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Rhizosphere inorganic carbon enhancement of crop production

Moshe Sagi & S. Herman Lips
Biostress Research Laboratory
J. Blaustein Institute for Desert Research
Ben-Gurion University of the Negev
Sede Boqer - Israel 84990

and

Flordeliza Faustino and Evelyn M. T. Mendoza
Institute of Plant Breeding
University of the Philippines at Los Baños
College, Laguna. Philippines

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TABLE OF CONTENTS

Cover Page	1
Table of contents	2
Executive Summary	3
Section I	4
A. Research Objective	4
B. Research Accomplishments	4
Research in Los Baños	4
Research in Sede Boqer	10
C. Scientific Impact of Collaboration	13
D. Description of Project Impact	13
E. Strengthening of Developing Countries institutions	13
F. Future Work	13
Section II	14
A. Managerial Issues	14
B. Budget	14
C. Special Concerns	14
D. Collaboration, Travel, Training and Publications	14
E. Request for A.I.D or BOSTID Actions	14

Section I

Executive Summary

Research on this project started immediately upon the beginning of funding in February 1999. Both teams of researchers advanced their work on the alleviating effects of HCO_3^- on plants subjected to salinity or fertilized with manure and ammonium-based fertilizers instead of nitrate. The working hypothesis, developed on the basis of joint work within the framework of **C12-157**, is that plant roots receiving larger than usual amounts of ammonium may find it easier to assimilate reduced inorganic nitrogen ions when using oxaloacetate (OAA) instead of oxoglutarate (OG) to renew the supply of glutamate required by GS (glutamine synthetase), the enzyme responsible for the assimilation of ammonium to glutamate to yield glutamine. The production of oxaloacetate depends on the level and activity of the enzyme PEPc that catalyzes the addition of HCO_3^- to phosphoenol pyruvate and the availability of HCO_3^- - that results in the desired OAA.

PEP + HCO_3^- --------(PEPC)----------□ OAA

NH_4^+ + GLU ----------(GS)----------□ GLN

OAA + GLN ---------(OAG-AT)---------□ 2 GLU

This mechanism to increase the supply of carbon skeletons for enhanced assimilation of ammonium turned out to be very important in salinity affected crops which have their usual large xylem transport of nitrate from roots to shoots blocked under these conditions. All of the relatively salt tolerant plants studied increase under saline conditions root reduction of nitrate to ammonium (by NR, nitrate reductase) together with PEPc and GS. This reflects in the nitrogen moving through the xylem which changes from mostly nitrate under non-saline conditions to mainly amides under saline conditions.

The practical advantages of HCO_3^- fertilization have been studied in Israel. Tomato and melon production under saline conditions in the presence of soil HCO_3^- increased considerable the quality and the subsequent price of the marketed products. The increase in price more than made up for the loss of yield caused by salinity.

The Philippines have plenty of rainwater – during the rainy season. However, this is not the case during the prolonged dry season, in which many fields are not cultivated for luck of water and irrigation system. We have shown together with our Philippino colleagues that slightly saline water from Laguna de Bay could be used for irrigation to obtain higher and better quality yields of peanuts, maize and other crops during the dry season (**C5-001, C11-131**).

The current Filipino team is studying the effect of bicarbonate fertilization on the productivity and fruit quality of several local crops such as mangustan

Another line of work we will start soon with potted plants will be to study the effect of limited irrigation and fertilization on the productivity of these desert plants.

Section 1

A. Research Objective

Overall aim:

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.

Specific objectives:

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 that 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. Research in Los Baños (Philippines)

1. Differential gene expression in salt tolerant and salt sensitive corn as affected by soil CO_2 levels

Mae Mendoza and Beth Garcia

The objective of these experiments was to identify specific genes that are expressed under conditions of salinity and enhanced dissolved inorganic carbon dioxide in the rhizosphere.

To clone and characterize salt -expressed genes

Transformation of salt-sensitive corn using the cloned genes.

Total RNA was extracted from the roots of salt sensitive Pi 11 and salt-tolerant Pi 31 exposed to 4 treatments namely: control: 0 salt + 0 CO_2; 100 mM NaCl + 0 % CO_2; 0 mM NaCl +0.1% CO_2; and 100 mM NaCl + 0.1% CO_2. The total RNA profile isolated from the 2 inbreds grown under these conditions is shown in Fig. 1. It is interesting to note that more total RNA was extracted when the

plants received 0.1% CO_2 under non-saline environment (Lanes 3 and 7). Pi 31 gave more brightly stained and thicker RNA bands than Pi 11. Within an inbred, increasing CO_2 level to 0.1% did not effect improvements in the amount of RNA transcripts in the presence of salt (Lanes 4 and 8). However, Pi 11 grown under 100 mM NaCl + 0.1% CO_2 showed more RNA transcripts than Pi31 similarly treated. **This suggested that the susceptible Pi 11 was favorably affected by rhizosphere CO_2 enrichment compared to Pi 31.**

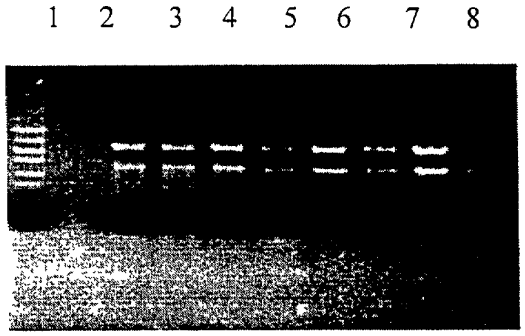


Figure 1. Total RNA extracted from roots of corn (14 days old). The roots were treated with different combinations of CO2 and salt. Lanes 1 to 4 were from accession number P11, treated with 1) 0 salt and 0 CO2, 2) 100 mM salt and 0 CO2, 3) 0 salt and 0.1% CO2, 4) 100 mM salt and 0.1% CO2. Lanes 5 to 8 were from accession number P31, treated with 5) 0 salt and 0 CO2, 6) 100 mM salt and 0 CO2, 7) 0 salt and 0.1% CO2, 8) 100 mM salt and 0.1% CO2.

1. The total RNA from Pi 31 grown under 0 salt +0.1% CO_2 was used in initial studies on the cloning of PEPC and Na+/H+ antiport. RT-PCR of the total RNA produced the cDNA template for subsequent PCR work. Primers were designed based on known sequences of the two genes and by using the Vector NTI computer program.

2. For Na+/H+ antiport gene, two primers were designed using the aligned sequences of the Na+/H+ antiport sequences. They were designated as NaH1 and NaH2. Their sequences are as follows:

NaH1 5' CTG CAG CTT GGT CAT CGT TTG 3'
NaH2 5' CGT CGA AGC TGT ATT GGT GC 3'

NaH1 was used in the subsequent PCR reactions. There were two PCR products obtained after the PCR reactions, a 500 bp band and 1.2 kb band (Fig. 2). These bands were isolated using the Agarose gel extraction kit (Fig. 3) and will be used in the ligation, transformation and sequencing procedures.

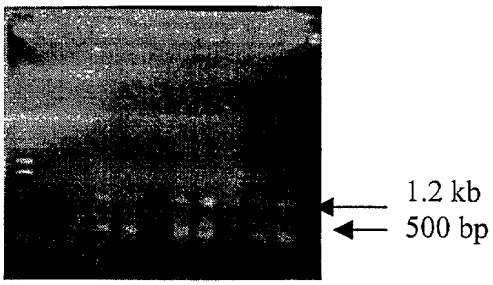


Fig.2. PCR amplification obtained using the NaH1 primer.

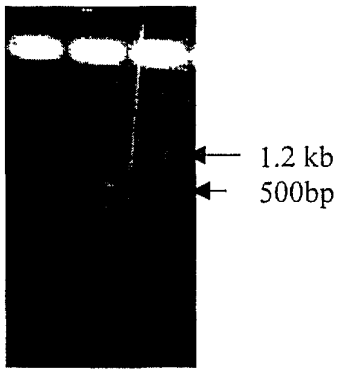


Fig.3. PCR products extracted from the agarose gel

1. For PEPC, seven forward primers were made using a Vector NTI program. The primer was designed based on the conserved sequences of known PEPC gene found in the roots. The two forward primers synthesized were pf5 and pf7. Alpha1 and alpha 2 were used as reverse primers.

   Primer 5 (pf5) : GGA TTA ATG GCA AGC AGG AG
   Primer 7 (pf7) : GTC TGA ACT GAG GGG CAA AC
   Alpha 1 : GAC TCG AGT CGA CAT CGA
   Alpha 2 : GAC TCG AGT CGA CAT CGA TTT TTT TTT TTT TTT TT

The total RNA was reverse transcribed using a Superscript II, an enzyme produced by Gibco BRL. After reverse transcription the cDNA produced was amplified by PCR using the designed primers. The PCR was done for 35 cycles. An amplified band approximately 1.6 kb was obtained using primer pf5 (Fig.4). The cDNA used came from corn treated with 0 salt and 0.1% CO_2. No PCR product was seen when primer pf 7 was used.

The 1.6 kb PCR product was re-amplified for another 35 cycles to produce more copies for ligation to pGEM-T vector.

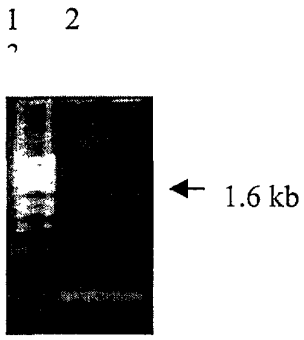


Figure 4. PCR product produced after 35 cycles using primer pf5. The size of the band in lane 3 is approximately 1.6 kb. Lane 1- MW.

Transformation and Plasmid Miniprep

DH5α competent cells produced by Gibco-BRL were used in the transformation experiment. After an overnight incubation at 37 °C of the transformed cells a colony was seen growing on the LB-ampicillin plate.
The plasmid was isolated using alkaline lysis method. After the plasmid had been isolated, it was cut by Eco-R1 to release the ligated PCR product. Two bands were seen after digestion of Eco-R1. The sizes of the bands were approximately 1.2 and 0.4 kb (Fig. 3). Like the PEPC gene the cloned gene has an internal ECO R1 site.

Sequencing and Sequence Analysis

The cloned gene was sequenced by Newcastle DNA Express Sequencing (NDES). The full length of the clone was hoped to be seen using primers SP6 and T7 (Table 1). The sequence results given by NDES has several N or unidentified nucleotide and thus it was difficult to see the true identity and characteristics of the cloned gene. Nevertheless the similarity of the clone gene with other known genes was searched using BLAST Sequence Similarity Program. The result of the search showed that the cloned gene was highly similar to wheat mitochondrial 26S rRNA gene with a score of 1041. A similarity with maize mitochondrial 26S rRNA gene also was also seen.

Based on the results the design primer has a complimentary sequence to the rRNA of corn, thus it is necessary to destroy the rRNA after reverse transcription. Treatment of RNAse A on the reaction mixture after transcription may help solve the problem.

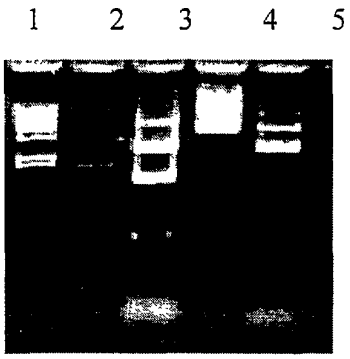


Figure 5. Electrophoresis of putative pepc gene ligated to pGEM-T . Lanes 1) Size marker 2) 1.6 kb PCR product, 3) pGEM-T with cloned gene digested by Eco R1, 4) pGEM-T with cloned gene, 5) pGEM-T only.

Table 1. Sequence results of cloned gene from corn.

SP6 PRIMER (707 bp)	T7 PRIMER (860 bp)
Alpha 1 GACTCGAGTCGACATCGAGGTGCCAAACGACTCCGT **Sac I** CGATAA**GAGCTC**TTGGGAGTCATCAGCCTGTTATCCC CGGCGTACCTTTGATCCGTTGAGCGAGAGCCCTTCCA CACGGGACTCCCGGATCACTATGGCCGACTTTCGTCT CTGTTCGACCAGTCGGTCTCACAGTCAGGCAGGCTTA TACCATTACGCTCACGAGCAGAATCAACGCTTGAGCC TACCTTCGCACACCTCCGTTACTCTTTAGGAGGCATCC GCCCCAGATAAACTACCCACCTCGCAGTGTCCCGCCC CCCCCCGAATGATCGGTGCGGCGGTTAGGCATCCTTA GACGAAAGAGTGGTCTTTCAGGATTGGTCGTTGTGTG TCACCACCTCCCACCTATCCTACACATTCGATCAAGGT TGTCACTGCGAAGCTATAGTCAAGGTGCACGGGGTCT TACCGTCTAGCCGTTGGTACTNCGCATCTTTNACGGA **Ecor I** **GAATTC**AATTTCACCGGGTCCATGTCGGGAACAGCGG GGCCAGTCGTTTACACCATTCGTGCAGGTNGCTACTT ATTGCGACAAAGGAATTTTCGCTACCTTTAGGACAGNT AGANTTACTGCCCGCCTTTTACCGGGGGCTTTTCATTC AAAGCNTTATTACACTTTTTTCTTTTCGACCTTTTCAGC ACCGGGCCAGGTGTCAGACTNTTTTNCATNCGGGGTA CCACTTAGT	**Pf 5 primer** GGATTAATGGCAATCANGAGGTGGGNTTGGAAGCAN TCATCNTTTGAAGAAAGCGTANNNGNTCANTGGNNTA GNTNCATGGCACCGAAAATGTCTCANAGGCTCAAGTG ATTNACCGAANCGACNAGNCCTTGAANGNTGCTNTNN CAAGTGTCATNANCGGGACGNTTTGTANATANGGGAA GGNTTTTGGTGACANGACCTGATGATNTNANAAGTNN NAANGCTGTCATGAGTNACNANAAAACCTGTGAAAAAC NCTGATNNCNTGGCAGTGGAAGGGTTTCTGCGNANAN TNAATNTACNCATNGTGANTCGNATTATAANGNACNCN NNAAAGGGTTTATGTACGNTGGGTACACAAAAGAGNN TAAATTGGTTGTGNCTANTAGAANNATGCCTGTANTGT NGNTNACGAATTGCGNNGATCNNNACNGANGGCTNGA CCTAATTTNANNNAGATTATATTTGATTTAATNTGTNAT CNTTATANNCATNANANACAGGTTTATTNANAAGGGNA NGGGNATTTGCTTGGACTNGANNAGNCNTTATTCTTNC TNNNTNNCTTCNCAANANAGGCCAATNNTTCTAGNTNT TTCTTCGGAACNCGANTGGANATNNNTTTTTGGGAGC GGTAGGGGGNTTTNTATACNACATCTTNCTGTTTNTTC NTNGGANTANGGGGTTTACCATATNTCTGANGNNNTA AAAAATCGCATNGATTAATTNTTNTTTNGTTNNNACAAA AANTNTTTNATTTNNGGTCTTTCTTGANGGTCATACNA AAAGTNAAAGGGCCTTTTGTTCATTTTATANANGATNA NAATTGNNTCANTTTTANATTTCNANACACACCG

Future Activities

- Continue differential gene expression between salt sensitive and salt tolerant inbreds.
- Continue cloning experiments for Na^+/H^+ antiport and PEPC genes.
- Characterization of cloned Na^+/H^+ antiport and PEPC genes

2. Crops responses

Flordeliza Faustino

Corn

1. Database search for phosphoenolpyruvate carboxylase and Na/H antiport genes from various sources was conducted. The homology of the sequences gathered was analyzed sing Vector NTI and forward primers for the genes were designed with the same program. Cloning conditions for PEPC and Na/H antiport are being optimized.

2. Corn seedlings were grown in the greenhouse under saline and enhanced rhizosphere inorganic carbon conditions. Improved growth of salt-treated plants ameliorated with 0.1% CO2 was demonstrated by both varieties, though growth enhancement was more pronounced in the salt-sensitive Pi-11. Enzyme analyses showed that 100 mM NaCl induced root NRA of salt-tolreant Pi 31 by as much as 140%. With the addition of 0.1% CO2 to the salt-treated pos, leaf and root NRA's of both Pi-11 and Pi 31 increased by 11% and 31%, respectively (averaged over two varieties and two replications), when compared over that of plants grown without salt and DIC. Extraction of total RNA fromt he roots is in process for the differential gene expression analysis of salt-tolerant Pi 31 and salt-sensitive Pi-11.

Mungbean

Optimizing the levels of CO_2 for growth of mungbean

Previuos pot experiment under greenhouse conditions had shown positive response of mungbean to added inorganic carbon in the form of bicarbonate equivalent to 0.1% CO_2. The present experiment was conducted to determine the level of CO_2 that would be optimum for growth of salt-sensitive Pag-asa 7 and relatively tolerant Pag-asa 9 under saline and non-saline conditions. Salinized environment was attained by the addition of 100 mM NaCl. Control plants were grown in pots without NaCl. The different CO_2 levels applied were the following: 0%, 0.1%, 0.2% and 0.3%. The CO_2 treatments were designated as C1, C2, C3 and C4 levels.

Effect of NaCl on mungbean growth.

Mungbean plants grew vigorously in the absence of NACl. However, in the presence of salt, plant fresh weight was reduced by about 13% (about 15% on a dry weight basis).

Effect of various levels of CO_2 on mungbean growth.

Under normal conditions, supplemental CO_2 at 0.1% increased the plant fresh weight (PFW) of Pag-asa 9 and PAg-asa 7 by about 19% when compared with those plants without added DIC. Inorganic carbon enhanced root growth of PAg-asa 9 at all levels of CO_2, withh the effect decreasing as the level of CO_2 was increased. Percentage increases in root fresh weight (RFW) ranged from 103% at 0.1% CO_2 down to 43% at 0.3% CO_2. Increases in stem fresh weight (SFW, 24%), leaf fresh weight (LFW, 37%) and the total PFW (21%) were also observed at 0.1% CO_2. However, as the levels of CO_2 were increased, SFW, LFW, and PFW decreased. Under saline environment, the addition of DIC produced better growth of the root. However, SFW, LFW as well as total PFW decreased with increasing CO_2 concentrations. Pag-asa 7 showed erratic response to the treatments.

Overall comaprison of the effects of added DIC on the growth of PAg-asa 9 and Pag-asa 7 showed that the salt induced loss of growth of mungbean plants could be negated by DIC supplementation around the roots. Plants grown under salt and supplemented with 0.1% CO_2 exhibited even higher PDW's than controls, those plannts grown under normal conditions, with or without added DIC, by as much as 24% and 13%, respectively. **These results are in agriment to our expectations that HCO_3^- fertilization can alleviate yield losses of leguminous plants affected by salinity by its possitive effect on dark CO_2 fixation by PEPc into OAA and the support of the latter of ammonium assimilation processes in the root.**

The optimal concentration of CO_2 suitable for growth of mungbean under saline and non-saline conditions apperaed to be around 0.1% CO_2. Higher levels of CO_2 were detrimental to mungbean plants grown under both conditions as shown by severe drying and ultimately death of plants.

Effects of 0.1% CO_2 on mungbean varieties grown at moderate and high salinity levels (on-going).

As a follow-up study, an experiment was set-up to determine the effect of 0.1% CO_2 on the salt-sensitive and salt-tolerant Pag-asa 9 grown in moderate (50 mM NaCl) and high salinity (100 mM NaCl) levels. The seeds were sown directly in salt-treated soil mixture while bicarbonate solution was added in samll amounts daily. Initial results on pod setting showed that the two varieties were significantly different in their ability to set pods under the above conditions. Regardless of salinity levels, highly significant differences in pod setting were observed amonng PAg-asa 9 plants grown with 0.1% CO_2, about 24% increase in pod setting over that of control plants without added DIC. Regardless of varieties, the effect of 0.1% CO_2 on percentage pod setting was significant only at high salinity level, with 32% increase in pod setting over that of control (0% CO_2).

II. Work in Sede Boqer (Israel)

Technical problems of HCO_3^- fertilization with drip irrigation
Alexander Fediuc and Herman Lips

The effects of HCO_3^- fertilization in crop production are controversial in several papers published during the last year. So are they in the reports and discussions of a COST network of the European Union. We are trying to find the explanation for the diversity of results reported. The equilibrium $HCO_3^- <> CO_2$ must be a factor of great importance since the affinity of PEPc is much larger for HCO_3^- than for CO_2. The pH of the nutrient solution and of the soil liquid phase determines to which side shifts equilibrium between these to inorganic carbon forms.

Using drip irrigation in greenhouses a pH around 7, bellow the soil pH of 7.5-8.0 would preserve most of DIC in the HCO_3^- form but, unfortunately, this pH also favors the formation of insoluble carbonate salts which clog the irrigation emitters. By lowering the pH of the irrigation using phosphoric acid instead of its Na^+ and K^+ salts will prevent to a large extent clogging of emitters and the determining factor may become the alkaline soil pH that will shift equilibrium back in favor of HCO_3^-. Experiments at this effects are presently under way

The effect of HCO_3^- fertilization on the capacity of crops to use ammonium instead of nitrate
E. Zdunek and H. Lips

According to our working hypothesis, the effect of HCO_3^- fertilization will be optimal when dark fixation of HCO_3^- will be required to boost ammonium assimilation in roots due to a relative shortage of the traditions carbon skeleton, oxoglutarate. For fast growing crops in the presence of NO_3^-, HCO_3^- fertilization would not improve crop production since carbon skeletons for ammonium assimilation in the root would not be required due to the immediate transport of NO_3^- taken up by root – to the shoots, where reduction and assimilation will take place.

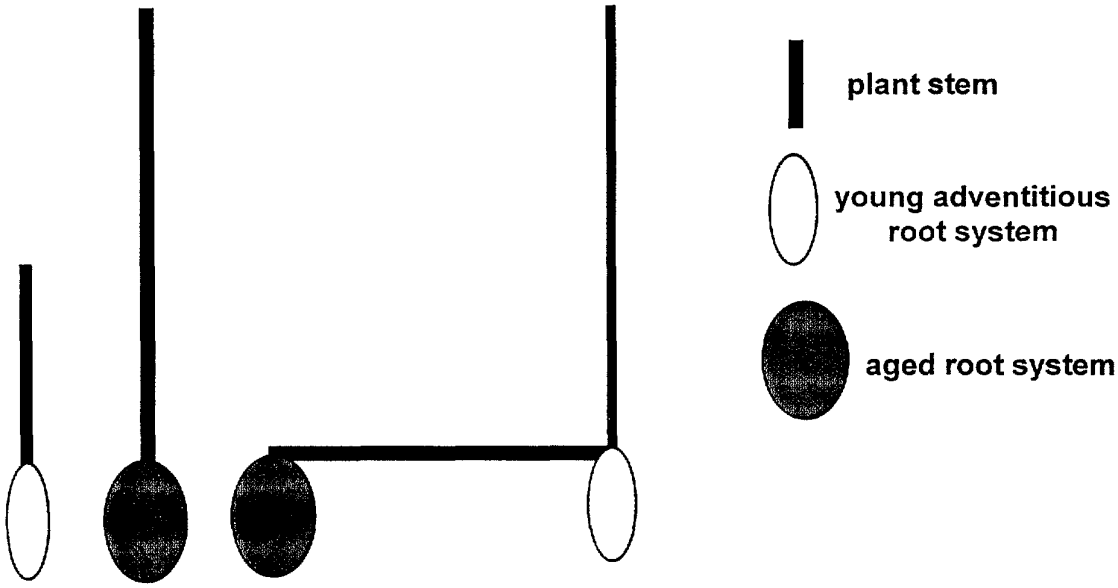
Consequently, the use of HCO_3^- may be most effective for plants under saline conditions, whose capacity to use NO_3^- is essentially inhibited, or for leguminous crops which generate their own ammonium from N_2 fixation. Following these ideas, experiments have been set up with peanut plants under field conditions irrigated with water with 1 or 8 dS m^{-1} (good quality and brackish water). The initial results, as was observed in the Philippines with more acid soils, are very encouraging.

HCO_3^- fertilization and the regeneration of adventitious roots in greenhouse tomatoes
A. Fediuc and H. Lips

Tomatoes are grown as an annual crop. This determines several months of inactivity in the greenhouse which lower the yearly productivity of the crop. During early experiments we have observed that old plants frequently create adventitious roots in soil areas kept moist by automatic drip irrigation. Since the tomato plants elongates under current agrotechniques up to 8 meters, the logistic supply of the productive part of the plant by the aging root system becomes more problematic and less efficient with time. The resistance to transport in both directions (shoot to root and root to shoot) increases with age affecting negatively the amount and quality of fruit.

Replacement of the aging root system by a young one was found effective in maintaining high productivity for a longer time and, in some cases, we managed to grow tomato plants as a "perennial" crop for about two full years. This prolonged productivity increased yearly yield by about 50% and saved a considerable amount of work and expenses implied in yearly replanting and limiting fruit production to only 7 months.

The generation of adventitious roots is greatly favored by HCO_3^- fertilization, presumably due to the need of a large investment of organic N compounds on the development of new root tissue



The effect of soil CO_2/HCO_3^- on gas exchange and growth rates of tomato.
M. D. Cramer and Richards

Stimulation of growth by elevated DIC has been observed previously in plants grown under conditions of salinity stress and was related to the site of NO_3^- reduction in salinity stressed plants (Cramer and Lips, 1995). Under high light intensity, however, the effect of DIC was greater in non-salinized than in salinized plants. Although enriched DIC may influence plant growth through anapletoric provision of C in the roots of enhanced uptake of NO_3^-, it is also possible that other more directly light-dependent mechanisms are operative.

Plants grown with elevated DIC at high RH (80%) and low light intensity exhibited higher stomatal conductance, higher water use efficiency and lower Jmax than plants grown with 360 μmol mol-1 aeration. Photosynthetic rates were, however, unaltered. This may be interpreted as indicating that with elevated DIC, the supply of carbon through the xylem partially met the demand for carbon for photosynthesis. A similar conclusion was reached by Bialczyk and Lechowski (1992). The would allow partial stomatal closure without significantly detrimental effects on growth.

The effect of enriched rhizospheric DIC on gas exchange may explain why growth effects on plants grown with elevated DIC are most readily observed in plants growing under high irradiances, salinity and drought stress or at high shoot temperatures.

The effect of dissolved inorganic carbon (DIC = CO_2 + HCO_3^-) on the growth and metabolism of young tomato (*Lycopersicon esculentum* (L.) Mill. Cv. 144) plants as affected by salinity was further studied in these experiments.

Enriched DIC increased biomass production by more than 200% on plants grown at more than 1500 μmol m^{-2} s^{-1} (Fig. 1). Plants grown under 1000 μmol m^{-2} s^{-1} increased biomass due to increased DIC only in the presence of salinity and high temperatures.

At high light intensities the photosynthetic rate, the CO_2 and light saturated photosynthetic rate (J_{max}) and the stomatal conductance of plants grown at high light intensity were lower in plants supplied with enriched than with ambient DIC (Table 1).

Labelled organic carbon in the xylem sap derived from root ^{14}C-DIC was found to be in the order of 1% of the photosynthesis of plants supplied with ambient DIC level or 10% of the photosynthesis of plants supplied with enriched DIC levels at the root level.

It is possible that organic C produced by DIC fixation by the roots may provide an emergency source of CO_2 assimilates to the shoots under conditions in where low stomatal conductance is advantageous such as salinity or drought, high shoot temperatures and high light intensity.

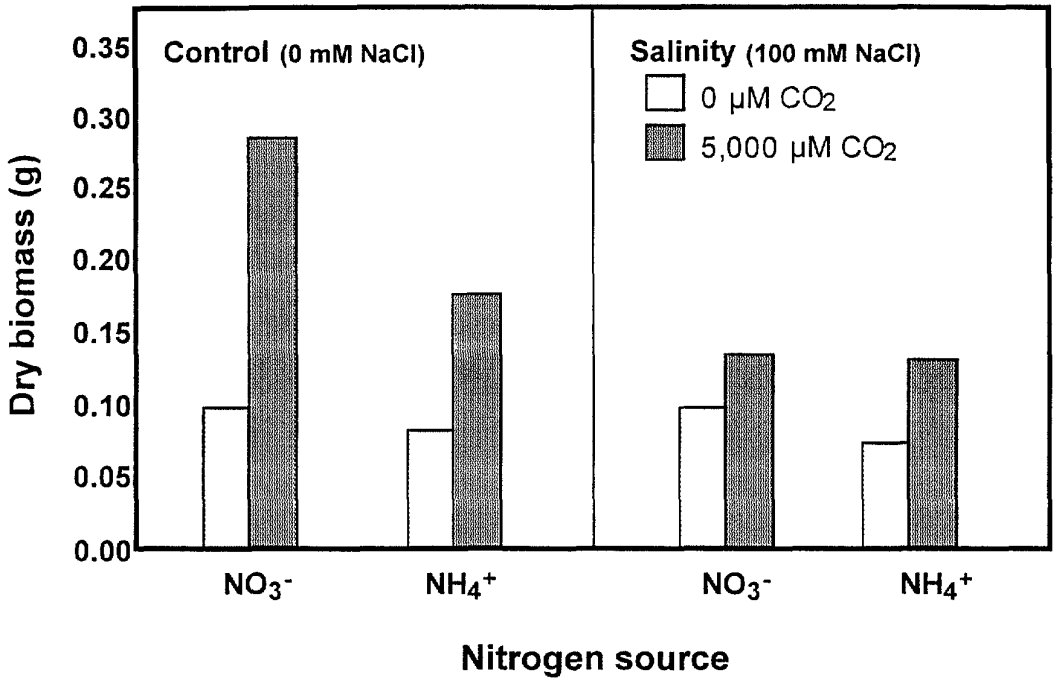


Figure 1: Biomass production by tomato plants as affected by salinity (0 and 100 mM NaCl, nitrogen source (NO_3^- or NH_4^+) and DIC (0 and 5,000 µM)

Table 1: Gas exchange variables (n=8) and variables from A:C_i curves (n=4) Plants were grown with controlled root temperature in a greenhouse with a maximum daily Irradiance of 1,000 µmol m^{-2} s^{-1} for 12-14 days after transfer into hydroponic cultures with 2 mM NO_3^-. Measurements were performed at an air temperature of 34.2+0.2 °C and with a saturating irradiance of 1,350±20 µmol m^{-2} s^{-1}

Photosynthesis parameters	Control		Salinity	
	360	5,000	360	5,000
A (µmol m^{-2} s^{-1})	17.5±0.2c	14.4±0.3b	13.6±0.7ab	11.9±0.7a
E (mmol m^{-2} s^{-1})	7.8± 0.3ab	8.1±0.2b	7.8±0.4ab	6.9±0.2a
C_i (µmole mol^{-1})	218±6a	221±2a	200±7a	201±9a
G_s (mmol m^{-2} s^{-1})	628±63c	405±17b	239±21a	207±15a
WUE (mmol mol^{-1})	2.2±0.1b	1.8±0.1a	1.7±0.1a	1.7±0.1a
A:C_i curve				
C_i at C_a = 360 (µmol mol^{-1})	227±6b	234±2b	183±7a	204±8a
A (µmol m^{-2} s^{-1})	17.8±07b	16.3±0.5b	11.7±0.8a	11.8±0.7a
A_0 (µmol m^{-2} s^{-1})	28.9±0.5c	25.5±0.5b	24.1±1.4b	19.7±1.2a
l	0.38±0.03a	0.36±0.01a	0.51±0.02b	0.41±0.01a
J_{max} (µmol m^{-2} s^{-1})	35.9±0.9c	31.2±0.7b	30.5±0.2b	25.1±0.3a
$V_{Rubisco}$ (µmol m^{-2} s^{-1})	0.14±0.01a	0.10±0.02a	0.15±0.02a	0.12±0.03a
r^2	0.99	0.99	0.95	0.95

Table 2: Effect of rhizospheric DIC concentration on the translocation of DIC and organic products of DIC incorporation in the xylem sap and the concentration of soluble products of DIC incorporation in the shoot and root (nmol g^{-1} FW)

	Rhizosphere CO_2 concentrations (μmole mol^{-1})	
	360	5,000
Xylem (nmol g^{-1} root FW h^{-1})	1.3±0.4 a	16.4±1.4b
Xylem (μmol m^{-2} s^{-1})	0.07±0.02a	0.88±0.09b
Shoot (nmol g^{-1} FW)	2.5±0.3a	18.4±2.3b
Root (nmol g^{-1} FW)	16.2±2a	158±8.b
Shoot:root	0.17±0.03a	0.12±0.01a

C. Scientific Impact of Collaboration:

During the present year cooperation was conducted mainly through email connection. Several drafts of papers in preparation have been exchanged and made ready for submission for publication.

D. Description of Project Impact

The main impact of the present project is the development of new and betters way to use irrigation water during the dry season in the Philippines, allowing local farmers to make use of local water resources affected by mild salinity (up to 4-5 dS m^{-1}). Although the use of HCO_3^- fertilization is just one way of alleviating deleterious salinity effects on crop production, research on this point may stimulate seeking additional ways to keep up crop production during the dry season through the use of intensive irrigation with a wide quality range of water resources.

E. Strengthening of Developing Countries Institutions

The AID/CDR grant is the basis for increased research activity, fellowships for graduate students, purchase of new equipment required for advanced modern methodologies, etc. The connection to the Israeli lab gives important scientific, technical and moral support to carry on and expand the activities of the Filipino group.

F. Future Work

Corn

1. Optimization of cloning conditions for PEPC and Na/H antiport
2. Extraction of roo total RNA for differnetial gene expression analysis of salt-tolerant Pi 31 and salt-sensitive Pi 11.
3. Effect of bicarbonate (0.1% CO2) on root absorption of major ions.

Mungbean & Peanut

1. Simulated field studies using big tanks on the effect of bicarbonate (0.1% CO2) on yield and its components, and other physiological parameters.
2. Monitoring of enzyme activities such as PEPC, NRA, BNF

Garcinia mangostana

On-going: acceleration of the vegetative and reproductive phases of growth of Garcinia mangostana.

Section II

A) Managerial Issues:
No outstanding issues at the moment.

B) Budget: No changes have been made or asked for

C) Special Concerns: Non

D) Collaboration, Travel, Training and Publications:

Briefly describe collaborative activities. Provide a list of completed travel and training since the last report. Indicate who was involved, and when and where activities occurred. Describe anticipated activities in next 6 months. Provide a list of project publications that have appeared since the last report.

E) Request for American Embassy Tel Aviv or A.I.D. Actions: No requests.