PN-ADI-364

Semi-Annual Report

Covering period from November 1st 2005 until April 1st 2006

Submitted to the U. S. Agency for International Development; Bureau for Global Programs, Field Support and Research; Center for Economic Growth

STUDIES OF STRESS TOLERANCE MECHANISMS IN PROSOPIS

Project Number C16-099 and Award Number TA-MOU-02-C16-099

Principal Investigator: Dr. J. E. Ephrath
Grantee Institution: Jacob Blaustein Inst. for Desert Res. Ben-Gurion University of the Negev
Address: Sede- Boqer Campus, Sede Boqer, 84990 Israel
Telephone Number: 972-8-6596756
FAX Number: 972-8-6596757
E-mail address: yoni@bgumail.bgu.ac.il

Co-Principal Investigator: Dr. Moshe Sagi
Institution: The institutes for Applied Research
Address: Bergmann Campus P. O. Box 653, Beer-Sheva 84105, Israel
Telephone Number: 972-8-6479312
FAX Number: 972-8-
E-mail address:gizi@bgumail.bgu.ac.il

Co-Principal Investigator: Ing. Nora Grados Quesada
Institution: Laboratorio de Quimica, University De Piura
Address: Ave Ramon Mugica 131 Apdto353 Piura-Per, Piura, Peru
Telephone Number: +51-74-328171
FAX Number: +51-74-328645
E-mail address: ngrados@udep.edu.pe

Administration Official:	*Sharona Rittberg*	Administration Official:	Prof. A. MABRES TORELLO, Rector
Institutional Affiliation:	Ben-Gurion University of the Negev	Institutional Affiliation:	Universidad de Piura Piura. Peru
Address:	P.O. Box 653, Beer Sheva 84105 Israel	Address:	Av. Ramon Mugica, 131, Aptdo 353 Piura-Peru
Telephone Number	972-8-6472443	Telephone Number	: +51-74-328171
FAX Number:	972-8-6472930	FAX Number:	+51-74-328645
E-mail address:	sharona@bgumail.bgu.ac.il	E-mail address:	

Section I: Technical Progress

A) Research Objectives:

Overall aim

Study the effect of the changes in the levels of N assimilation and salinity in the physiological response and in the purine catabolism of *Prosopis pallida* to lead the plant to counteract the salt stress effect.

Specific objectives:

(1) Determine the influence of nitrogen (KNO_3 and NH_4) (7.5 and 2.5 mM respectively), salinity (NaCl) (100 mM), KNO_3 (7.5 mM) + NaCl (100 mM), and NH_4 (2.5 mM) + NaCl (100 mM) on chlorophyll content in *Prosopis pallida*.

(2) Evaluate the physiological status of *Prosopis pallida* using the leaf chlorophyll content as indicator of the nitrogen nutrient status

(3) Describe the effect of salinity in seed, leaves and root of *Prosopis pallida* plants, on the enzymes of purine catabolism, when it grows in two nitrogen sources (7.5mM KNO3 and 2.5mM NH4), in salt treatments (0 and 100 mM), and in a combination of nitrogen with salinity (7.5mM KNO3 +100 mM NaCl and 2.5mM NH4 +100 mM NaCl), during the vegetative period.

(4) Clarify the role of the purine catabolizing enzymes in the production of ureides by comparing the relevant enzyme activities in plants with high and low ureide production.

(5) Describe the influence of salinity in the rate of germination; physiological parameters such as the reduction of growth resulted

from changes in shoot length, total fresh and dry weight and the relative water content (RWC), when *Prosopis pallida* plants grows in two nitrogen sources (7.5mM KNO3 and 2.5mM NH4), in salt treatments (0 and 100 mM), and in a combination of nitrogen with salinity (7.5mM KNO3 +100 mM NaCl and 2.5mM NH4 +100 mM NaCl), during the vegetative period

B) Research Accomplishments:

SCIENTIFIC BACKGROUND:

Plants will respond in different ways to multiple stresses such as salinity, drought, flooding and others that affected their growth and development during their plant's life cycle.

Injury-induced by salinity, includes inhibition of the vegetative and reproductive growth, the production and germination of seeds, and alters plant morphology and anatomy, killing non-halophytes (mostly crop) and limiting the agriculture over the world.

Salinity influences flowering, pollination, fruit development, yield, and fruit quality.

Plant's growth inhibition includes metabolic dysfunctions, such as the decrease of photosynthetic rates and chlorophyll content, changes in protein and nucleic acid metabolism and enzyme activity.

Chlorophyll is a good indirect indicator of the nutrient status because much of leaf nitrogen is incorporated in chlorophyll, essential pigment for the conversion of light energy to stored chemical energy.

One of the important biochemical tolerant mechanisms is the synthesis of nitrogen-containing bases, purine and pyrimidine nucleotides source of energy that drive most of the cell reactions.

Thus, the form in which N is supplied, transported and storage in salt-stressed plants influence the salinity response.

For some leguminous species, especially Prosopis, the principal compounds for transport and storage of nitrogen are the ureides allantoin and allantoic acid, end products of purine catabolism pathway.

Ureides are made up of four C and four N atoms, having a C:N ratio of 1.0 compared with 2.5 and 2.0 for Gln and Asn, respectively; required less C for translocation of fixed N as ureide.

In plants, an essential soluble enzyme, xanthine dehydrogenase (XDH) plays a central role in nitrogen assimilation, and in the production of the ureides allantoin and allantoic acid. Studies in soybean and cowpea nodules

about the metabolic pathway of ureide production refer the formation of ureides by ammonia assimilation through glutamine synthetase and glutamate synthase, followed by purine synthesis and oxidation. No data is known about the production of ureides in uninfected cells.

A useful technique to study proteins is Immunoblotting, which provides information about presence, molecular weight, and/or quantity of an antigen by combining protein separation via gel electrophoresis with specific recognition of antigens by antibodies. The use of imnunoblotting in this research played a vital role in the determination of the relationship of the purine catabolizing enzymes in the production of ureides; one of the objectives in this research.

The aim of this study was analize the effect of the changes in the levels of N assimilation and salinity in the physiological response and, in the purine catabolism of *Prosopis pallida* to lead the plant to counteract the salt stress effect.

B.1. MATERIAL AND METHODS:

B.1.1. Plant Material and Growth Conditions

Two sets of seeds of *Prosopis* were nicked on the blunt end with a knife, and then imbibed to two nitrogen sources KNO_3 and $(NH_4)_2SO_4$, and NaCl to the growth medium. The concentration used were 7.5mM, 2.5mM and 100mM of KNO_3, $(NH_4)_2SO_4$, and NaCl respectively with a control of fresh water. Seeds were putting in a bath at 35°C for 24 h. One of them were planted in planting mixture consist of vermiculite, perlite, tuff and peat at a ratio of 1:1:1:1, and the other one were wrapped in aluminum foil, frozen in liquid nitrogen, and stored at -80°C. Uniform *Prosopis* plants, were grown in five replications for each treatment in a completely randomized block design. The experiments were conducted in a greenhouse, with an average greenhouse temperatures during the growth period fluctuated from 18 to 25°C. Midday photosynthetic photon flux density in the greenhouse was 300–500 μmol m^{-2} sec^{-1}.

B.1.2. Salt and nitrogen source treatments and harvests

Once the *Prosopis* seedlings were well established 7 days after sowing (DAS) the plants were subjected to two nitrogen sources KNO_3 and $(NH_4)_2SO_4$, and NaCl to the growth medium. The concentration used were 7.5mM, 2.5mM and 100mM of KNO_3, $(NH_4)_2SO_4$, and NaCl respectively. Plants were harvested at 44 days after treatments (DAT). Three to five replicates were included per harvest. Samples of the first and fourth leaves, stems and root of each plant were used for the enzyme activity and ureides assay. At harvest, plants were separated into stems, leaves and roots fresh and dry weights were recorded after 48 h at 70 °C.

B.1.3. Tissue Extraction and Protein analysis

Samples of seeds, leaves and roots of *Prosopis* were wrapped in aluminum foil, frozen in liquid nitrogen, and stored at -80°C.

Tissue was macerated with acid-washed sand in an ice-cold extraction medium containing 250 mM Tris-HCl (pH 8.48), 1 mM EDTA, 1mM DTT, 5 mM L-Cys, 80 mM Na_2MoO_4, 100 mM PMSF, 10 mM GSH, 10µl aproteinine, 10µl leopeptine, 10µl pepstatine, and 250 mM sucrose.

Samples of 2-3g of leaves, roots and seeds were extracted in a ratio of 1:3, 1:4 and 1:3 w/v respectively).

The homogenized plant material was centrifuged at 15000 rpm in a **Hermle Z 233 MK-2** refrigerated centrifuge at 4°C for 20 min. The resulting supernatant was subjected to native polyacrilamide gel electrophoresis (PAGE).

Soluble proteins in the assays were measured (Bradford, 1976) using crystalline BSA as a reference.

B.1.4. Gel Electrophoresis and Analysis of Enzyme Activity

Native PAGE was carried out in a Bio-Rad Mini-Protean III slab cell (Bio-Rad, Richmond, CA, USA) with a discontinuous buffer system (Laemmli, 1970) in 7.5% (w/v) polyacrylamide separating gel and 4% (w/v) stacking gels. Native-PAGE was carried out using 1.5 mm thick slabs loaded with 50-200 µg seed, leaf or root proteins, respectively.

After electrophoresis, AO activity was detected by the addition of 1 mM cinnamaldehyde, and XDH activity, by the addition of 1 mM hypoxanthine and 0.5 mM xanthine as specific substrates (Mendel and Muller, 1976) in the presence of 50 mM Tris-HCl, pH 8.48, at 25°C.

The gels were scanned in an Arcus 1200 Scanner (Agfa, Mortsel, Belgium) and quantified by NIH Image Software (Version 1.6).

B.1.5. Western blot analysis:

Protein extraction will be carried out as described above. The extracts were centrifuged at 15 000 rpm at 4 °C for 20 min. Aliquots of the supernatants containing 300 mg leaf proteins will be combined with the same volume of sample loading buffer (125 mM TRIS –HCl, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue), and heated for 5 min at 95 °C before being subjected to SDS-PAGE.

Native-PAGE and SDS-PAGE were carried out in 7.5% polyacrylamide gels (Laemmli, 1970). The separated proteins were electrophoretically blotted onto a nitrocellulose membrane (0.2 mm pore size; Schleicher and SchuÈ ll, Dassel, Germany) at 380 mA for 2 h. Unbound sites on the membranes were blocked with 5% (w/v) non-fat dry milk (Blotting Grade Blocker, Bio-Rad, USA) in TRIS buffered saline, TBS (20 mM TRIS-HCl, pH 7.5, 150 mM NaCl).

Immunodetection of XDH was carried out with antibodies against XO. After overnight incubation of the blots with the primary antibodies at 4 °C, the membranes will be washed three times with TBS-T buffer (TBS containing 0.05% (v/v) Tween 20) and incubate for 1 h at room temperature with secondary antibody. As secondary antibodies, the alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma, USA) diluted 1000-fold in TBS will be used. The immune-complexes will be visualized by developing the alkaline phosphatase activity with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (0.36 mM) and nitro blue tetrazolium (NBT) (0.32 mM) in 100 mM TRIS –HCl buffer, pH 9.5, 100 mM NaCl, and 5 mM $MgCl_2$.

The molecular weight of XDH were estimated with a mixture of highly purified recombinant proteins of specific molecular weights (Precision unstained protein standards, BioRad, USA): 250 kDa, 150 kDa, 100 kDa, 75 kDa, 50 kDa, 37 kDa, 25 kDa, 15 kDa, and 10 kDa. SDS-PAGE was carried out with a Mini-Protean II Cell (Bio-Rad, USA); electrophoretic transfer was performed with a Mini Trans-Blot Cell (Bio-Rad, USA).

B.1.6. Ureide determination

The colorimetric method described by Vogels & Van der Drift (1970) was used for total ureide (allantoin and allantoate) determination in fresh and dry leaves, roots and shoots, using allantoin and allantoic acid as standard.

Tissues were macerated with alcohol 80% in a ratio of 1:4 w/v.

B.1.7. Chlorophyll determination

The total chlorophyll content of leaves were extracted into 80% acetone and centrifuged in the dark. The absorbance is measured at 652 nm and chlorophyll concentration given by the following expression:

$$\text{Chlorophyll}\,(\mu g/ml) = \frac{A_{652} * 1000}{34.5}$$

B.2. RESULTS:

B.2.1. EFFECT OF NITROGEN AND SALINITY IN THE TOTAL BIOMASS

Figures 1, 2 and 3 shows the effect of the two nitrogen sources (KNO_3 and NH_4) and salinity on the biomass content of *Prosopis pallida*.

The total biomass graphs in all the treatment drew a Gauss response may be because of the many small effects added together.

Both nitrogen sources (KNO_3 and NH_4) increased the biomass production in comparison with the contro; (figure 1 and 2), while salinity decreased it.

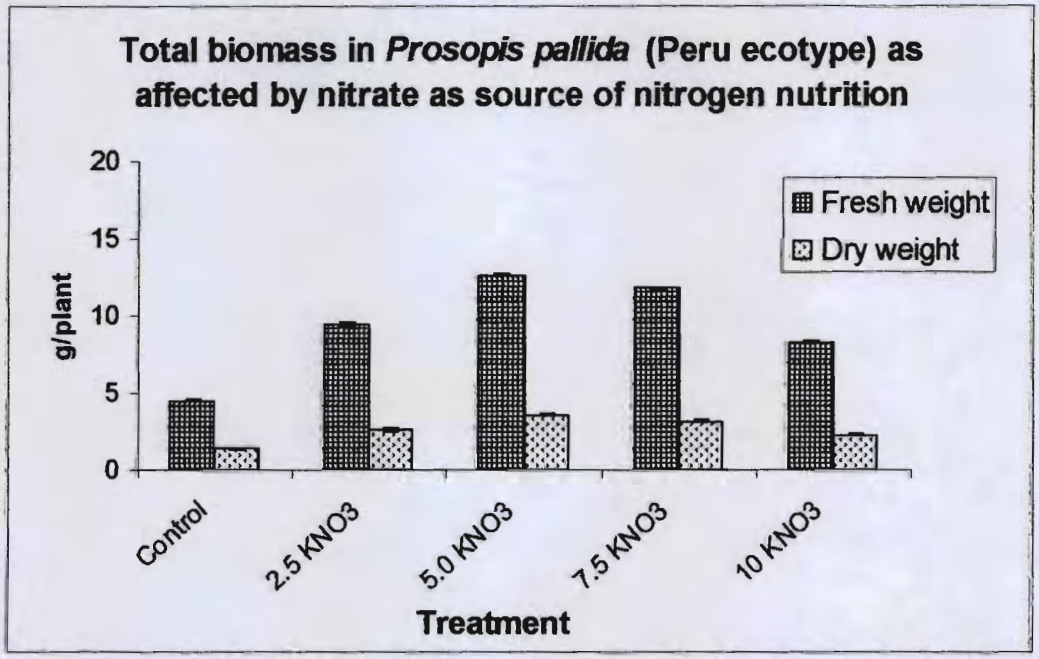


Figure 1: Total biomass in *Prosopis pallida* (Peru ecotype) affected by four concentration of nitrate (2.5, 5.0, 7.5 and 10 mM) as source of nitrogen nutrition. Plants grew in a greenhouse and they were harvested 44 days after the treatment.

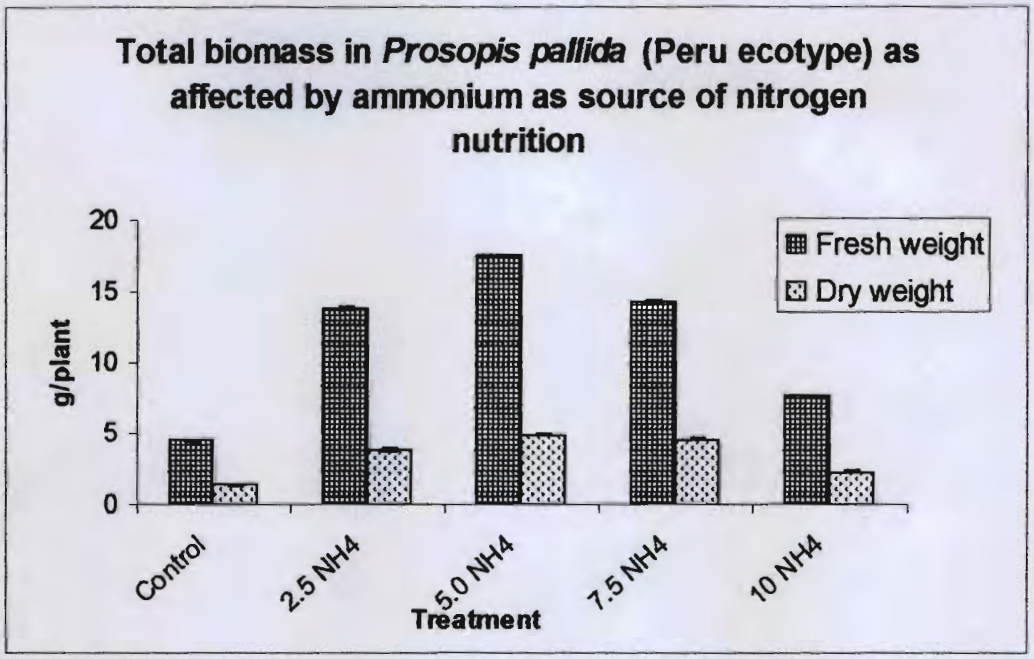


Figure 2: Total biomass in *Prosopis pallida* (Peru ecotype) affected by four concentration of ammonium (2.5, 5.0, 7.5 and 10 mM) as source of nitrogen nutrition. Plants grew in a greenhouse and they were harvested 44 days after the treatment.

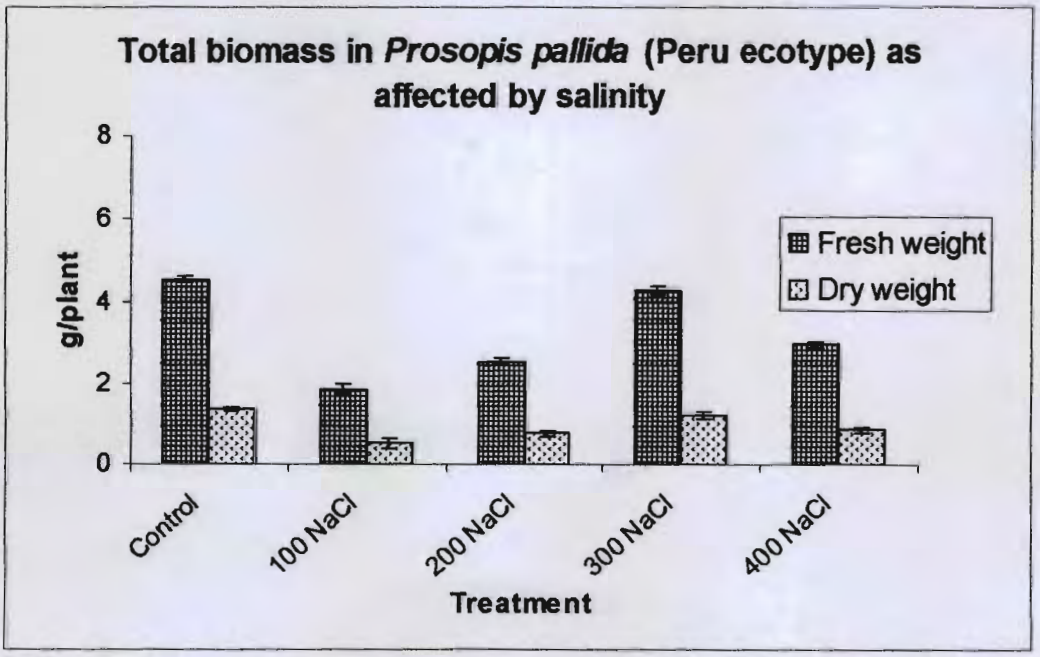


Figure 3: Total biomass in *Prosopis pallida* (Peru ecotype) affected by four concentration of NaCl (100,200, 300 and 400 mM). Plants grew in a greenhouse and they were harvested 44 days after the treatment.

B.2.2. NITROGEN AND SALINITY EFFECT IN UREIDES CONTENT.

Figures 5, 6, 7, 9, 10 and 11 shown the ureides, allantoin and allantoate, content in leaves, stems and roots affected by 2 nitrogen sources (KNO_3 and NH_4) and salinity (NaCl).

Ureides stems content were the highest of the three plant organs evaluated.

Both, allantoin and allantoate determination shown a high tendency of a Gauss distribution as those describe for biomass.

B.2.2.1. DETERMINATION OF ALLANTOIN IN DIFFERENT PLANT ORGANS.

Figure 5 and 7 shown that salinity and ammonium affected significantly, while figure 6 shown the effect of nitrate, which was not as significant as the other two.

The response was similar at those described in the biomass content.

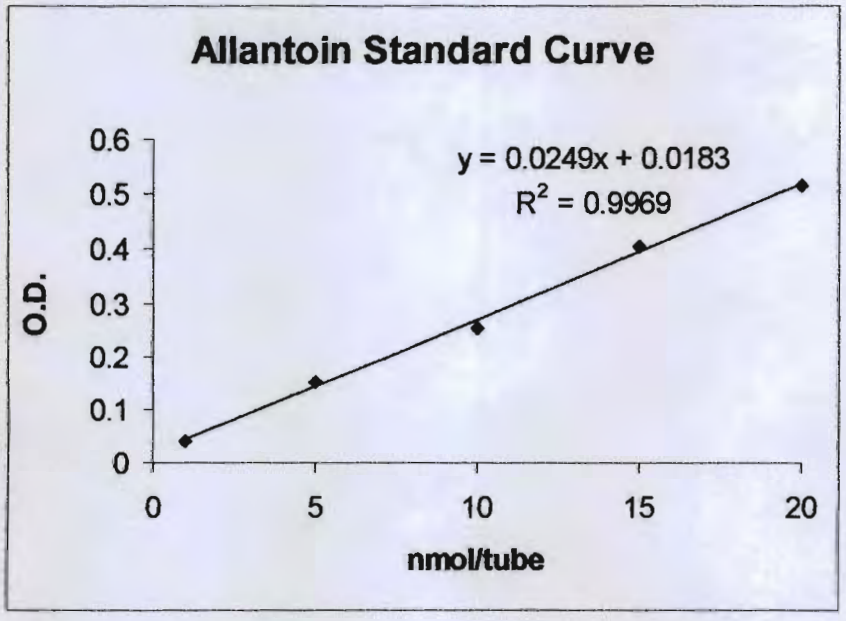


Figure 4: Allantoin standard curve

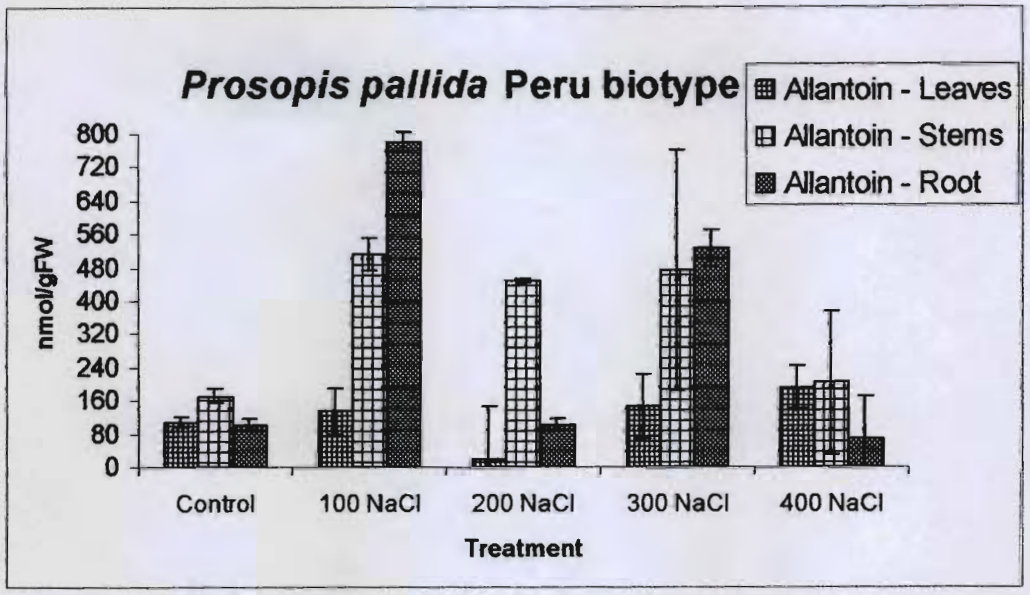


Figure 5: Allantoin determination in leaves, stems and root of *Prosopis pallida* (Peru ecotype) affected by four different concentration of NaCl (100, 200, 300 and 400 mM). Leaves, stems and roots were extracted in 80% ethanol in ratio of 1:5

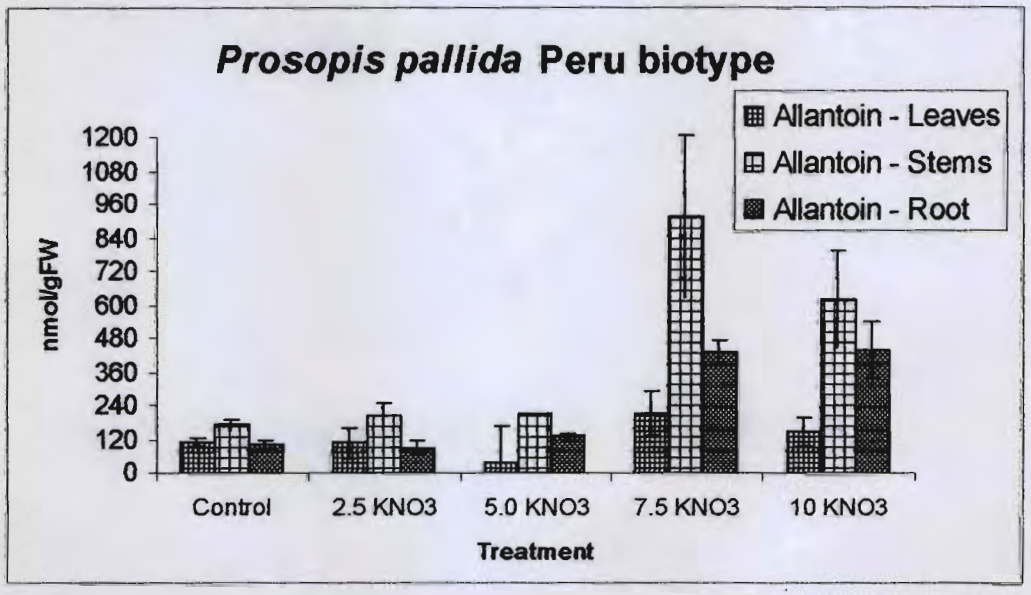


Figure 6: Allantoin determination in leaves, stems and roots of *Prosopis pallida* (Peru ecotype) affected by four concentration of nitrate (2.5, 5.0, 7.5 and 10 mM) as source of nitrogen nutrition. Leaves, stems and roots were extracted in 80% ethanol in ratio of 1:5

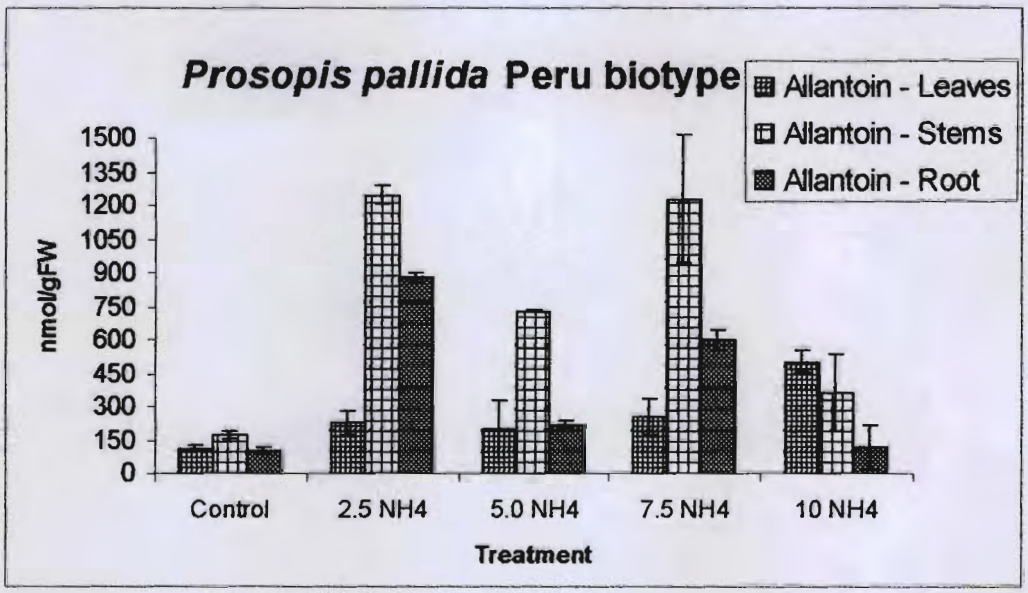


Figure 7: Allantoin determination in leaves, stems and roots of *Prosopis pallida* (Peru ecotype) affected by four concentration of ammonium (2.5, 5.0, 7.5 and 10 mM) as source of nitrogen nutrition. Leaves, stems and roots were extracted in 80% ethanol in ratio of 1:5

B.2.2.2. DETERMINATION OF ALLANTOATE IN DIFFERENT PLANT ORGANS.

Figure 10 and 11 shown that salinity and ammonium affected significantly, while figure 9 shown the effect of nitrate, which was not as significant as the other two.

The response was similar at those described in the biomass content.

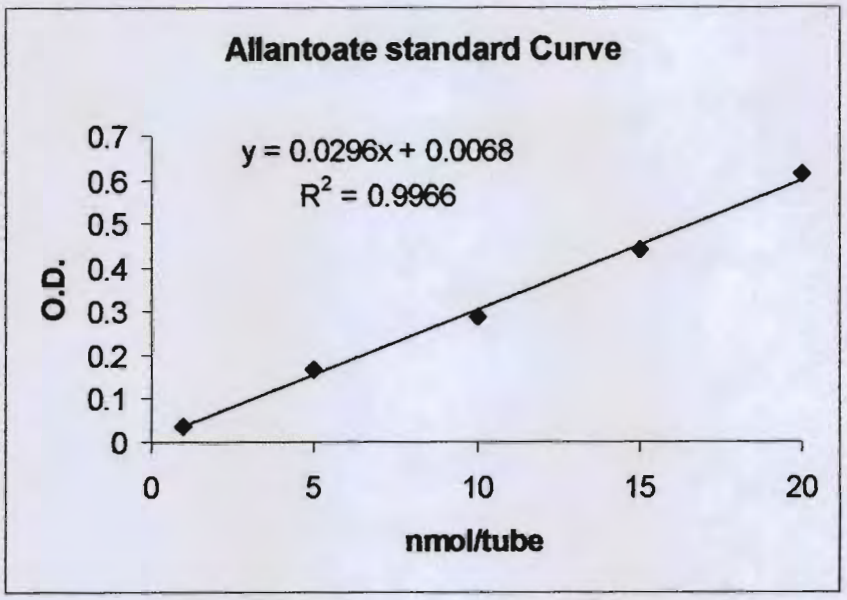


Figure 8: Allantoate standard curve

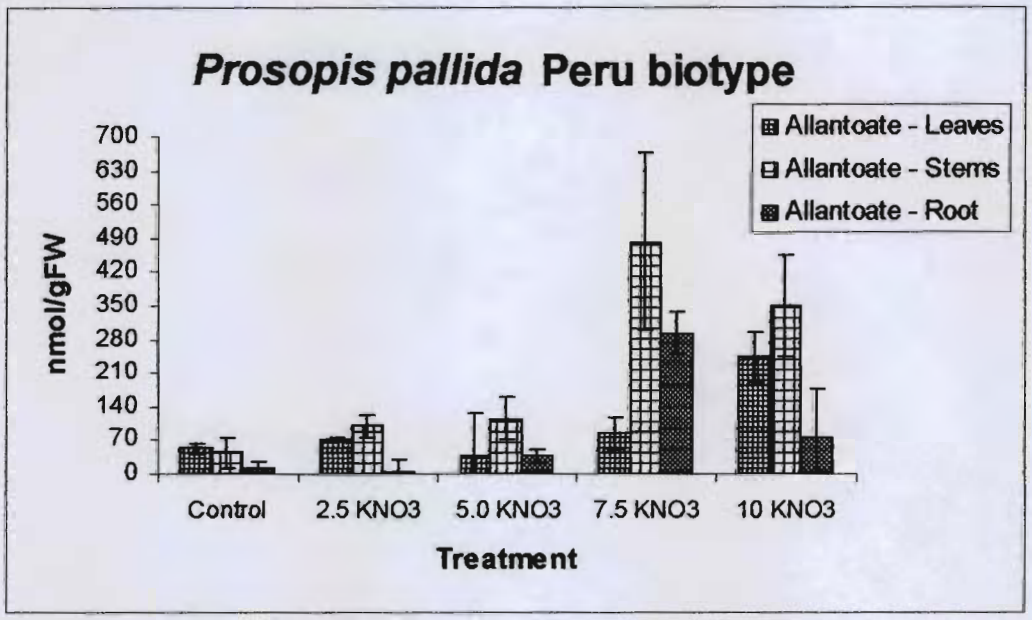


Figure 9: Allantote determination in leaves, stems and roots of *Prosopis pallida* (Peru ecotype) affected by four concentration of nitrate (2.5, 5.0, 7.5 and 10 mM) as source of nitrogen nutrition. Leaves, stems and roots were extracted in 80% ethanol in ratio of 1:5

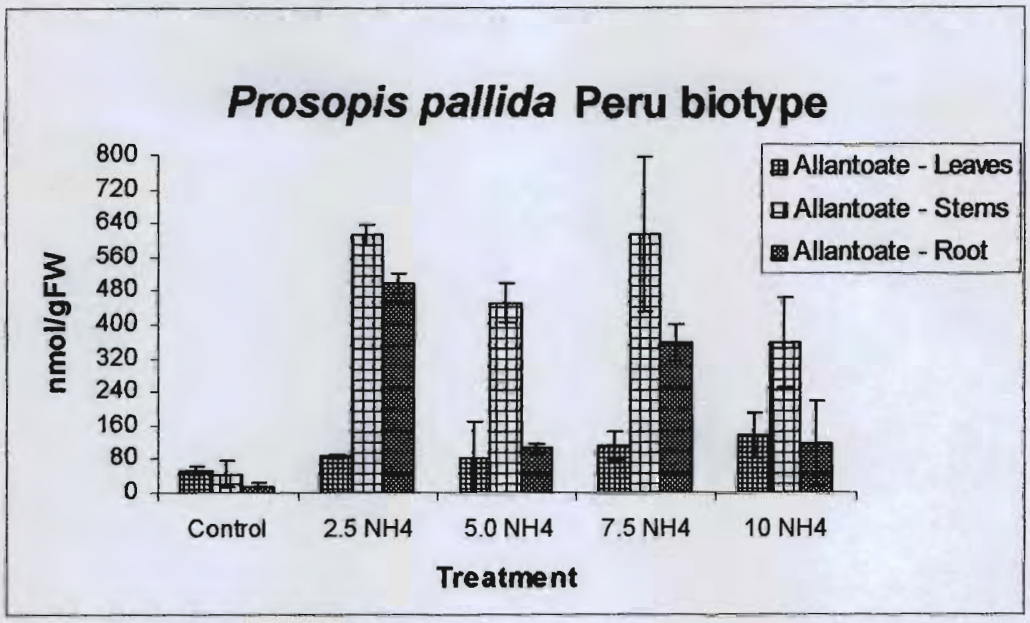


Figure 10: Allantoate determination in leaves, stems and roots of *Prosopis pallida* (Peru ecotype) affected by four concentration of ammonium (2.5, 5.0, 7.5 and 10 mM) as source of nitrogen nutrition. Leaves, stems and roots were extracted in 80% ethanol in ratio of 1:5

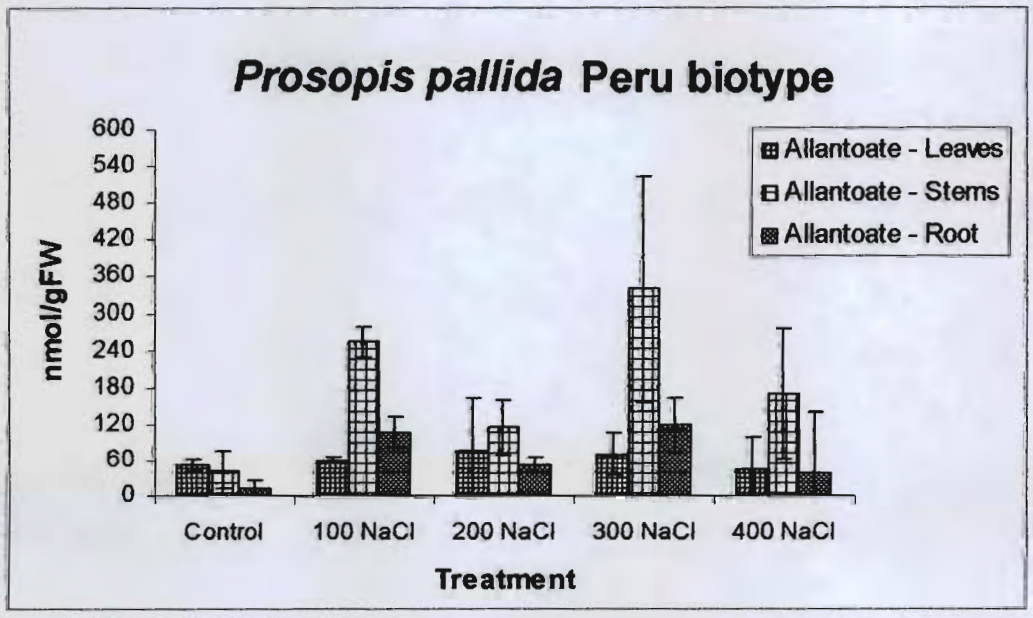


Figure 11: Allantoate determination in leaves, stems and root of *Prosopis pallida* (Peru ecotype) affected by four different concentration of NaCl (100, 200, 300 and 400 mM). Leaves, stems and roots were extracted in 80% ethanol in ratio of 1:5

B.2.3. EFFECT OF NITROGEN AND SALINITY IN THE PROTEIN ACTIVITY.

Figure 12 shown the AO and XDH protein activities of *Prosopis pallida* (Peru ecotype) seed as affected by two nitrogen sources and salinity.

The activity tends to increase with small concentration, reach a peak and then decrease with high concentration (Gauss tendency).

AO and XDH activities were determinate in those concentrations with highest activity and the activity of the combination of treatments.

Protein activity increased with the nitrogen supply and salinity. Salinity decreased the activity of NO_3^- and NH_4^+.

AO activity in leaves shown four bands while no activity was registed for roots in any of the treatments.

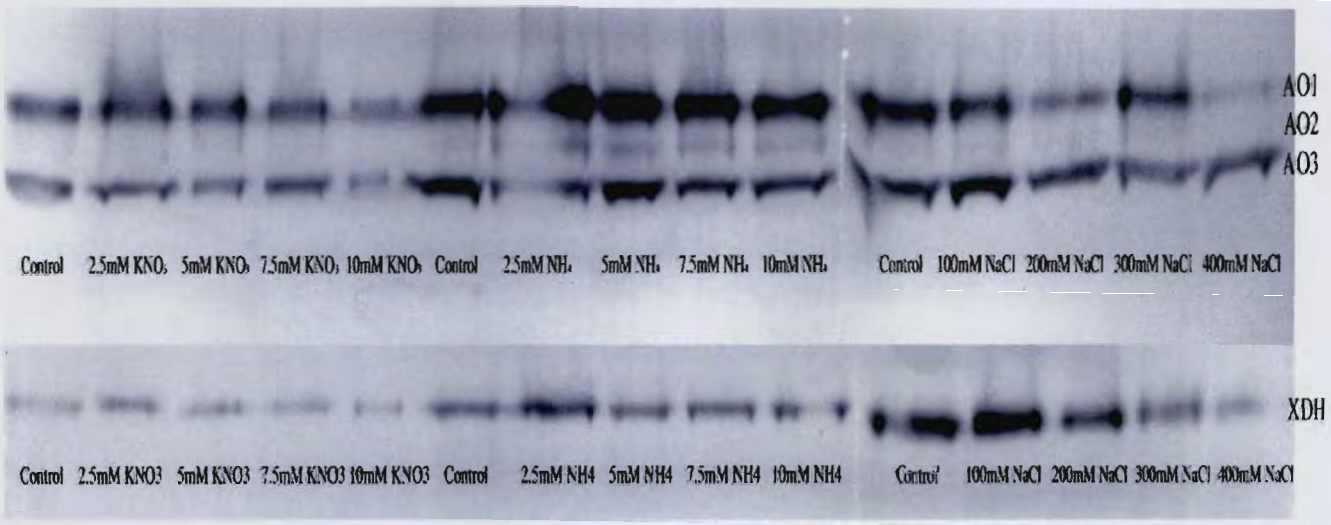


Figure 12: AO and XDH activities in *Prosopis pallida* (Perú ecotype) seeds after they were imbibitated in 2.5, 5.0, 7.5 and 10 mM NO_3^- and NH_4^+ and 100, 200 and 300 mM NaCl at 35°C for 24h of grown. Supernatant samples were subjected to native PAGE, followed by AO activity staining with cinnamaldehyde as substrate and XDH activity staining with hypoxanthine and xanthine as substrates.

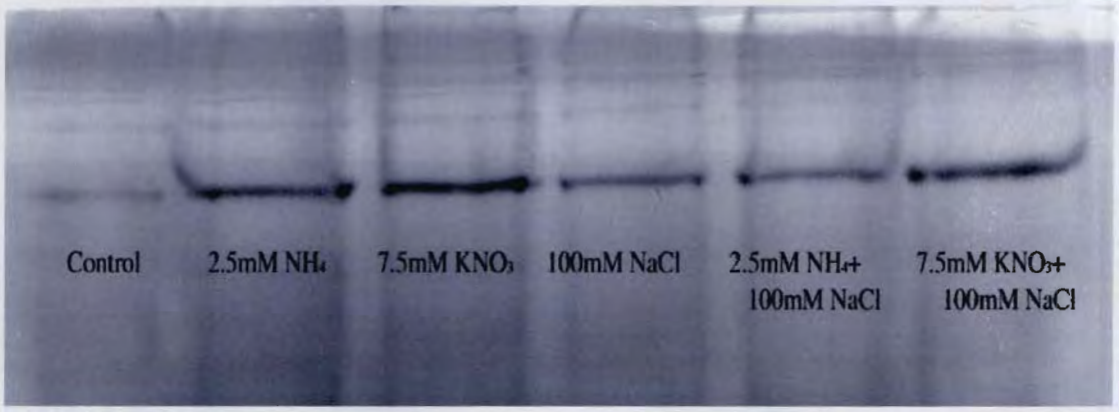


Figure 13: XDH activity in *Prosopis pallida* (Perú ecotype) seeds after they were imbibitated in 2.5 mM NH_4^+, 7.5 mM NO_3^-, 100 mM NaCl, 2.5 mM NH_4^++100 mM NaCl and 7.5 mM NO_3^-+100 mM NaCl at 35ºC for 24h of grown. Supernatant samples were subjected to native PAGE, followed by XDH activity staining with hypoxanthine and xanthine as substrates.

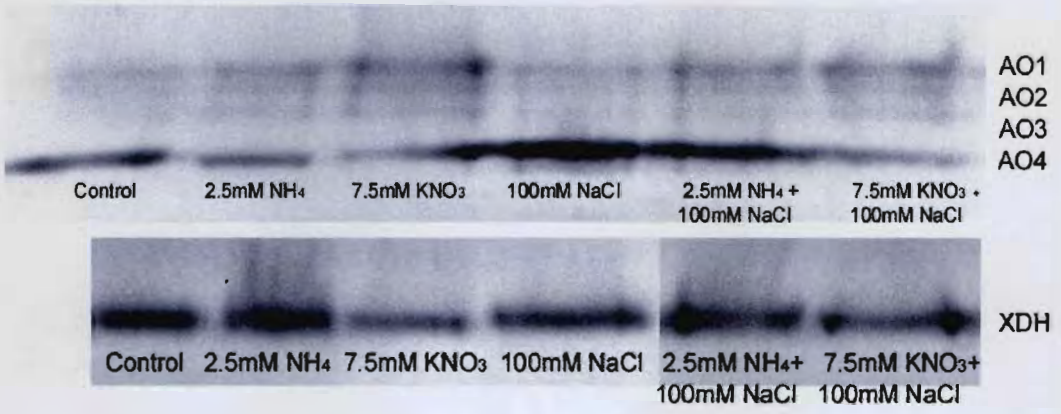


Figure 14: AO and XDH activities in *Prosopis pallida* (Perú ecotype) leaves after they were imbibitated in 2.5 mM NH_4^+, 7.5 mM NO_3^-, 100 mM NaCl, 2.5 mM NH_4^++100 mM NaCl and 7.5 mM NO_3^-+100 mM NaCl at 35ºC for 24h of grown. Supernatant samples were subjected to native PAGE, followed by AO activity staining with cinnamaldehyde as substrate and XDH activity staining with hypoxanthine and xanthine as substrates.

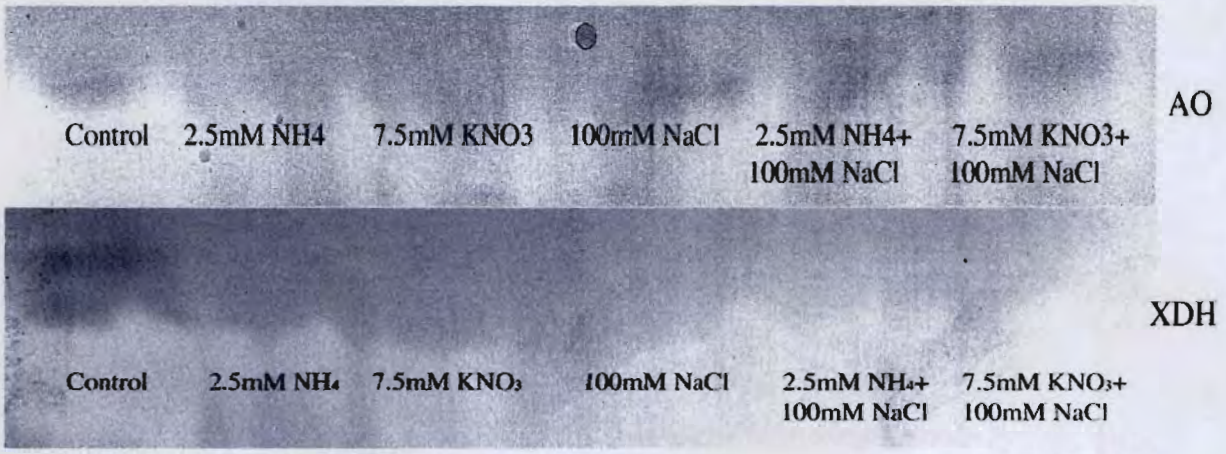


Figure 15: AO and XDH activities in *Prosopis pallida* (Perú ecotype) roots after they were imbibitated in 2.5 mM NH_4^+, 7.5 mM NO_3^-, 100 mM NaCl, 2.5 mM NH_4^++100 mM NaCl and 7.5 mM NO_3^-+100 mM NaCl at 35°C for 24h of grown. Supernatant samples were subjected to native PAGE, followed by AO activity staining with cinnamaldehyde as substrate and XDH activity staining with hypoxanthine and xanthine as substrates.

B.2.4. CHLOROPHYLL DETERMINATION

Chlorophyll content in both ecotypes was no significantly different.

Salinity increased the chlorophyll content in both ecotypes and increased the effect of ammonium in the plant.

Nitrate and ammonium effects were not significantly different to the control.

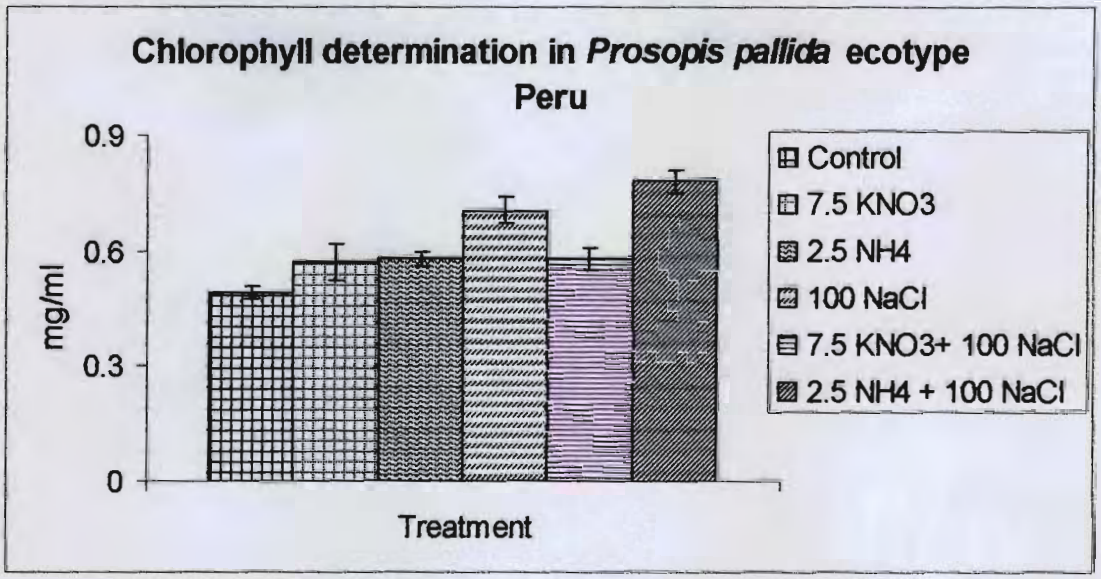


Figure 16: Chlorophyll determination in leaves of *Prosopis pallida* Peru ecotype as affected by 100 mM NaCl, and 7.5 mM NO_3^-, 2.5mM NH_4^+ as source of nitrogen nutrition, and the combination of it with salinity. Plants were harvested after 44 DAT.

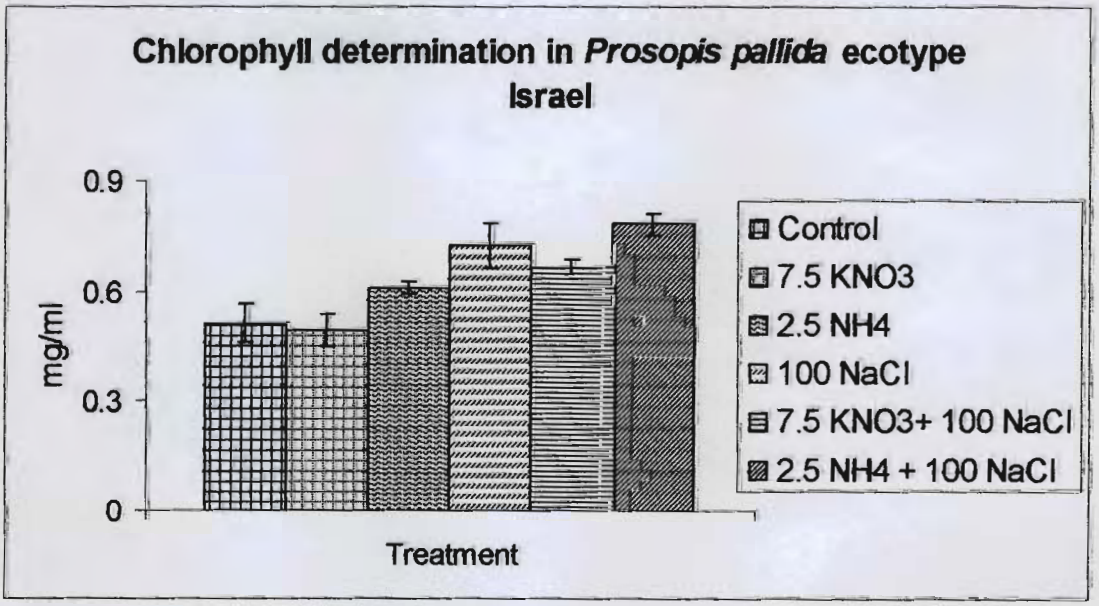


Figure 17: Chlorophyll determination in leaves of *Prosopis pallida* Israel ecotype as affected by 100 mM NaCl, and 7.5 mM NO_3^-, 2.5mM NH_4^+ as source of nitrogen nutrition, and the combination of it with salinity. Plants were harvested after 44 DAT.

B.2.5. IMMUNOBLOT ANALYSIS

Immunoblot analysis showed no difference between the activities of the treatments as XDH activity. This suggest the presence of other proteins different than XDH.

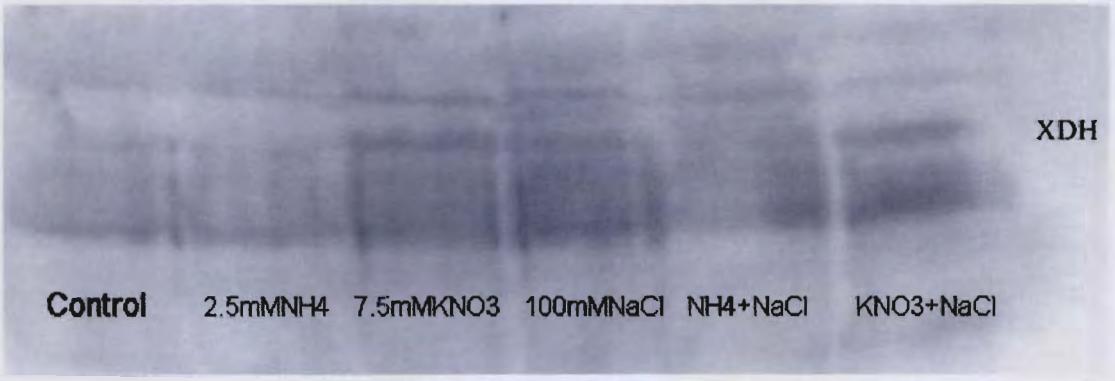


Figure 18: Immunoblot analysis of XDH. Crude extracts of seeds of *Prosopis pallida* plants were fractionated on Native-PAGE. The gel was loaded with approximately 120 µg of total soluble protein and examinated with anti-XO antibody. Membrane was developed with Horseradish Peroxidase.

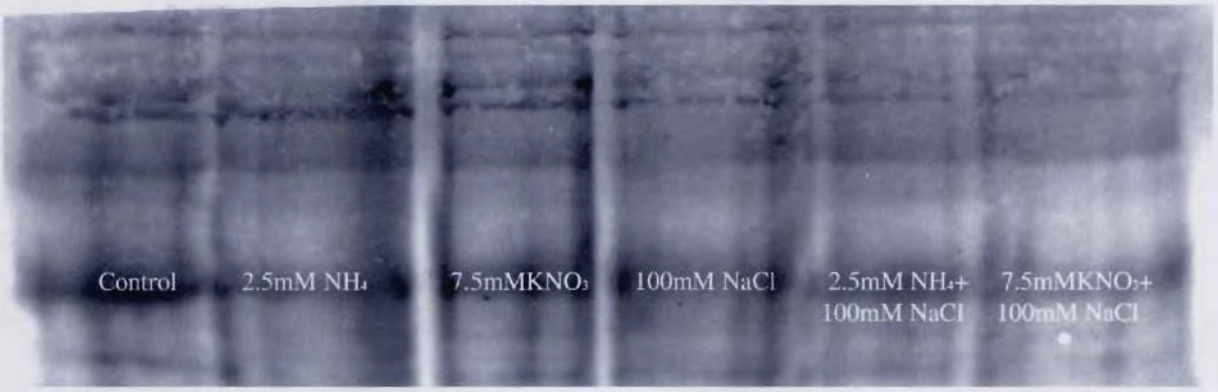


Figure 19: Immunoblot analysis of XDH. Crude extracts of seeds of *Prosopis pallida* plants were fractionated on Native-PAGE. The gel was loaded with approximately 120 µg of total soluble protein and examinated with anti-XO antibody. Membrane was developed with alkaline phosphatase activity with BCIP and NBT in developing buffer.

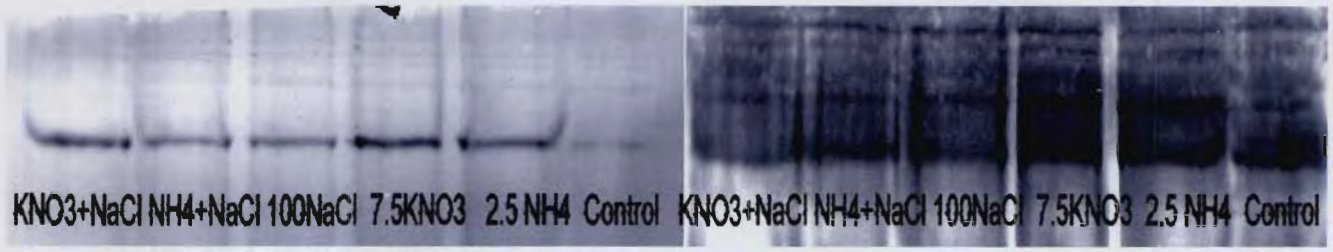


Figure 20: Activity and NBT- Immunoblot activity against XDH.

B-2 PERU

B-2-1 ACTIVITIES OF THE PERIOD

B-2-2 EXERIMENT 1: GENOTYPE COLLECTION

MONITORING OF THE SELECTED PROSOPIS GENOTYPES.

During this period - November 2005 - April 2006 - the selected genotypes located at Piura and Tumbes Departments, were monitoring in order to check out the annual main pod production which usually happened between January and March.

The pod production is important for the normal development of the study because the reproduction material (seeds) for the experiments come from the ripe Prosopis pods.

Until now the Piura Region has experimented a continuous dry weather causing the not production or a very low production of Prosopis pods. So we do not have the necessary amounts of seeds to carry out the experiments in Piura and Israel Research Laboratories.

The monitoring in this period shows the effects of the dry weather. Most of the genotypes Prosopis trees did not produce any pods; some of them gave a very low pod production. Some of them showed a slow flowering and fructification periods so up to day these trees do not have ripe pods and we can not collect any pods. As we explain in the prior report, some trees started the flowering period in October but this was not successfully, the flowers have failed to develop. Table 1 show the behavior of Prosopis genotypes regard their flowering and fructification periods.

Table 1: Behavior of Prosopis genotypes regards their flowering and fructification periods.

Genotype	Data of flowering	Data of fructification	Data of Harvesting	Fruit taste
Salinas 1	Absent	Absent	Absent	----------
Salinas 2	Absent	Absent	Absent	----------
Salinas 3	Absent	Absent	Absent	----------
Salinas 4	Absent	Absent	Absent	----------

Salinas 5	Absent	Absent	Absent	----------
Salinas 6	January	March	Not jet	----------
Ñapique 3*	Absent	Absent	Absent	----------
Ñapique 4	Absent	Absent	Absent	----------
Manglar 1*	January	March	April	Bite
Manglar 2	January	March	Marzo, Abril.	Bite
Parachique 1	January	March	Not jet	Sweet
Virrila 01	January	March	Not jet	----------
Virrila 02	January	March	March- April,	Sweet
Virrila 03*	January	March	March- April	Sweet
Virrila 04	January	March	Not jet	----------
Virrila 05	January	March	Not jet	----------
Virrila 06	January	March	Not jet	----------
Tumbes 01	January	March	April	Bite
Tumbes 02	January	March	Not jet	----------
Tumbes 03*	—	—	Absent	----------
Tumbes04	January	March	Not jet	----------
Tumbes 05	January	March	Not jet	----------
Tumbes 06	January	March	April	Sweet
Tumbes 07*	January	March	Not jet	----------
Tumbes 08	January	March	Not jet	----------
Tumbes 009	---	---	Ausencia	----------
Tumbes10	January	March	April	Bite
Tumbes 11	January	March	April	Bite
Tumbes 012	January	March	Not jet	----------
Tumbes 13	January	March	Not jet	----------
Tumbes 14	---	---	Absent	----------
Tumbes 15	January	March	April	Sweet
Tumbes 16	---	---	Absent	----------
Tumbes 17	January	March	April	Sweet
Mancora18	January	March	Not jet	----------
Mancora19	January	March	Not jet	----------

*: Genotypes for experiments.

B-2-2 EXPERIMENT 2:FIELD PLOTS OF TUMBES GENOTYPES

The growth parameters under salt and non salt treatments of the seedlings plants from Tumbes Prosopis genotypes were inform in the prior report until the 75 days of the edge of he plants. After this time, the plants were continuously evaluated and the records were not better than the Virrila 3, Ñapique 3 and Manglar 1 genotypes. The growth parameters on the 245th day are detailed in the Table 2.

Table 2: Growth parameters at 245 days edge of the seedling plants from Prosopis genotypes under salt treatment.

Edge (days)	Prosopis Genotype	Number of nodules	Height (cm)	Stem Diameter (mm)
245	Manglar 1	22,4	46,00	4,264
	Virrila 3	28,0	65,17	5,458
	Ñapique 3	20,3	40,89	4,193
	Tumbes 03	19,4	36,33	4,517
	Tumbes 015	28,6	39,66	4,816

B-2-3 DETERMINATIONS

Samples description

The determinations were carried out on the seedling plants that come from the Virrila (Virrila 3), Manglar de San Pedro (Manglar 1) and Ñapique (Ñapique 3) Prosopis genotypes. The seedlings plants were 11 months old. Salt treatment: 10-12 mS/cm, 4 L a week. The fertilizer formula: 17-17-17 g NPK, each 2 months.
Testify is a seedling plant without salt treatment but with the same watering and fertilization regime.

Biomass estimation

Materials and methods

The plants were extracted from the pots (3 plants for genotype). Each one was sectioned on root, stem and leaves. Roots and leaves were immediately measured and weighed. From this weigh we obtain the fresh biomass (FB).

The parts of the samples were arranged in paper bags in order to start the dry process in an electric stove (70C, over night). After this time, the samples were weighed to obtain the dry biomass.

pH and soil conductivity

Materials and methods

The soil simples were converted into paste so the pH and the electric conductivity can be measured.

B-2-4 ANALYSIS OF K+, Na+, Ca+2, Mg+2 ON SEEDLING PROSOPIS GENOTIPES PLANTS UNDER SALINE AND NON SALINE CONDITIONS (10-12 dS/m).

Materials and methods

Dry samples were milled and sieved (1mmmesh). A 1,5g of each sample was burnt to ashes (500C, 4 hours). The ashes were dissolved with acid (10mL) and distilled water until 100mL of volume. The ions determinations were carried out on the Spectrophotometer Perkin Elmer 1100. The AOAC 985.01 and EPA 7000[a] norms were the references for the analysis.

Results and Discussion

Table 3: Morphologic Characteristics of the samples seedlings.

Genotype	Sample	Stem (cm)	Pivot Root (cm)	Secondary root (cm)
Manglar 1	San Pedro 2	68	85.2	48
	San Pedro 3	59	72	45
	San Pedro 8	66	68.8	34
	Mean value	**64.33**	**75.33**	**42.33**
Virrila 3	Virrila 1	87	85	49
	Virrila 6	58.5	57.5	60
	Virrila 4	76	42.5	57
	Mean value	**73.83**	**61.67**	**55.33**
Ñapique 3	Ñapique 1	74	83	88
	Ñapique 2	62.5	103	45
	Ñapique 5	70	72	45
	Mean value	**68.83**	**86**	**59.33**
Witness	Witness 2	73	63	89
	Witness 6	90	121	94
	Witness 8	75.5	96	61
	Mean value	**79.50**	**93.33**	**81.33**

Table 5: Fresh and dry Biomass

Part of the pant	Genotype	Dry biomass		Moisture		Fresh biomass
		Mean value	%	Mean value	%	Mean value
	Virrila 3	28.23	49.61	28.98	50.39	57.20
	Manglar 1	5.71	41.30	8.15	58.70	13.86
	Ñapique 3	14.94	42.88	18.88	57.12	33.82
Stem	Witness	41.35	44.14	51.79	55.86	93.14
	Virrila 3	3.65	48.32	3.98	51.68	7.63
	Manglar 1	2.06	40.33	3.01	59.67	5.08

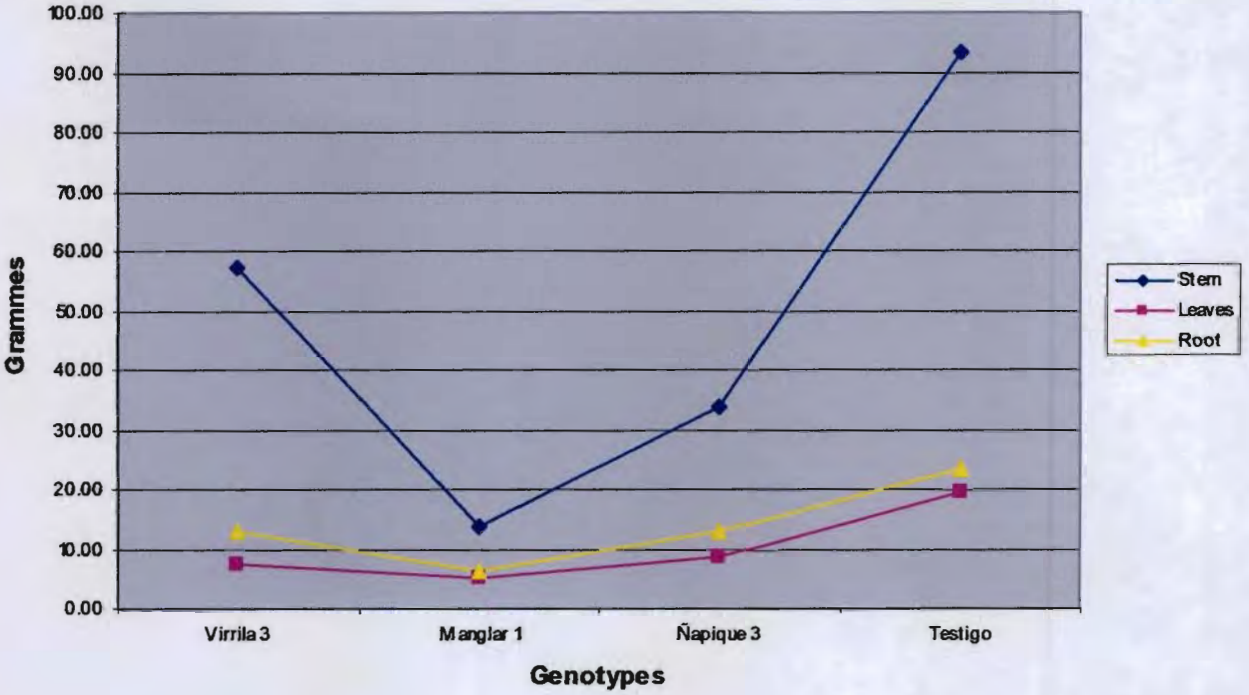
	Ñapique 3	3.82	44.55	4.88	55.45	8.70
	Witness	8.13	41.47	11.50	58.53	19.63
	Virrila 3	5.52	44.31	7.28	55.69	12.80
	Manglar 1	2.52	41.63	3.67	58.37	6.19
	Ñapique 3	4.47	34.79	8.32	65.21	12.79
Root	Witness	9.32	39.73	14.22	60.27	23.55

Ñapique 3, Virrila 3 show higher content of biomass (dry and fresh) than Manglar 1. These variations are present also in the measures of stem and root.

Table 4: pH and soil conductivity

Substrate Sample	pH	Conductivity (mS/cm)
Sample 1 salt T	9,13	18.43
Sample 2 salt T	9.23	20.13
Sample 3 salt T	9.1	21.5
Mean value	**9.17**	**20.02**
Sample 1 non salt T	8.12	3.45
Sample 2 non salt T	8.56	2.17
Sample 3 non salt T	8.37	2.4
Mean value	**8.35**	**2.67**

Fresh biomass



Dry biomass

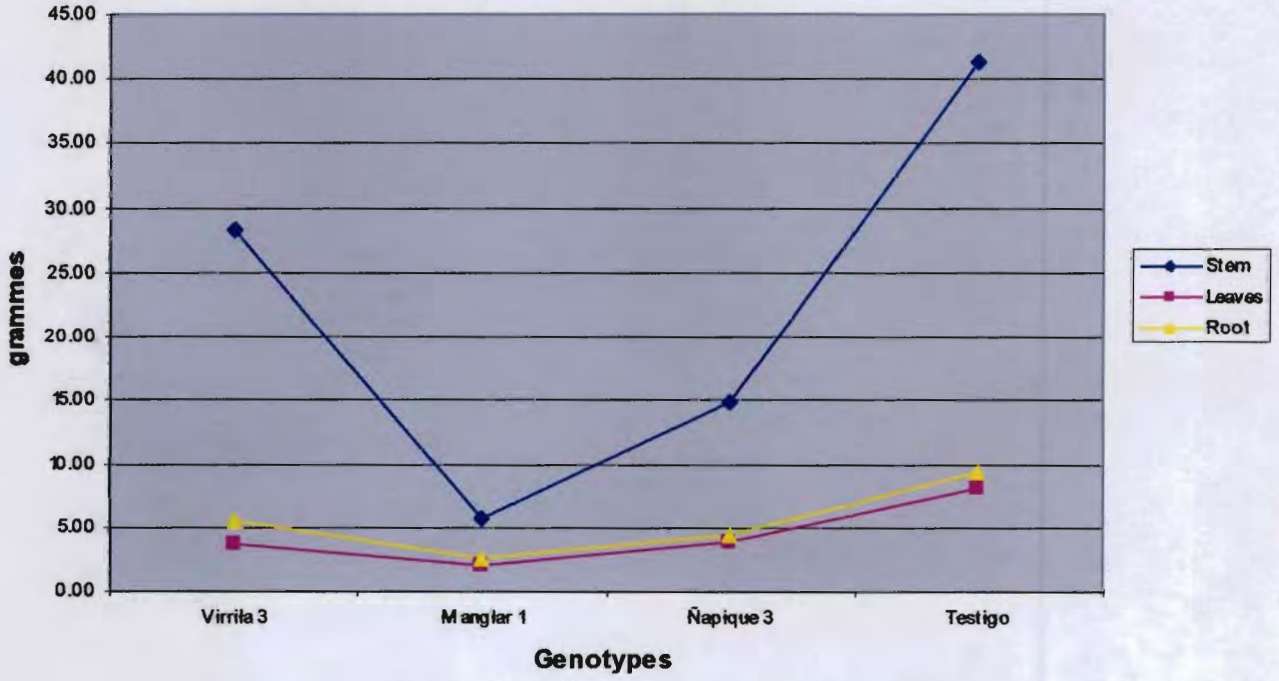


Table 6. Ions contents on the seedlings plants components

mol.Kg^{-1} DM= moles/kilograms of dry matter.

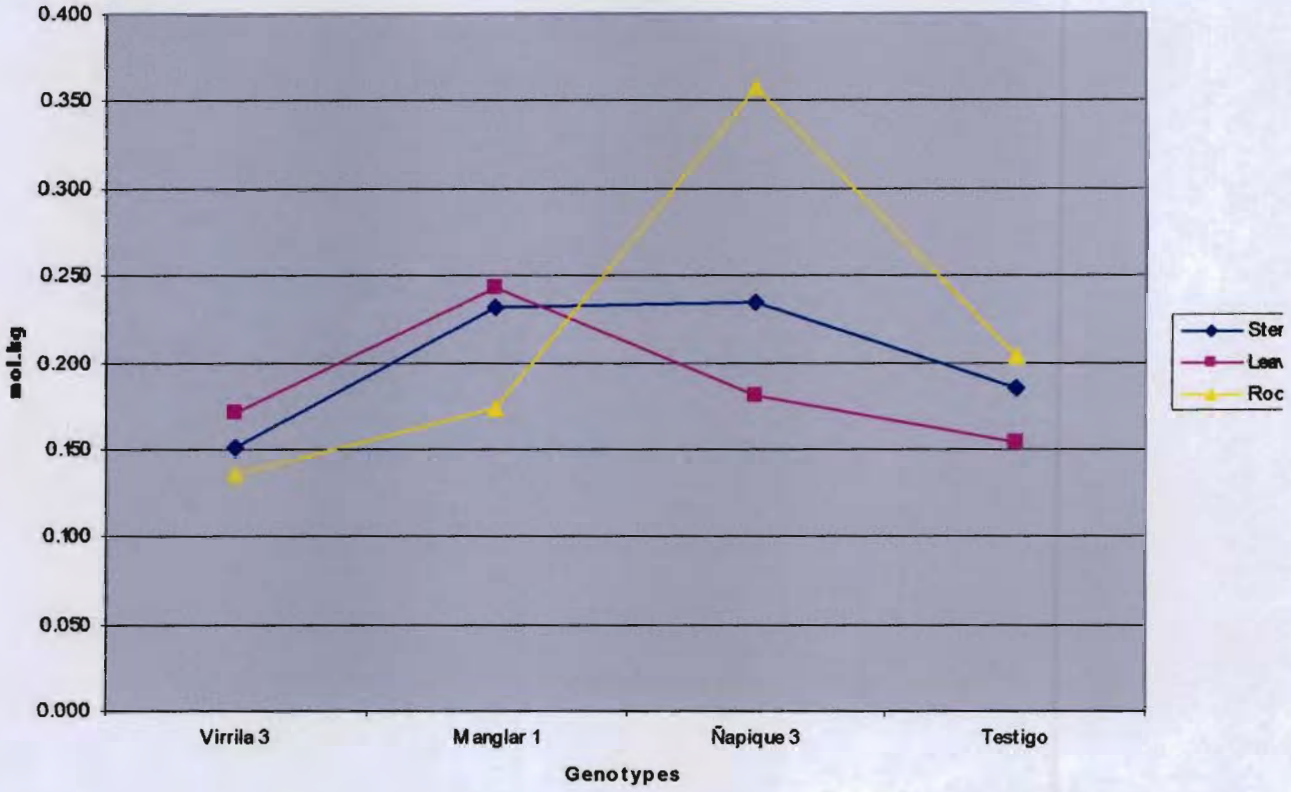
ORGAN		Ca^{++}		Mg^{++}		K^{+}		Na^{+}	
	GENOTYPE	%	mol.Kg^{-1} DM	%	mol.Kg^{-1} DM	%	mol.Kg^{-1} DM	%	mol.Kg^{-1} DM
	Virrila 3	0.603	0.151	0.078	0.032	1.301	0.333	0.280	0.122
	Manglar 1	0.930	0.232	0.127	0.052	1.317	0.337	0.372	0.162
	Ñapique 3	0.939	0.234	0.113	0.046	2.004	0.513	0.364	0.158
Stem	**Witness**	**0.742**	**0.185**	**0.117**	**0.048**	**2.019**	**0.517**	**0.250**	**0.109**
	Virrila 3	0.683	0.171	0.123	0.051	2.011	0.514	0.352	0.153
	Manglar 1	0.972	0.243	0.147	0.060	1.633	0.418	0.420	0.183
	Ñapique 3	0.723	0.180	0.141	0.058	2.117	0.541	0.278	0.121
Leaves	**Witness**	**0.615**	**0.154**	**0.143**	**0.059**	**1.786**	**0.457**	**0.251**	**0.109**
	Virrila 3	0.543	0.136	0.158	0.065	1.648	0.422	0.585	0.255
	Manglar 1	0.699	0.174	0.221	0.091	0.980	0.251	0.703	0.306
	Ñapique 3	1.433	0.358	0.325	0.134	1.517	0.388	0.753	0.327
Root	**Witness**	**0.815**	**0.203**	**0.230**	**0.095**	**0.923**	**0.236**	**0.405**	**0.176**

The contents of Mg^{++} and Na^{+} on the roots are higher than the leaves and stem content.

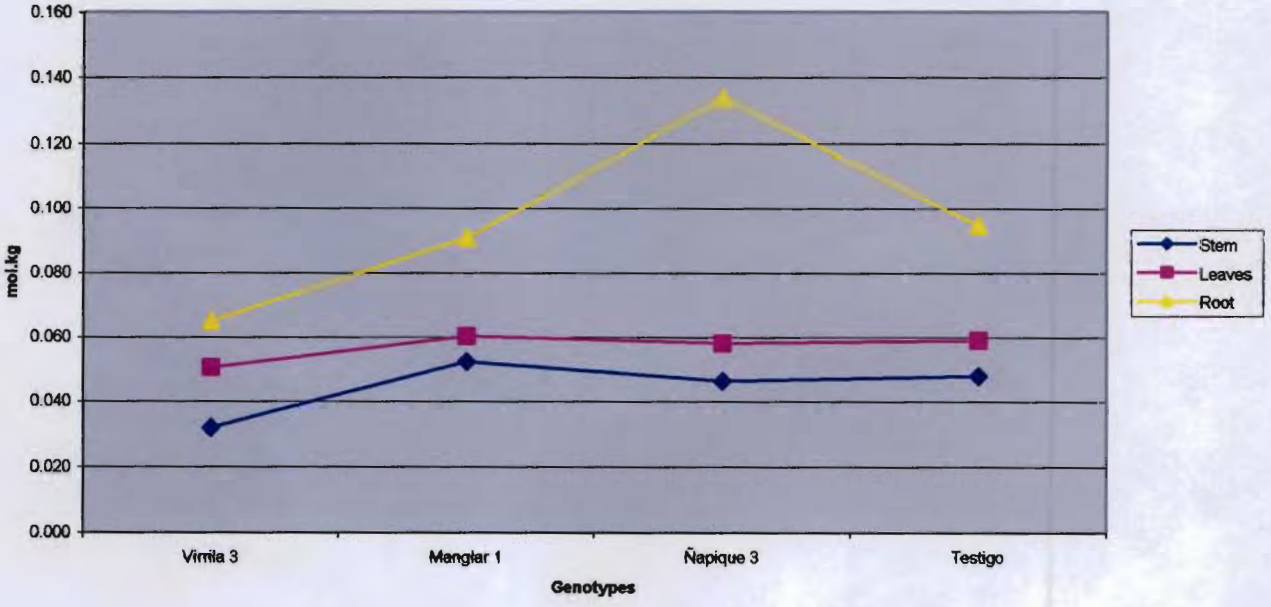
K^{+} content on the leaves and stem is higher than the roots. This value is one of the indicative mechanisms of the transpiration function that show prosopis tree under salt stress.

Ca^{++} content is not too much difference between the organs.

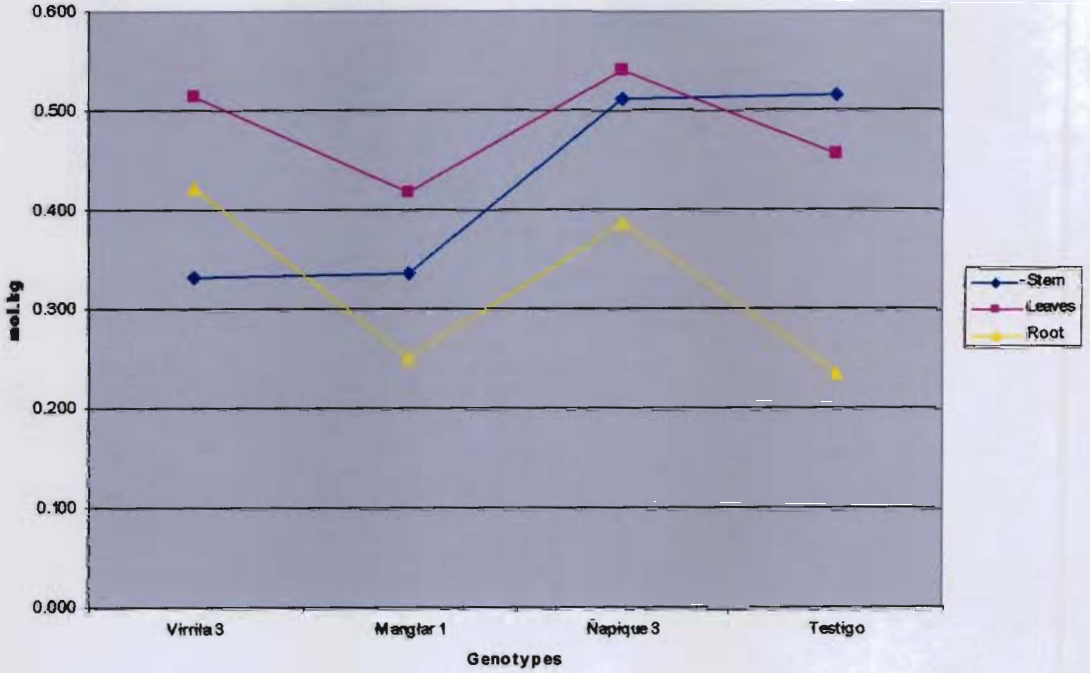
Amounts of calcium



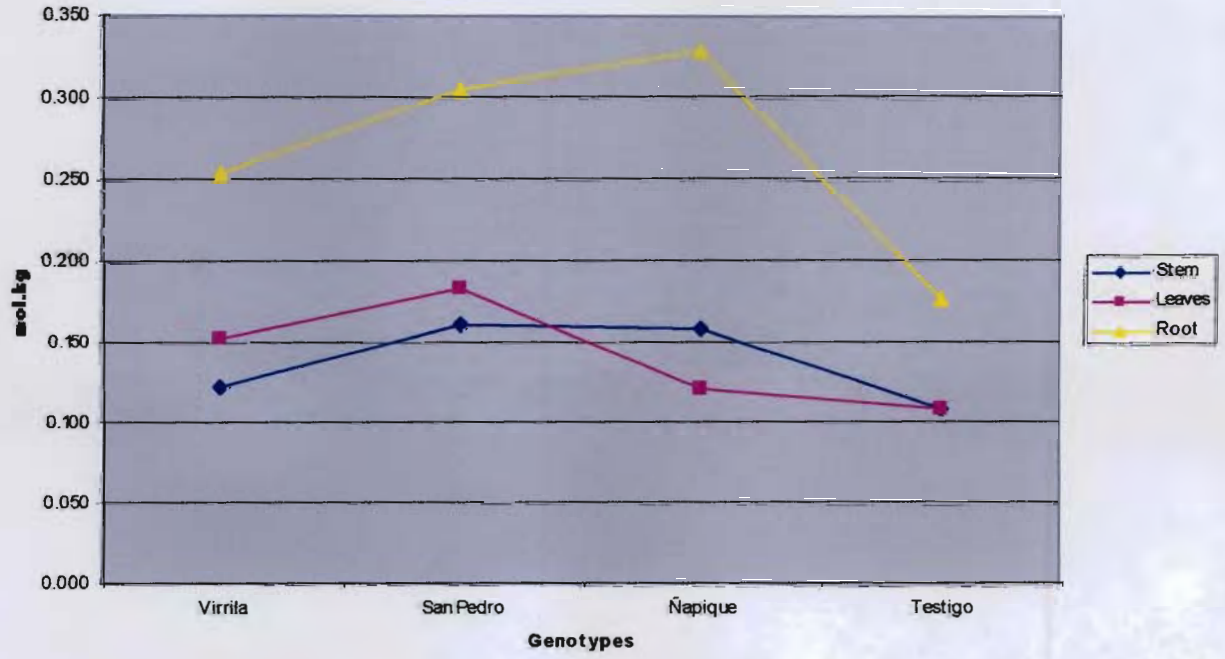
Amounts of magnesium



Amaunts of potassium



Amaunts of sodium



B-2-6 EXPERIMENT 3: MINERAL NUTRITION STUDIES ON PLANTS UNDER SALINE AND NON SALINE CONDITIONS .

Aim:

Determination of changes in the levels of N assimilation enzymes induced by salinity in Prosopis roots.

Activities:

It is just starting with the collects of the genotypes pods in order to extract the seeds to produce the correspondent seedlings. We will use two –three mont old seedlings.

B-2-7 EXPERIMENT 8: N2 FIXATION AS AFFECTED BY SALINITY, INORGANIC N IONS AND Mo

Aim:

To study the sensitivity of Rhizobium to salinity and inorganic nitrogen (ammonium or nitrate) to infect the roots and form active symbiotic nodules. Study of the amelioration of salinity inhibition of N assimilation by N and Mo levels in the nutrient solution.

Activities:

It is just starting with the collects of the genotypes pods in order to extract the seed to obtain the correspondent seedlings. Will be used one year old prosopis seedlings. The seedlings must be produced in 2005 but this was not possible as we explain above; the dry weather during the last 3 years in the Northern Coast of Peru has had not helped the trees to produce pods. So this experiment is late and we must wait for a year (2007).

C) STRENGTHENING OF DEVELOPING-COUNTRY INSTITUTIONS:

Mrs. Evelyn Farfan a M.SC student from Peru continues her studies at the Albert Katz Int'l School for Desert Studies at the Ben-Gurion University of the Negev, the Jacob Blaustein Institutesfor Desert Research under the supervision of Drs.Moshe Sagi and Jhonathan Ephrath.

D) FUTURE WORK:

Technical Work Plan for Peru and Israel (2004 – 2006)

Exp. 8: N2 fixation as affected by salinity, inorganic N ions and Mo

Aim:

Study the sensitivity of *Rhizobium* to salinity and inorganic nitrogen (ammonium or nitrate) to infect the roots and form active symbiotic nodules. Study of the amelioration of salinity inhibition of N assimilation by N and Mo levels in the nutrient solution

Activities:

The experiment will carry out in the 3rd year and about the following instructions we must be install this in 2005: potted plants will be used with one year old *Prosopis* seedlings.

Treatments: Nitrate (0 and 5 mM KNO3), Mo (0.2 and 3 µM) and NaCl (0, and 200 mM).

Experimental design: 8 treatments x 4 rep x 5 plants/treatment = 160 plants (pots of 30 L)

Determinations:

Nodulation: nodule number and fresh weight

N-fixation: methods suitable for the prosopis nodules will be determined

Nodule enzyme activity: Dissimilatory NR, GS, GOGAT, PEPc, free and bound MoCo.

Root enzyme activity: Assimilatory NR, NiR, GS, GOGAT, PEPc, free and bound MoCo.

Root: length, branching, nitrogen uptake areas along the root uptake: will be estimated from depletion of nitrate solutions driped through the pot

Organic N: in different parts of the plant

Mineral content: will be estimated in different parts of the plants - K+, Na+, Mg+2, Ca+2, NH4+, NO3-, Cl-, SO4-2, P.

Xylem sap composition: amides and amino acids, ureids, minerals, malate, cytokinins, absicic acid

Section II: Project Management and Cooperation

A) Managerial Issues: A student from Peru continues to do her M. Sc studies.

B) Special Concerns: No special concerns.

C) Collaboration, Travel, Training and Publications:

In February 2005, the student Evelyn Farfan started her two years masters study in the Albert Katz Intl' School for Desert Studies.

No publications were published yet from this project.

D) Summary of Requests for CDR Program Actions.

During this period, we requests from the U.S. Embassy in Tel Aviv and got the approval for the following:

1. We ask your approval to transfer an amount of US$ 8,500 from the item of salaries to the item of Materials.
2. To transfer US$2020 to domestic travel in the Peruvian budget.
3. To transfer an amount of US$ 7,500 from Salaries to International Travel (which will cover the two roundtrip tickets of Dr. Moshe Sagi and Dr. Jhonathan Ephrath to Peru and the return ticket of the M. Sc Student, Mrs. Evelyn Roxana Farfán).