

## Emergence capacity and seedlings early growth of four legumes in arid zones under NaCl-stress

Capacidad de emergencia y crecimiento inicial de plántulas de cuatro leguminosas en zonas áridas en condiciones de estrés por NaCl

Capacidade de emergência e crescimento inicial de plântulas de quatro leguminosas em zonas áridas sob estresse por NaCl

Francisco Higinio Ruiz-Espinoza<sup>1</sup>  

Juan José Reyes-Perez<sup>2</sup>  

Felix Alfredo Beltrán-Morales<sup>1</sup>  

Bernardo Murillo-Amador<sup>3\*</sup>  

Juan Carlos Rodríguez-Ortiz<sup>4</sup>  

Pablo Misael Arce-Amézquita<sup>1</sup>  

Rev. Fac. Agron. (LUZ). 2023, 40(2): e234020

ISSN 2477-9407

DOI: [https://doi.org/10.47280/RevFacAgron\(LUZ\).v40.n2.10](https://doi.org/10.47280/RevFacAgron(LUZ).v40.n2.10)

### Crop production

Associate editor: Dr. Jorge Vilchez-Perozo  

University of Zulia, Faculty of Agronomy  
Bolivarian Republic of Venezuela

<sup>1</sup>Universidad Autónoma de Baja California Sur, La Paz, Baja California Sur, Autónoma de Baja California Sur, México.

<sup>2</sup>Universidad Técnica Estatal de Quevedo. Av. Quito. Km 1 ½ vía a Santo Domingo. Quevedo, Los Ríos, Ecuador.

<sup>3</sup>Centro de Investigaciones Biológicas del Noroeste, S.C. (BMA). Avenida Instituto Politécnico Nacional No. 195. Colonia Playa Palo de Santa Rita Sur. La Paz, Baja California Sur, México. C.P. 23096.

<sup>4</sup>Facultad de Agronomía y Veterinaria-UASLP. Palma de la Cruz, Soledad G.S. San Luis Potosí, México.

Received: 11-04-2023

Accepted: 09-05-2023

Published: 02-06-2023

### Keywords:

Biomass

Growth

*Vigna unguiculata* L. Walp.

*Lablab purpureus* L. Sweet

*Clitoria ternatea* L.

*Canavalia ensiformis* L. DC.

### Abstract

Legumes are used as fodder and green manures, because of fix nitrogen biologically. The objective of this study was to determine the emergence capacity and the early growth of four legume species treated with different NaCl-stress concentrations. The experiment was established in a completely randomized design with a factorial arrangement, where the first factor was the four legumes' species (*Vigna unguiculata* L. Walp., *Lablab purpureus* (L.) Sweet, *Clitoria ternatea* L. and *Canavalia ensiformis* L. DC.) and the second factor was NaCl concentrations (0.25, 50, and 75 mM) with 16 treatments and four replications. The variables evaluated were emergence rate and percentage, stem and root length, fresh and dry weight of stem+leaves and root, stem and root length, stem diameter and the ratio of stems+leaves dry weight and roots dry weight (plant balance). The results showed that all variables expressed significant differences between species, NaCl and the species × NaCl interaction. A differential response between legumes to NaCl stress was observed. The most tolerant species to NaCl were *Vigna unguiculata* and *Canavalia ensiformis* showed a higher tolerance with respect to *Lablab purpureus* and *Clitoria ternatea*.

## Resumen

Las leguminosas se utilizan como forraje y abonos verdes, porque fijan biológicamente el nitrógeno. El objetivo de este estudio fue determinar la capacidad de emergencia y el crecimiento inicial de cuatro especies de leguminosas tratadas con diferentes concentraciones de NaCl. El experimento se estableció en un diseño completamente al azar con arreglo factorial, donde el primer factor fueron las cuatro especies de leguminosas (*Vigna unguiculata* L. Walp., *Lablab purpureus* L. Sweet, *Clitoria ternatea* L. y *Canavalia ensiformis* L. DC.) y el segundo factor las concentraciones de NaCl (0, 25, 50 y 75 mM) con 16 tratamientos y cuatro repeticiones. Las variables evaluadas fueron tasa y porcentaje de emergencia, longitud de tallo y raíz, peso fresco y seco de tallo+hojas y de raíz, longitud de tallo y de raíz, diámetro de tallo y la relación peso seco de tallos+hojas y peso seco de raíces (balance de la planta). Los resultados mostraron que, todas las variables expresaron diferencias significativas entre especies, NaCl y la interacción especies × NaCl. Se observó una respuesta diferencial entre leguminosas al estrés por NaCl. Las especies más tolerantes al NaCl fueron *Vigna unguiculata* y *Canavalia ensiformis* mostraron una tolerancia mayor con respecto a *Lablab purpureus* y *Clitoria ternatea*.

**Palabras clave:** biomasa, crecimiento, *Vigna unguiculata* L. Walp., *Lablab purpureus* L. Sweet, *Clitoria ternatea* L., *Canavalia ensiformis* L. DC.

## Resumo

As leguminosas são utilizadas como forragens e adubos verdes, por fixarem o nitrogênio biologicamente. O objetivo deste estudo foi determinar a capacidade de emergência e o crescimento inicial de quatro espécies de leguminosas tratadas com diferentes concentrações de NaCl-estresse. O experimento foi instalado em delineamento inteiramente casualizado com arranjo fatorial, onde o primeiro fator foram as quatro espécies de leguminosas (*Vigna unguiculata* L. Walp., *Lablab purpureus* L. Sweet, *Clitoria ternatea* L. y *Canavalia ensiformis* L. DC.) e o segundo fator foram as concentrações de NaCl (0,25, 50, e 75 mM) com 16 tratamentos e quatro repetições. As variáveis avaliadas foram taxa e porcentagem de emergência, comprimento de caule e raiz, massa fresca e seca de caule+folhas e raiz, comprimento de caule e raiz, diâmetro do caule e relação massa seca de caule+folhas e massa seca de raízes (equilíbrio da planta). Os resultados mostraram que todas as variáveis expressaram diferenças significativas entre as espécies, NaCl e a interação espécie × NaCl. Observou-se uma resposta diferencial entre as leguminosas ao estresse por NaCl. As espécies mais tolerantes ao NaCl foram *Vigna unguiculata* e *Canavalia ensiformis* apresentaram maior tolerância em relação a *Lablab purpureus* e *Clitoria ternatea*.

**Palavras-chave:** biomassa, crescimento, *Vigna unguiculata* L. Walp., *Lablab purpureus* L. Sweet, *Clitoria ternatea* L., *Canavalia ensiformis* L. DC.

## Introduction

The saline-stress is one of the main limiting factors of productivity in agricultural crops; therefore, going further in the study of saline tolerance is a priority for sustainable agriculture. The salinity is a

complex abiotic stress based on ionic and osmotic phenomena (Gul *et al.*, 2022;). The plants under salinity are affected from germination to more advanced development stages. The imbibition speed is reduced due to an osmotic effect in the seeds while cellular division, elongation, and mobilization of essential reserves for the germination process can also shows variations (Narejo *et al.*, 2023).

Soil salinity is one of the main degradation forms in arid and semi-arid regions where precipitation is low to keep regular salt percolation from the crop root system zone (Nachshon, 2018). The soils in arid and semi-arid regions contain high amounts of soluble salts, mainly NaCl and Na<sub>2</sub>SO<sub>4</sub> (Xu *et al.*, 2020), which allows a high sodium adsorption ratio (SAR) out of the soil solution. The excess of salts in drainage is often associated with sodicity problems (Mohanavelu *et al.*, 2021) since sodium occupies the exchangeable spaces.

The water quality deteriorates progressively due to the salt excess in addition to bad management of water and soil. The soil salinization gets hastened and worse when the salt-promoting accumulation factors are ignored. The recent studies show that, agricultural areas are being salt-affected worldwide areas including Mexico, where the distribution and extension of soils with salt problems have increased particularly in irrigation areas of arid zones (Negacz *et al.*, 2022).

The legumes used as green manure, besides their potential as nutrient source of livestock, offer other important advantages when are used in agriculture systems. The legumes promote biological nitrogen fixation (Mathesius, 2022) and this natural conversion of atmospheric N into available forms for the plant enhances economical and sustainable productivity (Daniel *et al.*, 2022). The objective of this study was to determine the emergence capacity and the early growth of four legume species, treated with different NaCl-stress concentrations.

## Materials and Methods

### Study area

The experiment was carried-out in the spring season of 2019 in the Universidad Autonoma of Baja California Sur (UABCS) in La Paz, Baja California Sur, Mexico, located at 24° 10' North latitude and 110° 19' West longitude. The study area has a climate BW (h') HW (e), that is, dry desert, warm, according to the Köppen climatic classification, modified by García (2004). The annual temperatures mean, maximum, and minimum in the study site are 29.6, 36.0 (in July), and 18.1 °C (in January), respectively. The annual precipitation is about 184.8 mm. The potential evaporation far exceeds precipitation, resulting in a water deficit of around 2472 mm per year and an average relative humidity of 62 % per month, while daily insolation is 8.5 h. The experiment was developed in a shadow mesh structure (40 % of shading, black color, model 20 mesh). The temperature inside the mesh structure ranged from 29 to 33 °C. The data of the climatological variables recorded during the study period were obtained from a portable weather station (Vantage Pro2® Davis Instruments, Hayward, CA, USA).

### Legumes species

The species used were (*Vigna unguiculata* L. Walp., *Lablab purpureus* L. Sweet, *Clitoria ternatea* L. and *Canavalia ensiformis* L. DC.). The seeds of these species were obtained from an organic plot in the experimental field of UABCS. The seeds were stored in the seeds collection bank at UABCS. The extract of *Ocimum tenuiflorum* (holy basil) was used to maintain the seeds free of insects and pathogens and were maintained stored at 10 °C.

### Experimental design

The experiment was developed using a completely randomized design with a factorial arrangement being the first factor, the four legumes (*Vigna unguiculata* L. Walp., *Lablab purpureus* L. Sweet, *Clitoria ternatea* L. and *Canavalia ensiformis* L. DC.), and the second factor, four NaCl concentrations (0, 25, 50 and 75 mM) using distilled water as control (0 mM NaCl) with 16 treatments and four replications per treatment.

### Experimental management

The seeds of each species were sown in 200 cells germination polystyrene trays containing Sunshine® an inert commercial substrate of Canadian sphagnum peat moss (Sun Gro Horticulture Distribution Inc. Agawam, MA, USA). The NaCl treatments were applied from the beginning of the experiment. The trays were irrigated every third day with 500 mL of water after the NaCl solutions treatments and let to drain to avoid salt accumulation in the substrate. The electrical conductivity (EC) readings were measured with a conductivity meter (Hach®, Model Sension+, Loveland, CO, USA) and taken after the preparation of the saline solution and subsequently to the drained liquid to compare the values of EC (prepared and drained), according with Murillo-Amador *et al.* (2007) No changes were detected in the drained solution.

### Emergence Rate (ER)

The seeds were considered to have emerged when the seedling emerged through the substrate surface. The percentage of emerged seeds was recorded daily (emergence rate), after the third day and once the application of NaCl treatments, and the total emerged seedlings counted after 11 and 21 days depending on the species. The emergence rate (M) is the speed that a seed germinate over a period (in days) was calculated using Maguire (1962) equation:  $M = n_1/n_2 + t_1/t_2 + \dots + n_{100}/t_{11 \text{ or } 21}$ ; where  $n_1, n_2, \dots, n_{100}$  is the number of germinated seeds in the times  $t_1, t_2, \dots, t_{11 \text{ or } 21}$  (in days).

### Emergence Rate Index (ERI)

The number of emerged seedlings was recorded daily, considering as the first day when the first emerged seedling was observed; the last observation was performed fifteen days after the establishment of the experiment. The emergence rate index was calculated according to the formula proposed by Maguire (1962):

$$ERI = \frac{\text{Number of normal seedlings}}{\text{Days at the first counting}} + \dots + \frac{\text{Number of normal seedlings}}{\text{Days at the last counting}}$$

### Seedlings growth

The emerged seedlings were maintained for 13 days for *L. purpureus*, 10 days for *V. unguiculata*, 11 days for *C. ensiformis*, and 21 days for *C. ternatea* and after this period, 10 seedlings per species, treatments and replication were selected; then, the roots and stems (stems+leaves) were separated, washed with running water and then with distilled water.

The fresh (g) and dry weight (g) of all tissues were determined using an analytical scale (Mettler Toledo®, model AG204, USA). The dry weight of roots and stems (stems+leaves) were obtained after being placed in paper bags and then in a drying oven (Shel-Lab®, FX-5, series-1000203, USA) at 70 °C until constant weight (about 72 h).

The stems height (cm) was measured from the base to the apex and after seedlings were harvested, while stem diameter (mm) was measured every three days during the experimental period using a digital Vernier (VWR® model 62379-531, S/N/61581129, Manufacturer Control Company, USA).

The roots length (cm) was measured using a graduated ruler and the measurements were taken from the base of the stem, where the root hairs begin, to the apex of the main root. The biomass balance plant was calculated dividing the stem+leaves dry weight by root dry weight.

### Statistical analysis

The data were subjected to analysis of variance using Statistica v. 13.5 (TIBCO Software Inc., 2018). The homogeneity of variance was determined using Bartlett's Box-test. The means comparison test was performed by Tukey HSD ( $p=0.05$ ). The emergence percentage data were transformed by arcsine according to Little and Hills (1989).

## Results and discussion

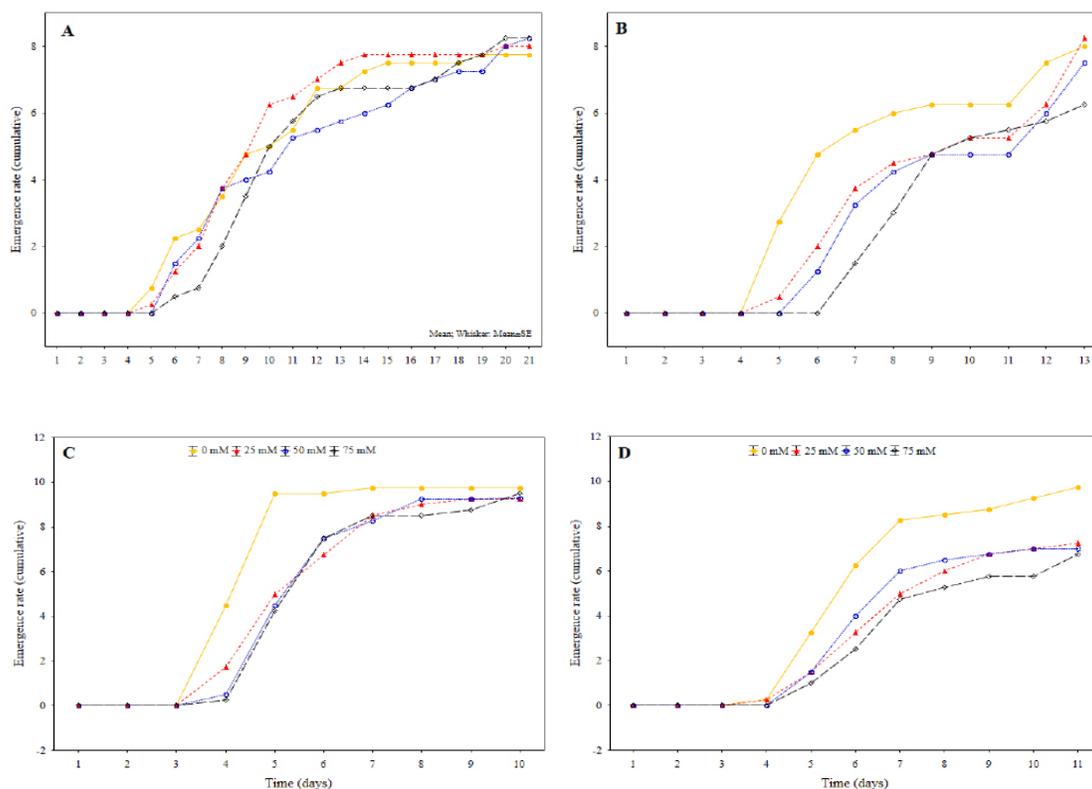
### Emergence rate and emergence percentage

The analysis of variance showed that emergence rate (Fig. 1) and emergence percentage, were affected by species, NaCl, and species  $\times$  NaCl interaction. This interaction indicates that NaCl effects were different between species. The NaCl concentrations affect the emergence rate of the species by delaying the emergence rate being more severely affected *C. ternatea* and less affected *V. unguiculata* (Figure 1).

The decrease in both rate and emergence percentage is the result of inhibition of embryo-axis growth because of delayed reserve mobilization and membrane disturbance caused by NaCl-stress and evidenced by increased leakage of materials from embryo-axis (Ibrahim, 2019). The results of seed emergence, according to the different NaCl concentrations, showed that the final emergence percentage (Table 1) of each species was influenced by NaCl.

The seeds of *Vigna unguiculata* L. Walp. showed a higher emergence percentage of 97.5 %, followed by *C. ensiformis*, *L. purpureus*, and *C. ternatea*, in that order. The emergence percentage was similar under 0 and 25 mM NaCl; nonetheless, an increment of NaCl concentrations reduced the emergence. The NaCl concentrations negatively affect the emergence of seeds of test plants. The salinity influences the germination percentage and the time in which this process occurs (Dehnavi *et al.*, 2020).

The results of the present study showed variability between species in the response to NaCl-stress of the four legume and therefore the germplasm can be considered for saline stress genetic improvement in legume species. Hence, efforts to identify saline-resistant germplasm are important goals to pursue. The results of the present study showed that most of the variables at the emergence stage exhibited a reduction as the salinity increased. Al-huraby *et al.* (2022) reported similar results in *P. vulgaris* L. when the number of germinated seeds decreased according to the media concentration. Likewise, Praxedes *et al.* (2020), reported that *V. unguiculata* seedlings were evaluated for emergence, vigor, salinity tolerance index, and dissimilarity. Increased salinity of irrigation water reduced emergence, vigor, and dry matter accumulation of cowpea varieties. The results showed that some cowpea varieties are most tolerant to salinity, while other varieties are most sensitive to salinity in the emergency and initial growth phase. Ravelombola *et al.* (2018) analyzed the salt tolerance index of 116 and 155 cowpea accessions at germination and seedling stages and found a substantial variation in salt tolerance index for germination rate, plant height reduction, fresh and dry shoot biomass reduction, foliar leaf injury, and inhibition of the first trifoliate leaf. Deng *et al.* (2019) investigated the relationship between osmotic regulators, stress resistance enzymes, and salt



**Figure 1. Effect of NaCl-stress in the emergence rate of four legumes, (A) *Clitoria ternatea*, (B) *Lablab purpureus* (C) *Vigna unguiculata*, and (D) *Canavalia ensiformis*. The values represent the mean  $\pm$  the error standard.**

tolerance of leguminous plants (*Vigna angularis* variety ‘Yuhongdou 2’ and traditional *Dolichos lablab*) under NaCl stress and found that, salinity stress inhibited the germination index of *V. angularis*. Then germination percentage, germination potential, germination index, and vigor index of *V. angularis* decreased with increasing NaCl stress and was significantly higher than that of *D. lablab*. 2) The relative salt damage rate of *V. angularis* and *D. lablab* increased with the increase in NaCl concentration.

#### Emergence Rate Index (ERI)

The analysis of variance showed that the emergence rate index was affected by species, NaCl, and species  $\times$  NaCl interaction. The table 1 shows that in general, the four species showed lower ERI values as NaCl concentrations increased. The ERI was higher at 0 mM NaCl except for *C. ternatea* which showed a high ERI at 25 mM NaCl; while *V. unguiculata* showed the highest in all NaCl concentrations followed by *C. ensiformis* whereas *L. purpureus* at 25, 50, and 75 mM NaCl showed the lowest ERI.

Similar results were reported by Ruiz-Ramírez *et al.* (2012) who evaluated the effect of different salinity levels with KCl in the ERI of five species of forage grasses, *Cenchrus ciliaris* L., *Chloris gayana* L., *Brachiaria brizantha* (A.Rich.) Stapf, *Brachiaria hybrid* cv. Mulato II and *Panicum maximum* Jacq cv. Tanzania concluded that ERI and other variables decreased as KCl levels increased.

The imbibition is the first stage of germination and affects the germination because of depends on the seed’s chemical composition, seed coat permeability, water potential difference, and the thickness of storage tissues (Fatokun *et al.*, 2022).

There is a specific association for maize seeds between the level of physiologic quality and imbibition in the sense that less germination corresponds to a higher imbibition percentage (Li *et al.*, 2022). In this

study, *L. purpureus* showed a similar response to maize seeds with the lowest ERI under 75 mM NaCl.

#### Seedlings growth

The analysis of variance showed that the seedling’s stem height and width, root length, stems+leaves fresh and dry-weight, root fresh and dry-weight, and plant balance were affected by species, NaCl, and species  $\times$  NaCl interaction (Table 1).

The stem height was higher at 0 mM NaCl in *C. ternatea* followed by the same specie at 50 and 25 mM NaCl. *Vigna unguiculata* at 0 mM also showed high stem height. The stem diameter was higher at 0, 25, 50, and 75 mM NaCl in *C. ternatea* followed by *L. purpureus* at 25, 50, and 75 mM NaCl. The lower stem diameter was at 50 mM in *C. ternatea*. *Canavalia ensiformis* showed the longest roots at 0 mM followed by *V. unguiculata* and *C. ternatea*. In general, *V. unguiculata* showed longer roots in all NaCl treatments (Table 1). According to Sheldon and Munns (2023) in saline media, substrate, or soil, with constant humidity through constant watering, salts are likely to stay in deeper layers and therefore roots decreased their length activating the formation of adventitious roots.

In the same sense, Muktadir *et al.* (2020) reported that high salinity generates a physiologic drought in plants. In accordance with this, the common bean doubles the number of secondary and tertiary roots with a modified architecture due to drought. For this reason, a system with widespread and deep roots is recommended to increase the productivity of alimentary legumes in drought and salinity conditions. Root length could be an important trait for *in-vitro* selection of bean varieties resistant to salinity with enhanced capacity to acquire water. Plant response to salinity varies according to species, cultivar, and distribution. This is evidence of the diverse strategies that plants have developed through their evolution. An

**Table 1.** Effect of NaCl-stress in the emergence and seedlings growth of four legumes, *Vigna unguiculata*, *Lablab purpureus*, *Clitoria ternatea* and *Canavalia ensiformis*.

Species	NaCl (mM)	Stem height (cm)	Stem diameter (mm)	Root length (cm)	Emergence (%)	Emergence rate index
<i>Clitoria ternatea</i>	0	8.50±0.33 bc*	1.83±0.20 h	8.01±0.52 ab	77.5±14.14 b	7.79±1.32 b
	25	6.32±0.45 ef	1.54±0.18 hi	6.85±0.40 bcd	80.0±12.58 a	7.90±1.68 b
	50	5.35±0.39 fgh	1.45±0.21 i	6.13±0.47 bcde	77.5±5.00 b	6.91±2.25 c
	75	4.10±0.74 h	1.59±0.27 hi	5.81±0.75 cde	82.5±5.00 a	6.68±1.57 c
<i>Lablab purpureus</i>	0	6.44±0.87def	2.96±0.13 fg	6.77±0.65 bcd	85.0±5.77 ab	7.85±1.13 bc
	25	5.79±0.27 fg	3.34±0.04 cd	7.07±0.68 abcd	82.5±5.00 b	5.50±1.12 e
	50	4.72±0.76 gh	3.29± 0.10cde	2.95±0.45 bcd	75.0±10.00 c	5.84±1.13 e
<i>Vigna unguiculata</i>	75	4.94±0.39 gh	3.45±0.39 bc	5.68±0.75 cde	67.5±18.92 d	5.19±1.30 e
	0	9.95±0.48 a	2.64±0.11 g	9.11±0.35 a	97.5±9.57 a	12.72±2.33 a
	25	8.26±0.23 c	2.96±0.23 efg	7.78±0.44 abc	87.5±5.00 a	9.75±0.30 b
	50	7.32±0.39 cde	2.96±0.04 efg	7.58±0.36 abc	82.5±9.57 b	9.72±1.57 b
<i>Canavalia ensiformis</i>	75	5.98±0.63 fg	3.07±0.12 def	6.61±0.65 bcd	82.5±9.57 b	9.46±1.30 b
	0	10.9±1.27 a	3.87±0.23 a	9.16±2.51 a	97.5±5.00 a	10.85±1.41 a
	25	9.66±2.01 ab	3.75±0.28 ab	7.27±0.63 abcd	72.5±5.00 cd	7.40±1.45 bc
	50	10.1±0.94 a	3.56±0.19 abc	4.57±0.23 e	70.0±18.25 d	7.75±0.81 bc
	75	7.62±1.59 cd	3.55±0.21 abc	5.16±0.62 de	67.5±22.17 d	6.35±2.30 d
		<b>Stems + leaves fresh weight (g)</b>	<b>Stems + leaves dry weight (g)</b>	<b>Root fresh weight (g)</b>	<b>Root dry weight (g)</b>	<b>Plant balance</b>
<i>Clitoria ternatea</i>	0	0.609±0.09 g	0.118±0.005 cdef	0.464±0.19 ab	0.052±0.005 b	0.254±0.017 a
	25	0.501±0.05 g	0.082±0.011 def	0.171±0.07 ef	0.043±0.005 b	0.479±0.053 a
	50	0.474±0.10 g	0.068±0.015 ef	0.097±0.049 fg	0.029±0.008 d	0.701±0.043 a
	75	0.347±0.07 g	0.062±0.012 f	0.033±0.014 g	0.021±0.006 e	1.878±0.976 b
<i>Lablab purpureus</i>	0	1.060±0.14 def	0.156±0.020 c	0.194±0.039 ef	0.039±0.009 bc	0.804±0.086 a
	25	0.967±0.09 ef	0.143±0.013 c	0.168±0.043 efg	0.035±0.005 c	0.851±0.092 a
	50	0.966±0.07 ef	0.140±0.002 cd	0.397±0.104 abcd	0.035±0.005 c	0.352±0.012 a
<i>Vigna unguiculata</i>	75	0.898±0.11 f	0.126±0.017 cde	0.349±0.117 bcd	0.029±0.008 d	0.361±0.032 a
	0	1.346±0.06 c	0.109±0.010 cdef	0.462±0.043 ab	0.046±0.006 b	0.235±0.054 a
	25	1.291±0.07 cd	0.101±0.009 cdef	0.398±2.053 abcd	0.033±0.001 c	0.253±0.065 a
	50	1.269±0.07cd	0.103±0.008 cdef	0.414±0.077 abc	0.032±0.003 c	0.248±0.032 a
<i>Canavalia ensiformis</i>	75	1.205±0.08 cde	0.101±0.014 cdef	0.300±0.093 cde	0.028±0.004 b	0.336±0.076 a
	0	3.695±0.25 b	0.612±0.017 b	0.522±1.29 a	0.099±0.021 a	1.172±0.983 a
	25	4.093±0.33 a	0.642±0.048 ab	0.372±0.096 bcd	0.090±0.005 a	1.725±0.873 a
	50	4.373±0.43 a	0.680±0.046 a	0.276±0.044 de	0.091±0.013 a	2.463±0.997 b
	75	3.782±0.32 b	0.661±0.044 ab	0.302±0.163 cde	0.054±0.015 b	2.188±0.983 b

\* The values represent the means ± standard deviation. Values with different letters in the same column are statistically different (Tukey HSD, p=0.05).

example of this is the higher salinity tolerances of the Poaceae family compared to Leguminosae, mainly due to their origin (Grigore and Vicente, 2023).

Plants that naturally inhabit saline soils tend to pose a higher capacity to extract water from the soil; however, halophyte plants not only need to be capable of absorbing water necessary to its development from a saline solution but also to absorb it with enough speed to maintain an adequate transpiration rate (Hasanuzzaman *et al.*, 2023). The NaCl treatments affected negatively stems and root fresh and dry weight, as the NaCl concentrations increased, fresh and root biomass decreased. *Canavalia ensiformis* L. DC. was less affected because of showed the highest values of biomass (fresh and dry) in the majority of NaCl treatments (Table 1). The reduction in biomass is because of the reduction of cell wall synthesis during stress which

affects the stem and root tissues causing a decrease in fresh and dry weight. Mubushar *et al.* (2022) showed that using growth biomass variables as indicator facilitate discriminate saline stress-tolerant genotypes and cultivars.

In the same context, Khan *et al.* (2023), mentioned that saline stress affects the structure and permeability of intracellular membranes, cell homeostasis, energy exchange reactions, DNA structure and functionality, and various enzymatic responses. These enzymes are mainly the ones related to metabolism during stress conditions and adaptive response, which protect from damage caused by stress.

Can-Chulim *et al.* (2014), reported that the emergence rate and germination of *P. vulgaris* (pinto bean), decreased by 54.7% at 9 dS.m<sup>-1</sup> and azufrado bean decreased 30.3%. Moreover, the differences in stem length between the control (T<sub>0</sub>) and the highest concentration

of salt treatment ( $T_0$ ) were 2.4, 4 y 3.2 cm for black, pinto, and azufrado bean, respectively. Higher electric conductivities resulted in germination reduction.

The smaller values of plant balance mean a better plant balance. Plant balance consists of the relation of stem+leaves dry weight over root dry weight. This relation was smaller for control plants because the seedlings showed lower weights of stem+leaves dry weight; however, with equal root weight as compared to the other NaCl treatments and species. The plant balance is very important for farmers because of has been observed that seedlings with a better plant balance have better establishment and development when transplanted to the field.

Furthermore, by getting a better plant balance in the field, better radicular growth could be obtained, thus, achieving better crop development. The NaCl salinity treatments reduced the plant balance due to the increment of osmotic pressure in the substrate solution in relation to the one in the root cells, affecting ion intake by the root hairs and, consequently, affecting plant nutrition and development (Acosta-Motos *et al.*, 2017). On the other hand, Li *et al.* (2017) concluded that a high concentration of Na is deleterious to the membrane selectivity and favors the passive accumulation of Na in roots and stems.

## Conclusions

This study showed a differential response of four legumes to NaCl concentrations. The species most tolerant to NaCl-stress were *Canavalia ensiformis* L. DC. and *Vigna unguiculata* L. Walp. In general, the emergence rate and emergence percentage, root length, root dry weight and stem height decreased as NaCl concentrations increased. Stem diameter, stem fresh and dry weight, and root fresh weight increased from 0 mM to 25 and 50 mM but in other case decreased from 50 to 75 mM, i.e., root fresh weight. Future experiments are needed to obtain information about the enrichment capacities of the legumes studied here; maybe, genotypes with lower emergence under high salinity could fix more atmospheric nitrogen than the tolerant ones.

## Literature cited

- Acosta-Motos, J.R., Ortuño, M.F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M.J., & Hernandez, J.A. (2017). Plant responses to salt stress: Adaptive mechanisms. *Agronomy*, 7, 18. <https://doi.org/10.3390/agronomy7010018>
- Al-huraby, A.I. & Bafeel, S.O. (2022). The effect of salinity stress on the *Phaseolus vulgaris* L. plant. *African Journal of Biological Sciences*, 4, 1, 94-107. doi: 10.33472/AFJBS.4.1.2021.94-107.
- Can-Chulim, A.A., Ramirez-Guerrero, L.G., Ortega-Escobar H.M., Cruz-Crespo, E., Flores-Román, D., Sánchez-Bernal E.I., & Madueño-Molina, A. (2014). Germinación y crecimiento de plántulas de *Phaseolus vulgaris* L. en condiciones de salinidad. *Revista Mexicana de Ciencias Agrícolas*, 5(5), 753-763. <https://doi.org/10.29312/remexca.v5i5.898>
- Daniel, A.I., Fadaka, A.O., Gokul, A., Bakare, O.O., Aina, O., Fisher, S., Burt, A.F., Mavumengwana, V., Keyser, M., & Klein, A. (2022). Biofertilizer: The future of food security and food safety. *Microorganisms*, 10, 1220. <https://doi.org/10.3390/microorganisms10061220>
- Dehnavi, A.R., Zahedi, M., Ludwiczak, A., Cardenas-Perez, S., & Piernik, A. (2020). Effect of salinity on seed germination and seedling development of Sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy*, 10, 859. <https://doi.org/10.3390/agronomy10060859>
- Deng, X., Ji, L., Wang, R., Liu, X., Yang, S., Guan, P., Wang, J. (2019). Response of seed germination and physiological mechanism of *Vigna angularis* and *Dolichos lablab* to salt stress. *Chinese Journal of Eco-Agriculture*, 27(8), 1218-1225. doi:10.13930/j.cnki.cjea.190227
- Fatokun, K., Beckett, R.P., & Varghese, B. (2022). A comparison of water imbibition and controlled deterioration in five orthodox species. *Agronomy*, 12, 1486. <https://doi.org/10.3390/agronomy12071486>
- García, E. (2004). Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía de la Universidad Nacional Autónoma de México. Ciudad de México. 97 p. <http://www.publicaciones.igg.unam.mx/index.php/ig/catalog/view/83/82/251-1>
- Grigore, M.N. & Vicente, O. (2023). Wild halophytes: Tools for understanding salt tolerance mechanisms of plants and for adapting agriculture to climate change. *Plants*, 12, 221. <https://doi.org/10.3390/plants12020221>
- Gul, Z., Tang, Z.H., Arif, M., & Ye, Z. (2022). An Insight into Abiotic Stress and Influx Tolerance Mechanisms in Plants to Cope in Saline Environments. *Biology*, 11, 597. <https://doi.org/10.3390/biology11040597>
- Hasanuzzaman, M., Zhou, M., & Shabala, S. (2023). How does stomatal density and residual transpiration contribute to osmotic stress tolerance? *Plants*, 12, 494. <https://doi.org/10.3390/plants12030494>
- Ibrahim, E.A.A. (2019). Fundamental Processes Involved in Seed Priming. In: Hasanuzzaman, M., Fotopoulos, V. (eds). Priming and pretreatment of seeds and seedlings. Springer, Singapore. [https://doi.org/10.1007/978-981-13-8625-1\\_4](https://doi.org/10.1007/978-981-13-8625-1_4)
- Khan, M., Ali, S., Al Azzawi, T.N.I., Saqib, S., Ullah, F., Ayaz, A., & Zaman, W. (2023). The key roles of ROS and RNS as a signaling molecule in plant-microbe interactions. *Antioxidants*, 12, 268. <https://doi.org/10.3390/antiox12020268>
- Li, Y., Liang, Y., Liu, M., Zhang, Q., Wang, Z., Fan, J., Ruan, Y., Zhang, A., Dong, X., Yue, J., & Li, C. (2022). Genome-wide association studies provide insights into the genetic architecture of seed germination traits in maize. *Frontiers in Plant Science*, 13:930438. doi: 10.3389/fpls.2022.930438
- Li, B., Tester, M., & Gilliam, M. (2017). Chloride on the move. *Trends in Plant Science*, 22, 3, 236-248, <https://doi.org/10.1016/j.tplants.2016.12.004>.
- Little, T.M. y Hills, F.J. (1989). *Métodos estadísticos para la investigación en la agricultura. México*. Edit. Trillas. 270 p.
- Maguire, J.D. (1962). Speed of germination-aid in selection and evaluation for seedling emergences and vigor. *Crop Science*, 2, 176-177. <https://doi.org/10.2135/cropsci1962.0011183X000200020033x>
- Mathesius, U. (2022). Are legumes different? Origins and consequences of evolving nitrogen fixing symbioses. *Journal of Plant Physiology*, 276, 153765, <https://doi.org/10.1016/j.jplph.2022.153765>.
- Mohanavelu, A., Naganna, S.R., & Al-Ansari, N. (2021). Irrigation induced salinity and sodicity hazards on soil and groundwater: An overview of its causes, impacts and mitigation strategies. *Agriculture*, 11, 983. <https://doi.org/10.3390/agriculture11100983>
- Mubushar, M., El-Hendawy, S., Tahir, M.U., Alotaibi, M., Mohammed, N., Refay, Y., & Tola, E. (2022). Assessing the suitability of multivariate analysis for stress tolerance indices, biomass, and grain yield for detecting salt tolerance in advanced spring wheat lines irrigated with saline water under field conditions. *Agronomy*, 12, 3084. <https://doi.org/10.3390/agronomy12123084>
- Muktadir, M.A., Adhikari, K.N., Merchant, A., Belachew, K.Y., Vandenberg, A., Stoddard, F.L., & Khazaei, H. (2020). Physiological and biochemical basis of faba bean breeding for drought adaptation-A Review. *Agronomy*, 10, 1345. <https://doi.org/10.3390/agronomy10091345>
- Murillo-Amador, B., Yamada, S., Yamaguchi, T., Rueda-Puente, E.O. *Ávila-Serrano*, N.Y., García-Hernández, J.L., López-Aguilar, D.R., Troyo-Diéguez, E., & Nieto-Garibay, A. (2007). Influence of calcium silicate on growth, physiological parameters and mineral nutrition in two legume species under salt stress. *Journal Agronomy Crop Science*, 193:413-421. doi.org/10.1111/j.1439-037X.2007.00273.x
- Narejo, G.A., Mirbahar, A.A., Yasin, S., Sirohi, M.H., & Saeed R. (2023). Effect of hydro and KNO<sub>3</sub> priming on seed germination of cotton (*Gossypium hirsutum* L.) under gnotobiotic conditions. *Journal of Plant Growth Regulation*, 42, 1592-1603. <https://doi.org/10.1007/s00344-022-10644-y>
- Nachshon, U. (2018). Cropland soil salinization and associated hydrology: Trends, processes and examples. *Water*, 8, 1030. <https://doi.org/10.3390/w10081030>
- Negacz, K., Malek, Z., Vos, A.D., & Vellinga, P. (2022). Saline soils worldwide: Identifying the most promising areas for saline agriculture. *Journal of Arid Environments*, 203, 104775, <https://doi.org/10.1016/j.jaridenv.2022.104775>.
- Praxedes, S.S.C., da Silva Sá, F.V., Neto, M.F., Loiola, A.T., Reges, L.B.L., Jales, G.D., & de Melo, A.S. (2020). Tolerance of seedlings traditional varieties of cowpea (*Vigna unguiculata* (L.) Walp.) to salt stress. *Semina: Ciências Agrárias, Londrina*, 41(5 suplemento 1), 1963-1974.
- Ravelombola, W., Shi, A., Weng, Y., Mou, B., Motes, D., Clark, J., Chen, P., Srivastava, V., Quin, J., Dong, L., Yang, W., Bhattarai, G., & Sugihara, Y. 2018. Association analysis of salt tolerance in cowpea (*Vigna unguiculata* (L.) Walp.) at germination and seedling stages. *Theoretical and Applied Genetics*, 131, 79-91, <https://doi.org/10.1007/s00122-017-2987-0>
- Ruiz-Ramírez, S., Valdés-Oyervides, A., Facio-Parra, F., y Arce-González, L. (2012). Efecto de diferentes niveles de salinidad en la germinación y vigor de semillas de cinco gramíneas forrajeras. *Agraria*, 9, 1, 7-13. <https://revista.uaaa.edu.mx/>
- Shelden, M.C. & Munns, R. (2023). Crop root system plasticity for improved yields in saline soils. *Frontiers in Plant Science*, 14, 1120583. doi: 10.3389/fpls.2023.1120583Shel
- TIBCO Software Inc. (2018). Statistica (data analysis software system), version 13. <http://tibco.com>.
- Xu, J., Li, Y., Wang, S., Wang, Q., & Ding, J. (2020). Shear strength and mesoscopic character of undisturbed loess with sodium sulfate after dry-wet cycling. *Bulletin Bulletin of Engineering Geology and the Environment*, 79, 1523-1541. <https://doi.org/10.1007/s10064-019-01646-4>