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Effect of NaCl on curarí (Tabebuia serratifolia) *seedlings under laboratory conditions*

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ABSTRACT: The objective of this work was to evaluate the effect of sodium chloride (NaCl) during the germination and initial growth of curarí (*Tabebuia serratifolia*) seedlings under laboratory conditions. The seeds were placed in five concentrations of NaCl: 2, 4, 6, 8 and 10 dS m^{-1}, and in distilled water (control), with an electric conductivity between 0,02 and 0,04 dS m^{-1}. A completely randomized experimental design was used, with five repetitions, and a variance analysis was applied. The germination percentage (GP) and germination rate (GR) were determined; and the stem length (SL), root length (RL), cotyledonal area (CA), fresh biomass (FB) and dry biomass (DB) were measured. The concentration of NaCl showed significant effects for the variables GP, GR, DB, SL, RL and CA. A germination of 79,6 % and a GR of 1,66 days were obtained when the seeds were subject to 6 dS m^{-1} of NaCl. The concentrations of 8 and 10 dS m^{-1} significantly decreased GP, SL and CA of the seedlings and delayed the GR. It is concluded that the *T. serratifolia* seedlings showed tolerance to the saline condition of NaCl up to 6 dS m^{-1}, for which this species can be used with agroforestry purposes in livestock production systems, as well as in the recovery of areas that show moderate salinity problems.

Key words: multipurpose trees, stress, survival

INTRODUCTION

In Venezuela there is a large diversity of wild trees with high utilization potential (Hoyos, 1992), including the agroforestry purpose (Ramírez *et al.*, 2012). Curarí (*Tabebuia serratifolia*) is a multipurpose species, native to the Amazonian rainforest of northern South America (Justiniano *et al.*, 2000), which has high economic, wood and ornamental importance in Brazil (Machado *et al.*, 2002) and it is considered optimum for agroforestry systems and plant recovery programs in degraded areas of Bolivia (Vargas *et al.*, 2000). However, in Venezuela it has not been given the importance it deserves, in spite of being a native species. At present it is still found in natural forests, rural zones and agricultural production systems of the country, under different environmental conditions (including the saline one), where it grows naturally. In the Zulia and Falcón states it is very common to observe it in livestock production units, in which it prevails and it is commonly confused with *Tabebuia chrysantha*. On the other hand, curarí is endangered today, due to felling –because its wood, of excellent quality and durability, is used in craftsmanship, furniture and supports, etc.–, burning and indiscriminate growth of urbanism, commercial activity and agricultural production without planning or environmental education. A simple way to rescue and include *T. serratifolia* in production systems is through the development of seedlings or small trees that grow naturally in the fields or forests.

Soil salinity is one of the oldest environmental problems that affect crop yield and plant distribution in nature (Camejo and Torres, 2000; Martínez *et al.*, 2010). Because of the constant increase of salinized soils, the establishment of fast and reliable criteria and laboratory methods to evaluate the tolerance of species to different salinity conditions is necessary, based on the affectations of germination, seedling growth and survival (González *et al.*, 2002).

In this sense, there is no information about the effect of salinity on the germination and initial growth of the seedlings. There is only one study conducted in Brazil, in which it is indicated that germination was fast at temperature ranges between 25 and 35 ºC, under laboratory conditions, with non-saline water (Machado *et al*., 2002). Nevertheless, in other species the effect of NaCl salts has been reported on germination (Meza *et al*., 2004; García *et al*., 2011), seedling height, root length and dry biomass accumulation (Argentel *et al*., 2006a). Based on these premises, as well as on the need of recovering this species and its inclusion in agroforestry systems, this work was conducted in order to evaluate the effect of different concentrations of NaCl during the germination and initial growth of *T. serratifolia* seedlings, under laboratory conditions.

MATERIALS AND METHODS

The trial was conducted at the plant propagation laboratory of the School of Agronomy, University of Zulia (LUZ). The *T. serratifolia* seeds were collected from trees located in the Jagüey de Monte sector, La Concepción parish (Jesús Enrique Lossada municipality, Zulia state).

Treatments and design. The seeds were extracted from the mature pods, before dehiscence. Afterwards, under laboratory conditions (25 ºC), they were placed on large and uncovered plastic trays until the seeds were detached from the fruit; the small or deformed ones were discarded, and the healthy ones were uniformly distributed on absorbent paper. Then, they were sprayed with the fungicide Vitavax® at 0,5 % (Ramírez *et al*., 2012), left to dry during four days (at 25 ºC), and were stored at 10 ºC for eight months.

After the storage period,the seeds were exposed to different saline concentrations of NaCl (2, 4, 6, 8 and 10 dS m^{-1}, adjusted by means of a conductivity meter (Copenhagen type CDM 2 dn.107361). Distilled water, with an electric conductivity range between 0.02 and 0.04 dS m^{-1}, was used as control.

Procedure. The seeds were placed on transparent plastic trays 12,5 cm wide, 15,5 cm long and 4,5 cm of height, with two sheets of absorbent paper and 15 mL of each solution, under continuous fluorescent light and at 25 ºC. Irrigation took place seven days later, with the corresponding saline solution in each treatment.

During eight days the number of germinated seeds was evaluated, to determine the germination percentage (GP) and the germination rate (GR) (Perozo *et al*., 2006). Fifteen days after setting the experiment, stem length (SL), root length (RL), cotyledonal area (CA), fresh biomass (FB) and dry biomass (DB) were measured. For such purpose, five seedlings were randomly selected from each repetition (those in the tray edges were discarded). SL was measured with a ruler, from the apex to the stem base; and RL, from the stem base to the apex of the main root; both variables were expressed in centimeters. To determine the CA, the cotyledons of each seedling were cut with a scalp and they were placed on a leaf area meter; while for the FB each plant was weighed on an analytical balance (Mettler Toledo, AB204-S/FAST). After this process they were dried in an oven at 74 ºC, during 48 hours, to obtain the DB (mg).

A completely randomized design was used, with five repetitions per treatment and 20 seeds as experimental unit, for a total of 100 seeds per treatment. A variance analysis was applied and Tukey's test, when significant effects were detected, for mean comparison (SAS, 2010).

RESULTS AND DISCUSSION

The variance analysis showed significant differences ($p < 0.05$) among the concentrations of NaCl for the GP in the *T. serratifolia* seeds. There were no significant differences between the control and concentrations 2, 4 and 6 dS m^{-1} of NaCl; but there were differences between this group and the other treatments (table 1). The conditions of 8 and 10 dS m^{-1} of NaCl had a similar performance, as well as the lowest germination percentages (46,56 and 55,2 %), which showed the inhibiting effect of such concentrations on the physiological and biochemical processes of germination: water imbibition, activation and synthesis of enzymes, digestion and translocation of substances towards the growth spots of the embryonic axis (Azcón and Talón, 2008). In tomato, the extreme concentrations of NaCl have caused physical and chemical changes in the seeds, as well as delay and decrease of germination (Camejo and Torres, 2000).

Seed tolerance to salinity during germination is a measure of the seed capacity to stand the effects of high concentrations of soluble salts in the environment, which decreases the hydric potential and causes lower water availability. Thus, they must generate sufficient osmotic potential to improve the hydric status of embryos and allow their growth (Cortés and Saavedra, 2007).

The high percentage (germination capacity) observed in the solutions up to 6 dS m^{-1} of NaCl (72,8-84,8 %) proved that *T. serratifolia* had a high degree of tolerance to salinity (fig. 1) and moderate tolerance in the highest concentrations (8 and 10 dS m^{-1} of NaCl). This result was considered promising for

Table 1. Effect of the concentration of NaCl eight days after the establishment of the trial.

Concentration of NaCl (dS m^{-1})	Germination (%)	Germination rate (days)
Control (0,02-0,04)	84,80^{a}	1,13^{b}
2	83,60^{a}	1,25^{b}
4	72,80^{a}	1,62^{b}
6	79,60^{a}	1,66^{b}
8	46,56^{b}	2,70^{a}
10	55,20^{b}	2,26^{a}
SE ±	6,63	0,25

Means with different letters in each variable statistically differ ($p < 0.05$).

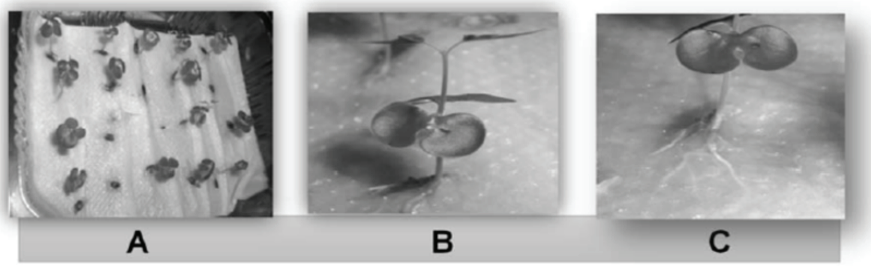

Fig. 1. A) *T. serratifolia* seedlings under saline conditions (6 dS m^{-1} of NaCl) fifteen days after the trial was established. B) seedling with cotyledons and first true leaves. C) root system of the seedling.

the species, because this type of information is not available. Some works conducted with seeds from other species show that each one has a different performance or tolerance to salinity, because concentrations of NaCl equal to or higher than 2,5 dS m^{-1} in *Manilkara huberi* (Meza *et al*., 2004), 6 dS m^{-1} in *Passiflora edulis* (Meza *et al*., 2007), 18 dS m^{-1} in *Thinopyrum ponticum* (Bazzigalupi *et al*., 2008), 34 dS m^{-1} in *Chenopodium quinoa* (Chilo *et al*., 2009) and 10 dS m^{-1} of KCl in the forage *Kochia scoparia* (García *et al*., 2011) decreased germination.

The concentration of NaCl caused significant differences in the germination rate (table 1) or average number of days required for germination. Two groups were detected: the first one constituted by the control and concentrations 2, 4 and 6 dS m^{-1} of NaCl; and the second one, formed by the concentrations 8 and 10 dS m^{-1} of NaCl. The last two concentrations prolonged the days for germination to occur. This response coincides with the observations made in *T. ponticum* (Bazzigalupi *et al*., 2008) and in *K. scoparia* (García *et al*., 2011). The reduction of the germination percentage, as well as the delay in the beginning of such process with the increase of saline stress, was related to the induced physiological drought, because with the increase of the salt concentration in the germination medium the osmotic potential and, consequently, the hydric potential, decrease.

No significant differences were observed among the concentrations of NaCl regarding the fresh biomass of the *T. serratifolia* seedlings (table 2), although a slight reduction was noticed from 4 dS m^{-1}, which could be associated to the fact that in most plants the absorption of water and important nutrients –such as nitrogen, sodium and potassium, essential for growth— decreases, in the presence of salts (Barkla *et al*., 2008).

According to the variance analysis, among the concentrations of NaCl there were significant differences in the accumulation of dry biomass in the *T. serratifolia* seedlings (table 2). This variable decreased significantly from 2 dS m^{-1} of NaCl, which was more marked in the concentrations 8 and 10 dS m^{-1} of NaCl. In addition, it constituted another indicator of the growth of *T. serratifolia* seedlings

Table 2. Effect of the concentration of NaCl on *T. serratifolia* seedlings, fifteen days after establishing the experiment.

Concentration of NaCl (dS m^{-1})	Fresh biomass (mg)	Dry biomass (mg)	Stem length (cm)	Root length (cm)	Cotyledonal area (cm^2)
Control (0,02-0,04)	69,17	$9{,}93^{a}$	$1{,}94^{a}$	$5{,}56^{a}$	$1{,}23^{a}$
2	60,93	$7{,}77^{b}$	$1{,}88^{a}$	$5{,}54^{a}$	$1{,}14^{a}$
4	56,16	$7{,}96^{b}$	$1{,}72^{a}$	$5{,}32^{ba}$	$1{,}08^{a}$
6	50,71	$7{,}81^{b}$	$1{,}60^{a}$	$4{,}10^{b}$	$1{,}09^{a}$
8	50,89	$6{,}11^{c}$	$0{,}71^{b}$	$1{,}44^{c}$	$0{,}68^{b}$
10	51,61	$5{,}16^{cd}$	$0{,}52^{b}$	$1{,}15^{c}$	$0{,}68^{b}$
SE ±	3,00	0,68	0,25	0,84	0,10

Means with different letters in each variable statistically differ ($p < 0{,}05$).

under saline stress. These results coincide with the reports for other species, such as *K. scoparia* (García *et al*., 2011), wheat (Argentel *et al*., 2006b) and guava tree (Casierra, 2006), in which there was a lower accumulation of dry biomass as the concentration of salts in the irrigation water increased. In the case of wheat, the dry biomass was the most sensitive indicator to the affectation caused by the NaCl salts (Argentel *et al*., 2006b). The tolerance of *T. serratifolia* to such salt is related to the fact that the tree is naturally developed under arid conditions (including the saline ones), which shows its adaptation capacity.

On the other hand, plants are capable of developing adaptive mechanisms to survive under high salinity conditions, among which is the accumulation and deposit of sodium along the growth axis, in a concentration gradient that tends to be higher in the young parts of the leaves, where it is isolated to prevent damage. This ion is deposited and absorbed into the central vacuole of plant cells, behavior associated to the sodium/protons (Na^+/H^+) exchanger which is activated by the proton pump of the tonoplast V-ATPase (Barkla *et al*., 2008).

Regarding stem length, significant differences were detected among the treatments (table 2). There were no significant differences between the control and concentrations 2, 4 and 6 dS m^{-1} of NaCl, but there were with regards to the other treatments. This indicates that the stem length was affected, due to the stress caused by saline irrigation from levels equal to and higher than 6 dS m^{-1} of NaCl. Such results have a certain similarity with those of a study conducted in lettuce (Lesmes *et al*., 2007), in which the inclusion of NaCl in the irrigation water inhibited shoot elongation. However, in tomato, the salinity-caused affectations on the stem have been irreversible (Morales *et al*., 2010). On the other hand, Barkla *et al*. (2008) reported that the accumulation of sodium in plant cells becomes toxic and affects a large variety of metabolic processes (including the enzymes that participate in photosynthesis). Thus, plant growth is inhibited since the stress begins due to the salts.

The variance analysis showed that there were significant differences among the treatments regarding root length (table 2). In the control and solutions 2 and 4 dS m^{-1} of NaCl, the seedlings showed a similar performance and reached the highest root length; while in 6, 8 and 10 dS m^{-1} of NaCl this variable decreased. Nevertheless, these last two concentrations differed from 6 dS m^{-1} and the roots showed little development and brown coloring, possibly due to the deterioration of the root system by the exposure to high saline concentrations, which has been reported in other studies (Morales *et al*., 2010; García *et al*., 2011). In beans (García *et al*., 2010), the most sensitive organ to saline stress was the root, in concentrations higher than 12 dS m^{-1} of NaCl.

In *M. huberi*, the root was the organ that accumulated the highest sodium quantity, as compared with the aerial part (Meza *et al*., 2004). Little salt-resistant plants are not able to aim sodium at the mature parts; for which they keep it in the roots. For such reason, in susceptible or little-tolerant plants the absorbed sodium tends to concentrate in the root cells, where toxicity is produced and the passage of water and the necessary nutrients for their growth is inhibited (Barkla *et al*., 2008).

The root length values obtained in the concentrations 8 and 10 dS m^{-1} of NaCl showed that the roots had little growth. This caused the existence of a lower surface of solute and water absorption, and, thus, low stem length and dry biomass values. This performance was associated to the fact that salts alter water absorption, affect the growth of plant organs and have toxic effects (Cortés and Saavedra, 2007; García *et al*., 2011).

In *Opuntia ficus-indica* –a CAM (crassulacean acid metabolism) plant–, as in the plants with C_3 and C_4 photosynthetic metabolism, the rise of salinity increased the content of proline in the chlorenchyma and hydroparenchyma of the cladodes and roots, as general response to the changes in the external osmotic potential. The increase of proline prevents the entrance of toxic ions to the tissues and/or maintains the functioning of the necessary pumps to exclude the excess of NaCl, with which enzymatic inhibition is avoided. Such increase could be due to the *de novo* synthesis and to the decrease in the synthesis rate of proteins and/or degradation of the proline-rich proteins (Franco and Véliz, 2007).

The concentration of NaCl had significant effects on the cotyledonal area (table 2); there were no significant differences between the control and the concentrations 2, 4 and 6 dS m^{-1} of NaCl. Nevertheless, under the conditions of 8 and 10 dS m^{-1} of NaCl the cotyledonal area was reduced, the cotyledons tended to wrinkle and, in some cases, they showed chlorotic spots; in addition, the seedlings did not develop true leaves.

In *M. huberi* there was a reduction of the cotyledonal area with the concentration 8,5 dS m^{-1} of NaCl, which is associated to the negative effect of salts on the aerial part of the plant (Meza *et al*., 2004). In two bean varieties (García *et al*., 2010), 8 dS m^{-1} of NaCl produced a drastic effect on the plants, with chlorosis and necrosis in the leaves at seven and 14 days of growth, which could have caused the decrease of the relative chlorophyll content and the increase of lipid peroxidation at leaf level. In *Carica papaya* (Parés *et al*., 2008), the concentration 8 dS m^{-1} of NaCl caused variations in the stomatal index, stomatal density and thickness of leaf tissues. The symptoms observed in the *T. serratifolia* seedlings under saline stress have certain coincidence with the reports for other species (Meza *et al*., 2004; García *et al*., 2010).

The results of this study represent an important contribution, because there are no others in this regard, and they also lay the foundations for further studies in this species. It is concluded that the concentration of NaCl significantly influenced the germination percentage, germination rate, stem and root length, cotyledonal area and dry biomass of *T. serratifolia*. These variables constitute excellent growth or early morphological markers of the tolerance of this tree to salinity by NaCl. The lower growth or length of the main root, as well as the brown coloring of the tissues, allowed to recognize quickly the symptoms of saline stress in this species. The *T. serratifolia* seedlings showed tolerance to the saline conditions of NaCl up to 6 dS m^{-1}, for which they can be used for agroforestry purposes in livestock production systems and in the reclamation of areas with moderate salinity problems.

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