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Salinity effects on germination and growth of *Prosopis chilensis*

Efectos de la salinidad en la germinación y crecimiento de *Prosopis chilensis*

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ABSTRACT

Prosopis chilensis, also named Chilean algarrobo, is a leguminous tree, described as highly tolerant to heat shock, water deficit, salinity and injury stress. The objective of this research was to investigate the germination and growth responses of seeds, seedlings and young plants of *P. chilensis* under increasing concentrations of NaCl. Six groups of *P. chilensis* seeds were germinated. One group was watered with distilled water (control group) and the other five were treated with 0.3, 0.4, 0.5, 0.6, and 0.8 M NaCl. Control seeds germinated 100% in 48 hours, while those imbibed in 0.3M and 0.4M NaCl reached 100% after 21 days of imbibition. In 0.5M NaCl there was 66% germination after 28 days of imbibition. Above 0.5M NaCl there was no germination. Seedlings of 0.5 cm long germinated and maintained for 8 weeks in the same NaCl concentration used for germination grew less than the control seedlings. Seedlings and young plants of algarrobo coming from seeds germinated and grown for 14 days in distilled water could grow a lot better in 0.3 to 0.8 M NaCl. The seedlings reached a 69% growth of the control seedlings in 0.8M NaCl after 21 days of treatment, while plants showed 58% growth of the control group after 8 weeks of treatment at this concentration. It was also observed that the seedling cotyledonary expansion was delayed by salinity. Under salt treatment the number of leaves per plant was reduced with a tendency to become significantly different to the control with 0.8M NaCl after 8 weeks of treatment. The conclusion of this research is that algarrobo can be consider as a *Prosopis* spp. highly tolerant to salinity because plants and seedlings can grow in 0.8M NaCl.

Key words: algarrobo, salinity, salt tolerance, imbibition, stress.

RESUMEN

Prosopis chilensis, llamado algarrobo chileno, es un árbol leguminoso, considerado tolerante al estrés térmico, al déficit hídrico, a la salinidad, y al estrés de heridas. El objetivo de este trabajo fue investigar la germinación y crecimiento de plántulas y plantas jóvenes de *P. chilensis* a diferentes concentraciones de NaCl. De seis grupos de semillas, un grupo fue regado con agua destilada (grupo control) y los otros 5 fueron tratados con 0,3, 0,4, 0,5, 0,6, y 0,8 M NaCl. Las semillas control germinaron 100% en 48 horas, mientras que las embebidas en 0,3M y 0,4M NaCl germinaron en un 100% después de 21 días de imbibición. En 0,5M de NaCl hubo un 66% de germinación a los 28 días de imbibición. Sobre 0,5M de NaCl no hubo germinación. Plántulas de 0,5 cm de longitud, provenientes de semillas germinadas en diferentes concentraciones de NaCl y mantenidas por 8 semanas en las concentraciones de NaCl usada en la germinación, crecieron mucho menos que las plántulas control. Plántulas y plantas jóvenes de algarrobo provenientes de semillas germinadas y crecidas por 14 días en agua destilada pudieron crecer mejor en los tratamientos de 0,3 a 0,8 M de NaCl. Las plántulas regadas con 0,8M de NaCl alcanzaron un 69% del crecimiento del control después de 21 días de tratamiento, y un 58% del crecimiento del control a las 8 semanas de tratamiento. La expansión de los cotiledones de las plántulas también se retrasó con el tratamiento salino. El número de hojas por planta tiende a disminuir con la salinidad existiendo una tendencia a ser significativamente diferente al control con 0,8M de NaCl después de 8 semanas. La conclusión de esta investigación es que algarrobo debe ser considerado como una especie de *Prosopis* altamente tolerante a la salinidad porque plantas y plántulas de algarrobo pueden crecer en 0,8M NaCl.

Palabras clave: algarrobo, salinidad, tolerancia a la sal, estrés.

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INTRODUCTION

Trees of the leguminous genus *Prosopis* grow in arid and semi arid regions of tropical and subtropical areas of the world. They are considered underused trees, in spite that the genus has potential for food, timber, fuel production and control of desertification (Felker et al. 1981, Torres 1985). Among *Prosopis* spp., *Prosopis chilensis*, a Chilean mesquite named algarrobo, occurs in the arid and semi arid region of Chile, from Copiapó at the North (27° S) to Talca (37° S)(Roig et al. 1993). Algarrobo has also been planted in the Pampa del Tamarugal as part of the reforestation program of Corporación Nacional Forestal (Torres 1985).

Algarrobo trees are considered to be tolerant to water stress, low and high temperatures (Felker et al. 1983, Medina & Cardemil 1993), wound stress (Rodríguez & Cardemil 1994, 1995) and high salinity (Arce et al. 1990).

Medina & Cardemil (1993) demonstrated that *P. chilensis* is highly tolerant to heat shock with a strong expression of heat shock proteins. Rodríguez & Cardemil (1994, 1995) found that seedlings responded to mechanical injury with a precocious development of secondary roots and increasing expression of cell wall proteins.

Arce et al. (1990) have reported the germination responses of three species of *Prosopis: P. chilensis, P. tamarugo* and *P.alba* when the seeds were imbibed in different concentrations of NaCl. Felker et al. (1981), have also studied the growth of different *Prosopis* spp. at a maximum concentration of 0.5 M NaCl with almost no growth of *P. chilensis* at this concentration. However, studies on growth of seedlings and plants of *P. chilensis* in concentrations of NaCl higher than 0.5 M have not been performed even when the seedlings have to establish and plants have to grow in saline soils such as that of the Pampa del Tamarugal. Here the soil has a crust of salt with a thickness ranging from 10 to 60 cm and containing sodium, magnesium, potassium and calcium (Briner 1985). Algarrobo along with other *Prosopis* spp. such as *P. tamarugo* y *P. alba*, was used from 1965-1970 by the Corporación Nacional Forestal (CONAF) in a reforestation program to recover the ecology of salt flats of the Atacama Desert. In this program, nearly 3136 ha of the Pampa del Tamarugal was reforested with algarrobo (Aguirre & Wrann 1985).

The objective of this research was to test the hypothesis that *Prosopis chilensis* is highly tolerant to salt and, therefore, can germinate and grow at salt concentrations higher than that of sea water (0.5M NaCl) since is able to live over a crust of salt at the Pampa of Tamarugal. This research is re-evaluating the germination and growth of young plants and seedlings, and the leaf number produced per plant at increasing concentrations of NaCl from 0.3 to 0.8M.

MATERIAL AND METHODS

Germination experiments

Seeds of *P. chilensis* were collected in Peldehue, Central Chile, in March 1993. For germination, the seeds were scarified for 20 min in concentrated H_2SO_4, and rinsed several times in distilled water. The seeds were germinated on trays covered with humid paper (with distilled water or different concentrations of NaCl) and kept under continuous light during all the germination period. Germination was monitored every 8 h during the first 48 h after imbibition, and considered positive when the radicle appeared. For the seeds germinated in saline solutions, the germination was also monitored 14, 21 and 28 days after the start of treatments.

For germination experiments, 150 seeds were scarified and germinated at 25°C as described above and divided into six groups of 25 seeds each to be imbibed in different concentrations of NaCl. The group 1 was imbibed in distilled water; the group 2 was treated with 0.3M NaCl; the group 3 with 0.4M NaCl; the group 4 with 0.5 M NaCl; the group 5 with 0.6 M NaCl, and the group 6 with 0.8 M NaCl. Each experiment was repeated four times and the percent of germination was determined during 28 days. The significance of differences of germination among groups treated with different NaCl concentrations was evaluated by Kruskal-Wallis test after saline treatment.

Seedling growth experiments

Seedlings of 0.5 cm long derived from seeds germinated at 25°C in humid paper with distilled water (control experiment) and from seeds germinated in the same substrate but irrigated with 0.3, 0.4, 0.5, 0.6 and 0.8 M NaCl, were grown for 8 weeks in the same saline concentrations used for germination. The length of the seedlings were measured at the end of the 8 week period. An ANOVA and Tukey Test were performed to evaluate the significance of differences between lengths of seedlings at different NaCl concentrations.

In another experiment, seeds were germinated at 25°C in humid paper with distilled water. After germination the seedlings were grown for 14 days in the paper irrigated with distilled water, then divided into 5 groups, one for each saline treatment: distilled water (control); 0.4 M NaCl; 0.5 M NaCl; 0.6 M NaCl; 0.8M NaCl. The experiments were run with three replicates of each treatment and the growth was determined by measuring the length of the seedling axes. Growth determinations were made after 7, 14 and 21 days of treatment. An ANOVA of repeated measurements was performed to evaluate the significance of differences between groups of seedlings at different NaCl concentrations.

Plant growth experiments

Two month old plants were used for growth experiments under saline treatments. The plants were obtained from seeds germinated at 25°C in humid paper with distilled water and the seedlings were maintained in the

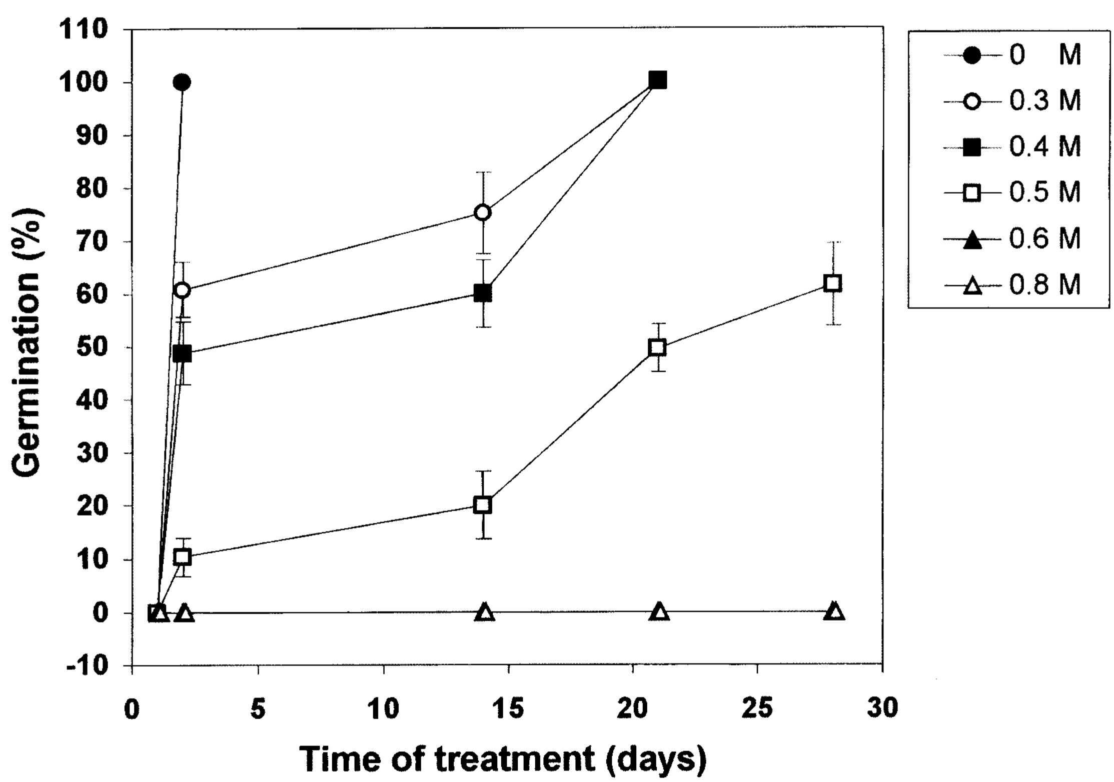

Fig. 1: Germination of seeds of *Prosopis chilensis* during 28 days of treatment at different concentrations of NaCl. The germination among treatments, after two days, showed significative differences (P<0.05; Kruskal-Wallis test). Vertical bars = standard deviations.

Germinación de semillas de *Prosopis chilensis* durante 28 días de tratamiento a diferentes concentraciones de NaCl. La germinación entre tratamientos, después de dos días, mostró diferencias significativas (P<0,05 ; Kruskal-Wallis test). Barras verticales = desviaciones estandard.

same substrate for 14 days into chambers at 25°C with a photoperiod of 16 h. After this period of time, the seedlings were moved to a perforated Styrofoam platform with the roots submerged into a recipient containing Hoagland II nutrient solution. After 4 weeks under this condition, the plants were transferred to pots with organic soil and separated in 6 groups irrigated with: distilled water; 0.3 M NaCl; 0.4 M NaCl; 0.5 M NaCl; 0.6M NaCl and, 0.8M NaCl. During the treatments the plants were kept in a greenhouse at a maximum temperature of 30°C in the day and a minimum of 18°C at night. Plant growth was monitored for two months by measuring the length of the stem and determining the number of leaves of the main stem. During the 8 week period of the experiment the branches were not developed. Therefore, they were not considered as a growth parameter. An ANOVA of repeated measurements was performed to determine the significance of differences of the length and number of leaves between groups of different saline treatments. Leaf area was estimated by multiplying the length by the width of the leaf. All the statistical tests were performed using the SYSTAT software.

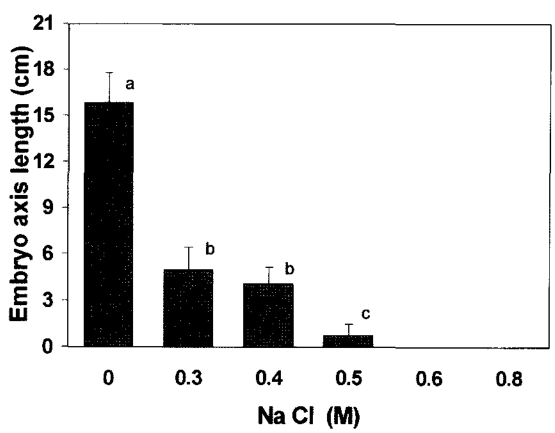

Fig. 2: Growth of seedlings after 8 weeks of treatment at different concentrations of NaCl. The seedlings were 0.5 cm long at the beginning of the experiment and came from seeds germinated at the same concentration of NaCl of the growth treatment. The significance of differences (noted by different letters) was determined by the ANOVA and Tukey test ($P<0.05$). Vertical bars = standard deviations.

Crecimiento de las plántulas después de 8 semanas de tratamiento a diferentes concentraciones de NaCl. Las plántulas tenían 0,5 cm de longitud al comienzo del experimento y provinieron de semillas germinadas a las mismas concentraciones de NaCl en que fueron crecidas. Las diferencias sig≠nificativas (señaladas por diferentes letras) fueron determinadas por la ANOVA y test de Tukey (P<0,05). Barras verticales = desviaciones estandard.

RESULTS

Germination rate decreased with increased NaCl concentration (Fig. 1). Thus, while the germination of the control group was 100% those treated with 0.3 and 0.4 M NaCl only reached 50% and 40% of germination, after two days respectively. However, after 14 days, these seeds reached 75% and 68% germination; 100% germination was achieved after 21 days of treatment in these two NaCl concentrations. Seeds treated with 0.5 M NaCl germinated 10% after 7 days, 20% after 14 days and, 68% after 28 days of treatment. The seeds imbibed in 0.6 and 0.8 M NaCl did not germinate.

Seedlings of the same age (0.5 cm long at the beginning of the experiment) derived from seeds germinated in 0.3, 0.4, 0.5, 0.6 and 0.8 M NaCl and grown for 8 weeks in the same saline concentrations used for germination, grew a lot less than the control seedlings which derived from seeds germinated and grown in distilled water (Fig. 2). After 8 weeks, the control seedlings had a length of 16 cm, while the seedlings germinated and irrigated with 0.3, 0.4, and 0.5 M NaCl were 5.5, 4.1 and 1.15 cm long, respectively. Thus, the last treatment showed a significative difference with the control group ($P<0.05$; ANOVA and Tukey test). Seeds treated with 0.6 and 0.8 M NaCl did not germinate either during these 8 additional weeks of imbibition.

Seedlings derived from seeds germinated and grown thereafter germination for 14 days in distilled water, were able to grow in all NaCl concentrations, although slightly less than the control plants (Fig. 3). The growth differences between control and experimental seedlings increased in all concentrations over time, showing a significative difference ($P<0.05$) between the length of the control seedlings and the length of those irrigated with 0.6-0.8 M NaCl after

21 days of treatment. The high tolerance of *P. chilensis* to salinity was evident when seedlings watered with 0.8 M NaCl were able to grow one cm (from 8 to 9 cm) during the period going from 7 to 21 days of treatment.

The cotyledonary expansion was also affected by high NaCl concentrations. Eleven out of 14 seedlings expanded their cotyledons when treated with water or 0.3, 0.4 and 0.5 M NaCl; 8 out of 25 when treated with 0.6 M NaCl and, 6 out of 25 when treated with 0.8 M NaCl.

When 6 weeks old algarrobo plants were irrigated with different NaCl concentrations for 8 weeks, no significant differences in the length of the stems of plants were found during the first 4 weeks of salt treatment. The same was observed with respect to the length of the stems between salt-treated plants and control plants (Figure 4). After this time, the differences in growth between control plants and those treated with 0.8 M NaCl became significantly different ($P<0.05$). At the end of the treatment, the plants irrigated with 0.8 M NaCl had grown in average 7 cm being 13 cm shorter than the control plants.

Considering the number of leaves present in the stem of the plants used in the experiment of Figure 4, this increased in all concentrations of NaCl, with not significant differences respect to the control plants after six weeks of saline treatment (Fig. 5). The number of leaves however, tends to increase slowly with time of treatment in all concentrations of NaCl. At the eighth week, the number of folioles between control plants and those treated with 0.8 M NaCl have a statistical difference of $P=0.094$ with a tendency to become significantly different if the control period of the experiment is extended beyond 8 weeks. The leaf area was also affected being smaller in the salt treated plants. The leaf area was reduced to 1/2 in 0.5 M NaCl and to 1/3 in 0.8 M NaCl compared with the leaf area of the control plants. At the end of the treatment, all the leaves were turgid and showed an intense green color even those in 0.6 and 0.8 M NaCl.

The salt treated plants were able to recover the stem length, the leaf growth and leaf number when returned to be irrigated with tap water.

DISCUSSION

Studies showing salt tolerance in the genus *Prosopis* have been performed in *P. tamarugo* (Leoncini & Zamora 1969), in *P. farcta* (Bazzas 1973, Dafni & Negbi 1978), in several species of *Prosopis* including

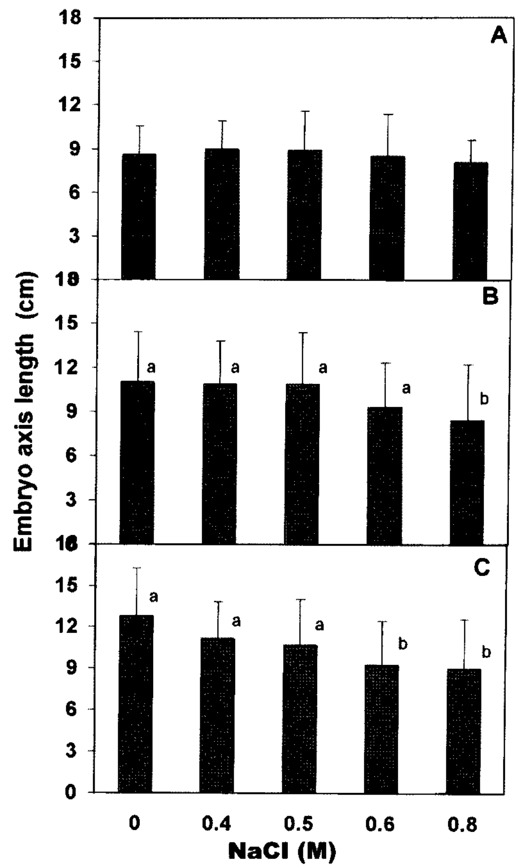

Fig. 3: Growth of seedlings after 7 (A), 14 (B) and 21 (C) days of treatment at different concentrations of NaCl. In this experiment, the seedlings came from seeds germinated in distilled water. The significance of differences (noted by different letters) was determined by the ANOVA of repeated measurements with a $P<0.05$. Vertical bars = standard deviations.

Crecimiento de plántulas después de 7 (A), 14 (B) y 21 (C)días de tratamiento a diferentes concentraciones de NaCl. En este experimento, las plántulas provinieron de semillas germinadas en agua destilada. Las diferencias significativas (señaladas por letras diferentes) fueron determinadas por la ANOVA de mediciones repetidas con un P<0,05. Barras verticales = desviaciones estandard.

P. chilensis (Felker et al. 1981) and, in *P. chilensis*, *P. tamarugo* and *P. alba* (Arce et al. 1990). However, none of these works report experiments performed in seedlings and plants of *Prosopis* spp. with concentrations of NaCl higher than in sea water, which is approximately 0.5 M NaCl, as done now in this work.

The results demonstrate that *P. chilensis* is a tree highly tolerant to salt stress because:

a) Although salinity in general delays the onset of germination there is 68% germination in 0.5 M NaCl after 28 days of treatment.

b)The seedlings can grow in 0.8 M NaCl, being more salt tolerant than germinating seeds.

c) Plants can grow in 0.8 M NaCl for 8 weeks with not significant differences in growth respect to the control plants for the first 4 weeks of treatment.

d) Similarly, the number of leaves of the plant stem increased in all NaCl concentrations during the 8 weeks of treatment with not statistical differences in relation to the control plants. However, there is a tendency in the plants to increase the differences of the leaf number among the groups over time of treatment and, probably, a significative difference will be found between the control group and that treated with 0.8 M NaCl if the period of the experiment is extended beyond 8 weeks.

e) The plants can reassume the rate of growth of the control group and the size of

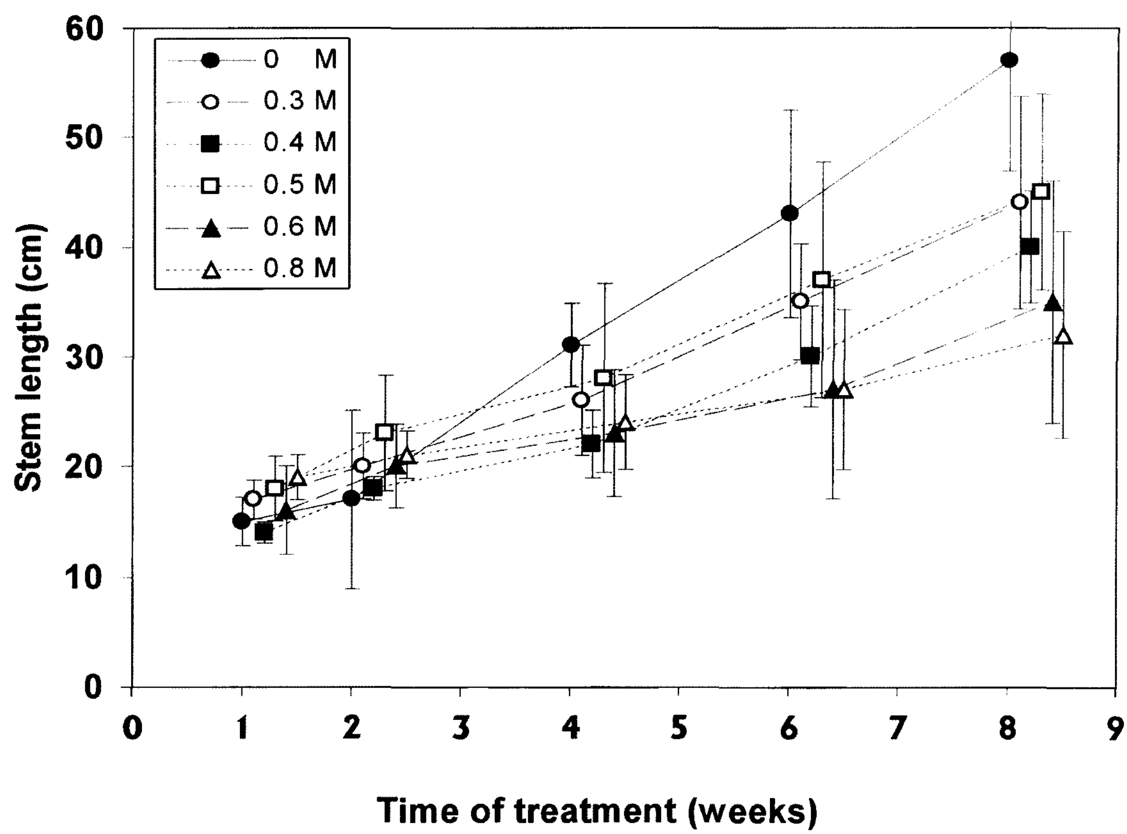

Fig. 4: Stem growth of plants during 8 weeks of treatment at different concentrations of NaCl. The Significance of Differences for growth af the stems among groups treated with different salt concentrations, were evaluated by an ANOVA of repeated measurements. A P<0.05 was found between control plants and those treated with 0.6 and 0.8 M NaCl after the sixth week of treatment. Vertical bars = standard deviations.

Crecimiento del tallo de plantas tratadas por 8 semanas con diferentes concentraciones de NaCl. Las Diferencias Significativas para el crecimiento de los tallos entre grupos tratados con diferentes concentraciones de sal, fueron evaluados por una ANOVA de mediciones repetidas. Un P<0,05 se encontró entre el grupo control y aquellos tratados con 0,6 y 0,8 M de NaCl después de la sexta semana de tratamiento. Barras verticales = desviaviones estandard.

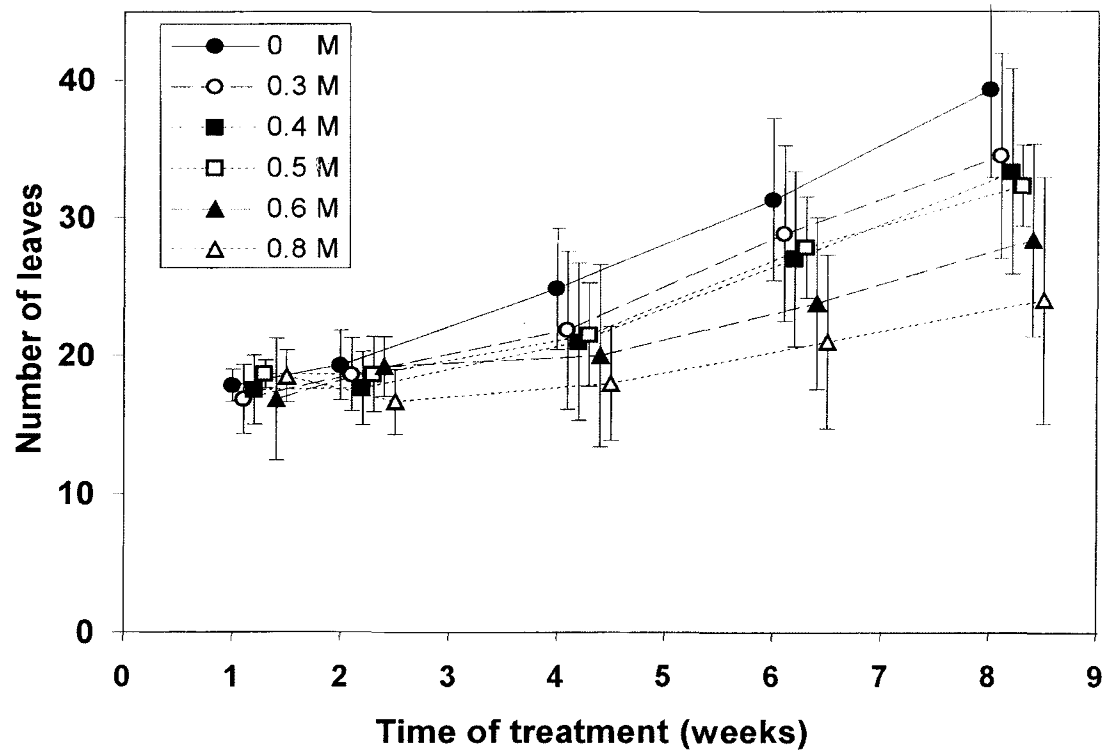

Fig. 5: Effect of NaCl treatment on the number of leaves of the main stem of *Prosopis chilensis* plants. The number of leaves was determined during 8 weeks for each treatment. The plants are the same used in the experiment of Figure 4. The significance of differences were also evaluated by an ANOVA of repeated measurements. A $P=0.094$ was found between the control group and that treated with 0.8 M NaCl at the eigth week of treatment. Vertical bars = standard deviations.

Efecto del NaCl en el número de hojas del tallo principal de plantas de *Prosopis chilensis*. El número de hojas fue determinado durante 8 semanas para cada tratamiento. Las plantas fueron las mismas usadas en el experimeto de la Figura 4. Las diferencias significativas fueron evaluadas por una ANOVA de mediciones repetidas encontrándose un $P=0{,}094$ entre el grupo control y el grupo tratado con 0.8 M de NaCl a las 8 semanas de tratamiento. Barras verticales = desviaciones estandard.

the leaves recovers if they are returned to be irrigated with tap water.

Prosopis chilensis seems to increase its thermotolerance when the seeds are germinated at 35°C (Medina & Cardemil 1993). In contrast, the tolerance of the seedlings to salinity is decreased when the seedlings came from seeds germinated in 0.3, 0.4 and 0.5 M NaCl. Indeed, seedlings coming from these seeds grew less than those treated with salt but coming from seeds germinated in distilled water (see Fig. 2 and 3). From these results we can conclude that during growth and development of the seedlings of algarrobo acquires tolerance to salinity. However, more research needs to be done in *Prosopis chilensis* to find out the molecular mechanisms of this acquired salt tolerance throughout seedling development (Winicov & Bastola 1997, Flowers et al. 1997).

The length of the stem is affected earlier and by a lower concentration of NaCl (0.6 M) than the leaf number. In addition, the size of the leaf area is also affected by NaCl becoming smaller under treatments from 0.5 to 0.8 M. Thus, the effect of NaCl on the length of the stems and number of leaves of plants seems to indicate that the salt treatment affects more the growth of organs than the differentiation of them in *Prosopis chilensis*.

We expected to have a large statistical dispersion of the data among individuals of the same group, since there is a high genetic variability in *Prosopis chilensis* as occurs in all *Prosopis* spp. due to the obligate cross pollination for seed production (Felker et al. 1981, Rodhes & Felker 1988). This was true for the experiments of seedling coming from seeds germinated in distilled water and for those with plants grown in Hoagland´s solution II. However, this statistical dispersion was rather low in the germination experiments and in those where the growth of seedlings was evaluated after irrigation with the same NaCl concentrations used for germination (Figs. 1 and 2). Probably this is due to the fact that seeds acquire a synchronized growth given by germination at different salt concentrations, as it does other stress conditions.

Felker et al.(1981) observed less growth of *P. chilensis* plants than those of *P. articulata*, *P. pallida* and *P. tamarugo*, with almost full losses of leaves, using 0.25 and 0.5 M NaCl added to a nitrogen-free nutrient solution. From these experiments they concluded that *P. chilensis* is less salt tolerant than the other *Prosopis* spp. in spite that *P. chilensis* is able to grow on soils covered with salt.

In spite of the difference between their data and ours, our results seems to favor that *P. chilensis* is a tree highly tolerant to salinity. One might speculate that algarrobo is more salt tolerant than most of the salt tolerant terrestrial plants reported so far, such as the genera, *Chenopodium*, *Suaeda*, *Salicornia*, *Atriplex* (Somers 1979), *Phillyrea latifolia* (Gucci et al. 1997) and *Mesembryanthemum crystallinum* (Yen et al. 1997). However, without analyses and quantification of the salt contents of the algarrobo plants grown in saline conditions and without knowing the physiological and molecular mechanisms of the salt tolerance of algarrobo, it is not possible to compare, in this respect, *Prosopis chilensis* with other species (Winicov & Bastola 1997, Yen et al. 1997).

In any case, the high tolerance of algarrobo can explain why it has been possible for CONAF(Corporación Nacional Forestal) to reforest part of the "Pampa del Tamarugal" with *P. chilensis*, where the seeds of *Prosopis* spp. such as *P. tamarugo* germinate and seedlings have to grow and settle in a soil covered with a crust of salt. It will be possible then to consider *P. chilensis* as a tree that can provide food, fuel, timber and to halt desertification in the coastal deserts of the world using sea water for irrigation. Because salinity in soil affects 7% of the land´s surface and about 5% of the cultivated land with a 20% of irrigated land suffering from secondary salinization, (Flowers et al. 1997), *Prosopis chilensis* can be considered as well as a genetic resource for salt tolerance.

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