- /organic N ratio in the xylem sap also results in better fruit quality – higher level of soluble sugars and other parameters of fruit quality
E. Bicarbonate fertilization increases the capacity of the plant to use reduced inorganic N such as NH_4^+ while reducing the use of oxidized inorganic N such as NO_3^-. This results in lower pollution of water resources by leached and run off unused nitrate which binds little to soil colloids. It also decreases the undesirable accumulation of nitrate in agricultural produce.

E) Strengthening of Developing Country Institutions:

Addition to the research facilities of the Institute of Plant Breeding (Los Baños) during the period of the proyect covered by the present report:

1. Renovation of a cubicle (20 ft x 20 ft) which serves as the Crop Stress Biotechnology Unit of the Plant Physiology Laboratory
2. Acquisition of a Fischer pH meter Model 50 with capability to measure ion concentration in samples using ion selective electrodes (ISE's)
3. Acquisition of several selective electrodes for uptake studies of major nutrients and ions (salt)
4. Acquisition of computer and computer table to aid data analysis
5. Acquisition of an air conditioner for the abovementioned laboratory
6. Acquisition of a freezer for sample storage used in enzyme/molecular studies

7. Acquisition of portable pH meter for greenhouse hydroponic studies
8. Repair and calibration of our defective LICOR photosynthesis meter at USA
9. Repair of our old model LICOR porometer and purchase of new battery and sensor
10. Acquisition of glass dryer

(F) Future Work:
Work is well within schedule. However, using different crops of interest in the Philippines requires some optimization experiments which are somewhat time consuming. The work plan remains essentially unchanged although some changes in timing may be unavoidable. In addition the work of Mae Mendoza and Beth Garcia in differential gene expression of some crops is a recent addition to the original plan.

Maize:
1. Cut out DNA fragments induced by salt and CO_2 treatments, reamplify and Northern blot analysis.
2. Cloning of differentially expressed genes, linking to changes in aldehyde oxidase, xanthin dehydrogenase, PEPc, AS, etc.
3. Determine PEPC activity levels and isoforms

Legumes, mangosteen:

1. Conduct more trials under greenhouse and field conditions to confirm previous results. Our recent experiment on the mapping of CO_2 levels for optimum mungbean growth were faced with ineffective foliar control of white flies, mites and leaf miner. In the future set up, furadan will be added to the root medium.
2. Since we have already identified appropriate varieties of mungbean,peanut for our studies, we plan to elucidate the physiological and biochemical (ion uptake) and molecular framework on the bases of salt tolerance and DIC enhancement effect in mungbean/peanut, under the optimum level of CO_2.
3. To monitor/differentiate the expressions of the different enzymes involved according to the working hypothesis of the project.
4. Continue physiological studies in mangosteen.

Section II

A) Managerial Issues: There was a temporary break in communications originating from the changes in phone and email systems in Los Baños. This situation has been overcome now and the email system, although still slow (a message may take about 24 h to arrive) is greatly improved, which reflects in the progress of the experimental work and the transfer of data.

B) Budget: None

C) Special Concerns: None

D) Collaboration, Travel, Training and Publications: Prof. Mae Mendoza and Dr. Flor Faustino visited Sede Boqer in 1998. Beth Garcia, a graduate student of Prof. Mendoza, has spend about six months of training in Sede Boqer. Prof. Lips and Dr. Sagi will visit Los Baños this year (2000) in order to discuss experimental results and consider future experimental work during and after the time schedule of the present project.

E) Request for American Embassy Tel Aviv or A.T.D. Actions. None