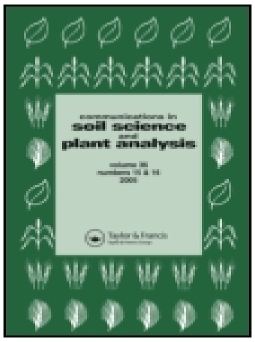

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Induced Salinity and Supplementary Phosphorus on Growth and Mineral Content of Frijolillo

A. Madueño-Molina,[1] J. D. García-Paredes,[1] J. Martínez-Hernández,[2] R. Bugarín Montoya,[1] and J. I. Bojórquez-Serrano[1]

[1]Autonomous University of Nayarit, Tepic, Nayarit, México
[2]College of Postgraduates, Montecillos, Edo. de México, México

Abstract: A greenhouse pot experiment was carried out using pumice material to investigate the response of frijolillo [*Rhynchosia minima* (L.) DC] grown at high salinity to supplementary P (P). Plants were tested during a period from germination to vegetative growth stage. Four levels of sodium chloride (NaCl; 0, 25, 50, and 100 mM) combined with two levels of P (4 and 8 meq L^{-1}) were tested in a factorial arrangement with four replications. This cultivar was tolerant to salinity stress up to 50 mM of NaCl and its growth was not affected. However, with high salinity (100 mM of NaCl), growth of both stem and root was reduced. Concentration of potassium (K) and P was affected adversely. The increment of P in the saline solution results in a greatest accumulation of biomass and in a better response to the osmotic adjustment of this wild specie. The amount of NaCl was correlated negatively with the amount of K and calcium (Ca) and positively correlated with P and magnesium (Mg).

Keywords: Growth, NaCl, phosphorus, *Rhynchosia minima* L., salt stress

INTRODUCTION

Frijolillo [*Rhynchosia minina* (L.) DC] is a wild legume widely distributed along the coastal plains in Nayarit, México. This area represents the

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Address correspondence to Diego García-Paredes, Secretaría de Investigación y Posgrado, Universidad Autónoma de Nayarit, Tepic, Nayarit, México. E-mail: juandieg@nayar.uan.mx

greatest biological diversity of this species, although this plant may be found from 0 to 1350 m above sea level (McVaugh 1987). This legume is used by cattle as fresh forage during the dry season, even in places where salinity conditions exist. However, this plant has not received enough study regarding its agronomic characterization and nutritional status.

Stress in plants can be defined as any factor that inhibits their growth and development. High concentrations of salts may cause a reduction of water in plants, accumulation of ions to toxic levels, and a reduction of nutrient availability. Furthermore, when salt concentration in soil solution increases and water potential in plants decreases, the water potential in cells also decreases and cell division and elongation may cease. Under these conditions, stomatal closure occurs with a reduction in photosynthetic rate (Ashraf 1994).

Phosphorus (P) is an important nutrient for plants. The total content can reach 0.2% of its total dry weight. It is an element present in compounds such as nucleic acids, adenosine triphosphate (ATP), and phospholipids. Therefore, plants suffering from P deficiency are retarded in growth and the shoot–root ratio is usually low (Shachtman, Reid, and Ayling 1998). Phosphorus is also involved in the control of enzymatic reactions as well as the regulation of metabolic paths (Theodorou and Plaxton 1993). Some studies have correlated sodium chloride (NaCl) stress with nutrient deficiencies. Adams (1991) as well as Sonneveld and de Kreij (1999) found that high concentrations of NaCl induce P and potassium (K) deficiencies in tomato and cucumber. Therefore, an optional strategy for coping with high concentrations of NaCl could be to attempt to supplement with P where the growth condition is known to be saline.

The experiment was conducted to evaluate the effect of NaCl salinity on growth and development of frijolillo with two levels of P and to investigate the effects of salinity on mineral content in leaves of this legume.

MATERIALS AND METHODS

A pot experiment, filled with washed pumice material and sieved through a 0.4- × 0.4-mm mesh, was conducted under greenhouse conditions at the Facultad de Agricultura of the Universidad Autónoma de Nayarit in Mexico. The study was developed in a hydroponics system using frijolillo (*Rhynchosia minima*) as a plant indicator.

Experimental Design, Treatments, and Plant Materials

The experiment had a factorial A × B arrangement, in a complete randomized design with four replications. The factor A was four levels of NaCl and factor B was two levels of P (Table 1). The nutritional solution was that used by Hewitt and Smith (1975), which was modified to obtain two solutions with

Table 1. Combinations of NaCl and P evaluated

Treatment	P (meq L^{-1})	NaCl (mM)	CE (mS cm^{-1})
1	4	00	1.8
2	4	25	4.6
3	4	50	7.2
4	4	100	12.4
5	8	00	2.0
6	8	25	4.8
7	8	50	7.4
8	8	100	12.6

4 and 8 meq L^{-1} of P. These two levels of P were combined with four levels of NaCl (0, 25, 50, and 100 mM) for a total of eight treatments. The pH from the solution was adjusted to a level of 5.8 with sulfuric acid (0.5 mL for each 5 L of solution) to avoid making this factor a constraint on nutrient absorption. The pots were watered twice a day.

The duration of the experiment was 6 weeks with five seedling samples harvested every week to assess biomass yield and agronomic characteristics. These characteristics were defined as follows.

Length of Main Stem (LS)

This was the average length from five plants measured every week and was used to evaluate the dynamic of plant growth.

Leaf Area in Centimeters^2 (LA)

This characteristic was calculated using the equation obtained by Navejas (1995):

$$LA = (L \times A)(0.6667) \quad (1)$$

where LA is the leaf area in cm^2, L is maximum length, A is maximum width, and 0.6667 is a constant value used as a correction factor.

Leaf Weight (LW), Stem Weight (SW), and Dry Root Weight (RW)

The average weight, in grams, was calculated from five plants in each experimental unit, which were oven dried at 55°C until a constant weight was reached.

Dry Matter of Stem (DMS)

This was calculated by $LW + SW$.

Stem–Leaf Ratio (SLR)

This was calculated by LW/SW.

Shoot–Root Ratio (S-R)

This was calculated by RW/DMS.

Salinity Tolerance Index (STI)

A salinity index for stem and root was calculated according to the equation proposed by Maiti et al. (1996):

$$STI = \frac{\text{Dry weight of shoot under stress}}{\text{Dry weight of shoot in control}} \tag{2}$$

Chemical Analyses

At the end of the study, chemical analysis were performed to determine contents of P, K, calcium (Ca), magnesium (Mg), and sodium (Na) in leaves. Phosphorus was estimated by colorimetric methods. Potassium and Na contents were determined by flame photometry. Calcium and Mg were estimated volumetrically.

Statistical Analyses

The data collected were evaluated with an analysis of variance and mean comparison (Tukey, 0.05), using SAS version 6.12 software (SAS Institute, Inc., Cary, N.C., USA).

RESULTS AND DISCUSSION

The analysis of variance showed statistical differences between levels of P and NaCl concentrations for length of stem (starting from 28 days for P factor, 14 days for NaCl factor, and 21 days for the interaction), leaf area, and dry-matter (DM) production. The range of the coefficient of variation was 1.52 for leaf area sampled to 42 days to 7.0% for length of stem sampled at 7 days (data not shown).

Table 2 shows the effects of the main factors NaCl and P. The levels of P affected, in a positive way, the length of stem, the leaf area, and dry-matter production of stem and root. Similar results were found by Kaya, Higgs,

Table 2. Effects of phosphorus and NaCl on growth and development of frijolillo

Factor	Length of stem (cm)	Leaf area (cm^2)	DM stem (g)	DM root (g)
Phosphorus (meq L^{-1})				
4	70.0 b	186.0 b	6.7 b	4.7 b
8	74.9 a	211.9 a	7.9 a	5.7 a
NaCl (mM)				
0	89.5 a	265.0 a	8.6 a	6.3 a
25	84.8 b	230.3 b	7.2 b	6.0 b
50	65.8 c	172.0 c	7.2 b	4.7 c
100	49.7 d	128.6 d	5.8 c	3.9 d

Note. Within each column, the same letter indicates no significant difference between treatments ($P < 0.01$).

and Kirnak (2001), which observed that foliar spray of monopotassium phosphate (KH_2PO_4) alleviated the adverse effects of high salinity on plants. As expected, induced salinity with NaCl affected negatively these parameters of growth and development. The length of stem decreased 5.3, 26.5, and 44.5% with 25, 50, and 100 mM of NaCl, respectively, while leaf area was reduced by 13.1, 35.1, and 51.5%. These results are in agreement with those found by Volkmar, Hu, Steppuhn (1998) and Munns et al. (1988).

The interaction between salinity and P nutrition of plants is dependent, among others factors, "upon the composition and level of salinity and the concentration of P in the substrate" (Grattan and Grieve 1999). When compared the amount of P, at the same level of induced salinity, the values for length of stem, leaf area, DM of stem, and DM of roots were higher with the higher

Table 3. Effects of phosphorus and NaCl on growth and development of frijolillo, mean comparison (Tukey, 0.05%) for the interaction effects of NaCl and phosphorus factors

Treatment P/NaCl	Length of stem (cm)	Leaf area (cm^2)	DM stem (g)	DM root (g)
4/0	81.3 c	223.7 c	8.6.a	6.1 a
4/25	88.4 b	227.6 bc	6.3 c	5.5b
4/50	64.0 d	163.8 e	6.4 c	4.0 c
4/100	46.4 f	128.8 f	5.4 d	3.4 d
8/0	97.7 a	306.2 a	8.6 ab	6.6 a
8/25	81.1 c	232.9 b	8.1b	6.5 a
8/50	67.6 d	180.3 d	8.0 b	5.3 b
8/100	52.9 e	128.3 f	6.3 c	4.3 c

Note. Within each column, the same letter indicates no significant difference between treatments ($P < 0.01$).

value of P. However, for leaf area, when the concentration of NaCl was 100 mM, there were no significant differences between levels of P (Table 3).

Salinity conditions affected significantly DM production of stem and root. Yield of DM of main stem ranged from 8.59 g to 5.84 g for the levels of 0 to 100 mM NaCl. Dry matter production of root varied from 6.34 g to 3.86 g with the level of NaCl. In both cases, the production of DM with control was higher. There was a gradual reduction in DM for the treatment with NaCl, with a lineal trend. With respect to the main effect of P, the negative influence of NaCl was ameliorated by the high level of P. However, with the two levels of P, there was a negative trend (Table 3).

In this study, it was observed that the general trend of growth for main stem was similar with the levels evaluated through the saline gradient. The interaction between 8 meq L^{-1} of P, with 0 mM of NaCl was statistically superior with a length of main stem of 97.7 cm, followed by the interaction between 4 meq L^{-1} of P with 25 mM NaCl, with a length of main stem of 88.4 cm.

The effect of P showed higher values with 8 meq L^{-1} in comparison with 4 meq L^{-1} up to 50 mM of NaCl; however, when the concentration of NaCl was 100 mM, there were no significance differences between the levels of P (Table 3).

An exponential response for the length of main stem was shown for all levels of salinity tested (Figure 1). However, this response decreased when the salinity concentration increased. The length of stem at 42 days was 89.5 cm with the control and 49.7 cm for the treatment with 100 mM of NaCl. This difference

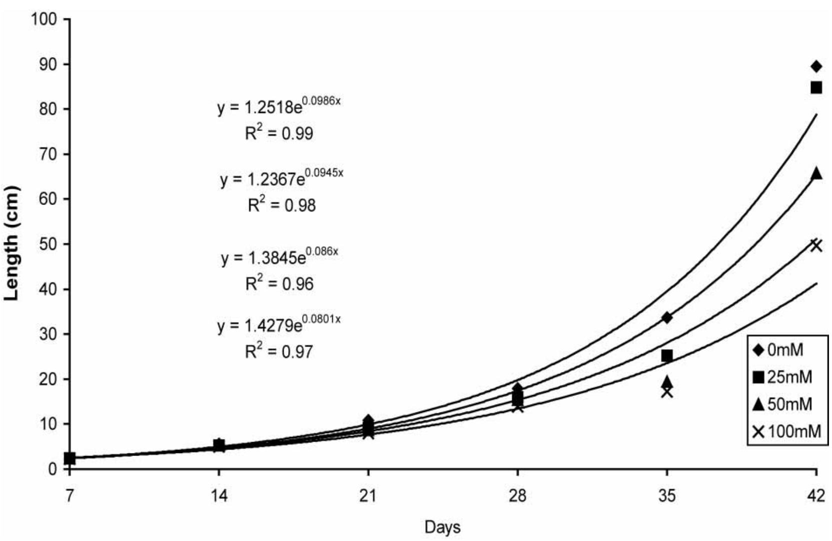

Figure 1. Growth rate of main stem of frijolillo in a salinity condition with NaCl.

represented a diminution of 44.5%. The negative effect of the concentration of NaCl on development of main stem was more evident with time.

The response of leaf area had a trend similar to the length of main stem. It had an average increment from 8.0 cm^2 (7 days) up to 199.0 cm^2 (42 days). An exponential response with all levels of salinity evaluated was observed with time (Figure 2); however, this response decreased according to the increment in saline concentration. The leaf area at 42 days was 265.0 cm^2 with the control, but with 100 mM of NaCl was only 128.6 cm^2, which was a reduction of 48.5%. The negative effect of NaCl concentration on leaf area was more evident with time. This leaf area reduction was because of the accelerated senescence of leaves caused by sodium accumulation in tissues.

The senescence process for salinity started with a light chlorotic condition until the leaf turned completely yellow and ended with leaf abscission. This process, combined with a retarded growth of new leaves, caused lower leaf area as observed at 42 days. On the other hand, new buds as well as meristematic parts showed small turgor losses during the hottest hours of the day (30°C ± 4).

The relationships between the ratios of leaf–stem and aerial biomass–root were examined. The relationship between leaf and stem was affected by salinity. The value for this ratio decreased from 2.03 with control to 1.54 with 100 mM of NaCl (Table 4). However, in all cases, those values were greater than 1.0. This proves that beside the negative effect of salinity, which was shown with leaf area reduction, the plant still is able to produce a fair amount of foliage. The aerial biomass–root ratio decreased from 0.74 to 0.66 in the same gradient. These results also prove that roots suffered greater damage with salinity compared to aerial biomass. Similar results were found by Murillo-Amador et al. (2001), who reported a decrease in the root–stem ratio of *Opuntia* with an increment of salinity.

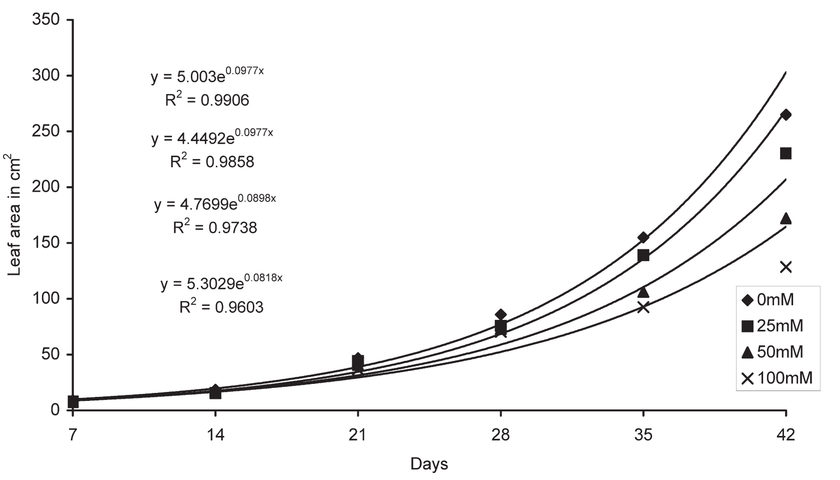

Figure 2. Leaf area growth of frijolillo under NaCl salinity condition.

Table 4. Indices of tolerance for shoot (ITS), root (ITR), and total biomass (ITB), shoot–root ratio (SRR), and leaf–stem ratio (LSR) in frijolillo under NaCl salinity

	NaCl in mM			
Variable	0	25	50	100
ITS	1.00	0.83	0.83	0.68
ITR	1.00	0.95	0.74	0.65
ITB	1.00	0.88	0.82	0.65
SRR	0.74	0.83	0.65	0.66
LSR	2.03	2.01	1.96	1.54

The tolerance index was evaluated. In this study, salinity affected in a negative way DM production, but yield of root, stem, and total biomass were more than 80% when the saline concentration was 50 mM of NaCl or less and only 35% with 100 mM of NaCl.

Table 5 shows the values of correlation coefficients for the agronomic variables. The aerial biomass–root ratio was associated in a positive and significant way. However, both variables were correlated in a negative way with the rest of the variables. This finding proves that the negative influence of salinity affects the whole plant, and because roots are in direct contact with salinity environment they have a greater influence.

Mineral Contents

The mineral contents in leaves were significant. The analysis of variance showed significant differences for mineral content in leaves with levels of P and NaCl and their interaction, except for N (data not showed).

The results for the main factors are in Table 6. The amount of P increased significantly from 0.06 to 0.10% with 0 to 100 mM of NaCl and 0.05 to 0.11% with the two levels of P.

Higher values in leaf tissues were observed with the interaction of 8 meq L^{-1} of P and 100 mM of NaCl reaching 0.14%, which was statistically significant compared to the rest.

The amount of K decreased gradually from 2.68 to 2.15% with 0 to 100 mM of NaCl (Figure 3). However, its absorption was favored with higher doses of P, with an increase from 2.37 to 2.58%. It is known that the negative effect of Na on K is because both ions are similar; however, if there is no equilibrium between the balances in both ions in the soil solution, then the selectivity of the membrane is broken, causing Na, instead of K, to go into the cells. Studies reported by Grattan and Grieve (1994) have shown that K concentration in plant tissue declines as the Na salinity is increased.

Table 5. Correlation coefficients for agronomics variables with NaCl salinity stress (Tukey, 0.05)

Variable	NaCl	LS	LA	DMS	DMR	ITS	ITR	ITB	SRR
LG	−0.981								
AF	−0.979	0.989							
MSV	−0.954	0.886	0.918						
MSR	−0.969	0.997	0.994	0.874					
ITV	−0.951	0.883	0.917	0.999	0.873				
ITR	−0.969	0.997	0.994	0.875	1.000	0.873			
ITB	−0.996	0.964	0.875	0.976	0.954	0.975	0.954		
SRR	0.689	−0.817	−0.786	−0.477	−0.842	−0.475	−0.843	−0.642	
LSR	−0.924	0.888	0.848	0.847	0.847	0.840	0.848	0.902	−0.559

Table 6. Effects of phosphorus and NaCl salinity on mineral content in leaves of frijolillo

Factor	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
Fósforo (meq L^{-1})					
4	0.05 b	2.37 b	1.03 a	1.27 a	0.49 b
8	0.11 a	2.58 a	0.97 b	1.23 b	0.67 a
NaCl (mM)					
0	0.06 c	2.68 a	1.06 a	1.19 b	0.07 d
25	0.08 bc	2.59 b	1.05 a	1.28 a	0.22 c
50	0.08 bc	2.48 c	0.97 b	1.25 a	0.72 b
100	0.10 a	2.15 d	0.93 d	1.28 a	1.32 a

Note. Within each column, the same letter indicates no significant difference between treatments ($P < 0.01$).

The content of Ca decreased from 1.06 to 0.93% for the effect of 100 mM of NaCl, and from 1.03 to 0.97% for the influence of P. Concentrations of Mg increased significantly in leaves from 1.19 to 1.29% with 0 to 100 mM of NaCl. However, leaf P concentration decreased in increasing NaCl concentration in nutrient solution. Similar findings were reported by Kaya, Higgs, and Kirnak (2001).

The amount of Na in leaves increased from 0.07 to 1.32% with 0 and 100 mM of NaCl. The sodium content in leaf tissue had a lineal trend

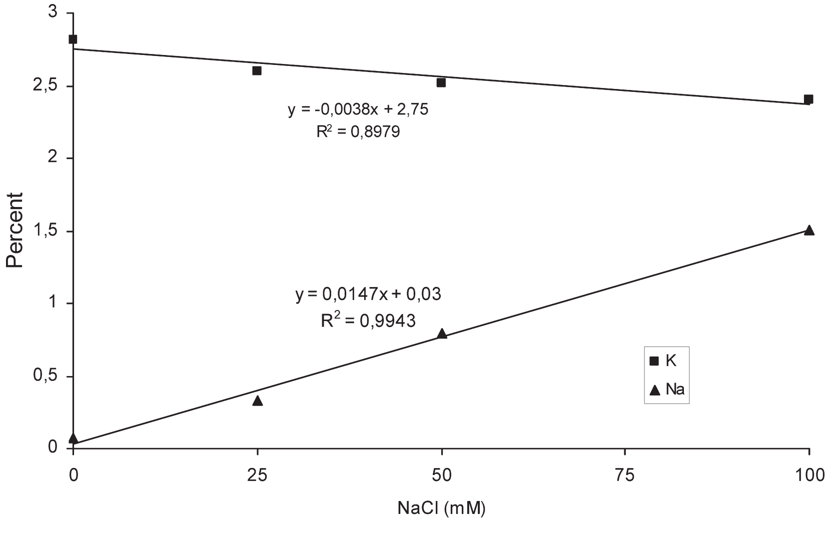

Figure 3. Effect of levels of NaCl in K and Na content in leaves tissue.

Table 7. Effects of the interaction between factor phosphorus and NaCl on mineral content in leaves of *Rhynchosia minima*

Treatments						
P (meq)	NaCl (mM)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
4	0	0.04 e	2.55 b	1.14 d	1.06 a	0.07 f
4	25	0.05 e	2.57 b	1.33 a	1.03 ab	0.12 f
4	50	0.05 e	2.45 cd	1.31 ab	1.01 b	0.65 d
4	100	0.06 de	1.91 e	1.31 ab	1.03 ab	1.13 b
8	0	0.08 cd	2.81 a	1.25 bc	1.06 a	0.07 f
8	25	0.11 bc	2.60 b	1.23 bc	1.06 a	0.33 e
8	50	0.12 ab	2.52 bc	1.19 cd	0.93 c	0.79 c
8	100	0.14 a	2.40 d	1.26 abc	0.84 d	1.51 a

Note. Within each column, the same letter indicates no significant difference between treatments ($P < 0.01$).

(Figure 3), increasing from 0.07 to 1.32% with 0 to 100 mM of NaCl; in the same way, its contents increased significantly with the levels of P from 0.49 to 0.67%.

Table 7 presents the effects of the interaction between factor P and NaCl on mineral content in leaves of *Rhynchosia minima*. The percent of P in leaves of frijolillo was higher when the amount applied of this element was 8 meq L^{-1}. The highest content of K in leaves was found in the treatment with 8 meq L^{-1} of P and 0 mM of NaCl. The lower content of Ca was found in the treatment with 4 meq L^{-1} of P and 0 mM of NaCl; however, for Mg the lowest content in leaves was obtained with the treatment with the highest content of P and NaCl. Finally, the response of Na was correlated in a direct way with the amounts applied of NaCl.

Table 8 shows the correlation for the elements and NaCl. There was a negative relationship between Na with K and Ca, which proved that these elements decrease the negative influence of Na. On the other side, a positive relationship was detected with P and Mg.

Table 8. Correlation coeficients for mineral content under NaCl salinity conditions

Variable	NaCl	P	K	Ca	Mg
P	0.985				
K	−0.990	−0.964			
Ca	−0.957	−0.917	0.926		
Mg	0.706	0.817	−0.652	−0.569	
Na	0.989	0.952	−0.982	0.603	−0.980

CONCLUSIONS

In conclusion, this wild bean shows tolerance to salinity stress up to 50 mM of NaCl because its growth was not affected. However, with high salinity (100 mM of NaCl), growth of both stem and root was reduced. The concentration of K and P was affected adversely. The increment of P in the saline solution results in the greatest accumulation of biomass and in a better response to the osmotic adjustment of this wild specie. The amount of NaCl was correlated negatively with the amount of K and Ca and positively correlated with P and Mg.

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