SEED GERMINATION AND SEEDLINGS GROWTH OF SOYBEAN (*Glycine max* (L.) Merr.) UNDER SALT STRESS

GERMINAÇÃO DE SEMENTES E CRESCIMENTO DE PLÂNTULAS DE SOJA (Glycine max (L.). Merr.) SOB ESTRESSE SALINO

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ABSTRACT: Seed germination and seedling growth of soybean (*cv*. BRS-133) were examined on paper towels pre-moistened with a range of saline solutions (0, 50, 100, 150 and 200 mM NaCl). Seeds were evaluated by the standard germination test and speed of germination-index while seedlings growth was evaluated by the measuring of root and shoot lengths and fresh and dry biomass. The results showed that salinity stress reduced both seed germination and seedlings growth. The germination percentage of the control group was 61% in average, while for the higher salt concentration (200 mM) the germination was reduced to 5%. As salt concentration increased, the shoot and root length and the fresh and dry biomass decreased significantly. The increase of the salt concentration also reduced the development of secondary roots. The results are discussed on the basis of the effects of salinity stress on germination and seedling growth of soybean.

UNITERMS: *Seedling growth, Seed germination, Soybean, Salinity stress.*

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural status of the environment. Among these, salinity is a severe problem that affect 2 million km^2 of land used for agricultural production. Hence, this is a major limitation to legume production in many areas of the world. The increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (ESSA, 2002; WANG; VINOCUR; ALTMAN; 2003).

Effects of salinity on plant water relations, nutritional imbalance, and ion toxicity are responsible for the inhibition of growth and as consequence decrease in plant yield (FRANCO et al., 1999). The response of plants to saline stress is complex since it involves changes in their morphology, physiology and metabolism, and may be expected to vary in different growth stages of plant species (HILAL et al., 1998). This fact has been shown by Chartzoulakis and Klapaki (2000) in pepper (*Piper nigrum*); Chartzoulakis and Loupassaki (1997) in eggplant (*Solanum melongena*) and by Dumbroff and Copper (1974) in tomato (*Lycopersicon esculentum*). Rice (*Oryza sativa*) young seedlings and plants at the flowering stage seem to be more sensitive than in mature stages (LUTTS; KINET; BOUHARMONT, 1995). Furthermore, these responses also may vary in different cultivars of the same plant. For example, Essa (2002) reported that germination percentages of soybean (*Glycine max* (L.) Merr.) were significantly reduced with increasing salinity levels. The Lee cultivar was less affected by salinity stress than Coquitt and Clark63 cultivars.

Taking into account the earlier reports, and since germination consists in an important factor to the development of several crops, the purpose of the present work was to investigate the effects of sodium chloride on soybean (cultivar BRS-133, Embrapa) seed germination and seedling growth.

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MATERIAL AND METHODS

A commercial soybean (*Glycine max* (L.) Merr.) seed lot, cultivar BRS-133, was used to assess seed germination and seedlings growth involved in the presence of NaCl using concentrations of 0 (control), 50, 100, 150 and 200 mM. The NaCl solutions were prepared with deionized water and the pH was adjusted to 6.0.

The germination test was performed with 50 seeds on three paper towels (Germitest® CL-060) moistened with an amount of water or saline solution equivalent to three times its dry weight. After planting, the rolled paper towels were placed vertically in plastic containers, containing a fine layer (±3 cm) of water or the solutions of the NaCl. The containers were wrapped in transparent plastic bags, making a closed system, and placed in a Mangelsdorf germinator at 25±2°C. The speed of germination-index was monitored daily while the standard germination test was determined after eight days. The results were expressed in percentage following the Rules for Seed Analysis (BRASIL, 1992).

To evaluate seedling growth, 20 seeds were distributed longitudinally along two straight lines on the upper third of the germination towel. The towel was moistened with deionized water (control) or with different concentrations of NaCl. The upper seed line was about 2 cm from the top edge of the towel. The seeds were placed on the paper with the micropyle facing the lower edge. The paper towels were rolled up and placed in germinator at 25±2°C for seven days, in darkness (NAKAGAWA, 1999). The seedling and radicle length were measured. The seedling length was measured as the full distance while the radicle length was determined as the distance between the differentiation region to its tip (NAKAGAWA, 1999).

The fresh and dry biomasses of seedlings were obtained after the length assessments, using the same methodology, but discarding the seedling cotyledons. The fresh biomass was obtained using precision analytic scales. After, the seedlings were placed in labeled paper bags and dried at 80°C for 24 hours, cooled and reweighed to obtain the dry biomass (NAKAGAWA, 1999).

The experimental design was completely randomized with four to ten replications. The relationship of seed germination, speed of germination-index, seedling length, root length, fresh and dry biomasses with levels of NaCl were analyzed by regression. The results are given in the text as P, the probability values, and Pd”0.05 was adopted as criterion of significance. The data analysis was carried out with the assistance of Sigmaplot® package, version 1.01 (Jandel Corporation, USA).

RESULTS AND DISCUSSION

When soybean seeds were germinated during 8 days at 25ºC in the presence of NaCl (Figure 1A), the results showed that at 50 mM, this salt did not influence germination percentage in comparison to control (62%). Nonetheless, NaCl affected seed germination significantly (Pd”0.05), i.e, the percentages were close to 37, 18 and 5% at 100, 150 and 200 mM, respectively. The same figure (1B) shows that NaCl (50 and 100 mM) did not affect the speed of germination-index (Pd”0.05) in comparison to control (48%). On the other hand, NaCl (150 and 200 mM) reduced significantly (Pd”0.05) this variable at approximately 41%. Another aspect is that visual differences in the roots were apparent. The increase salt concentration reduced the number and length of secondary roots, which were stunted and less flexible.

The data revealed that NaCl also decreased seedling and primary root lengths at all concentrations (Figure 2). Seedling lengths were reduced from 14.3 cm (50 mM) to 3.99 cm (200 mM) after 8 days of NaCl treatment, in comparison to control seedlings (23.9 cm). Likewise, primary root lengths decreased in contrast to control (14.7 cm). Root diminished from 8.33 cm (50 mM) to 2.61 cm (200 mM) under salt stress. It is clear from Figure 3 that NaCl reduced significantly (Pd”0.05) seedling fresh (Figure 3A) and dry (Figure 3B) biomass. Seedling fresh biomass diminished from 0.37 g to 0.065 g and seedling dry biomass diminished from 0.03 g to 0.008 g in comparison to controls (0.56 g for fresh and 0.039 g for dry biomass).

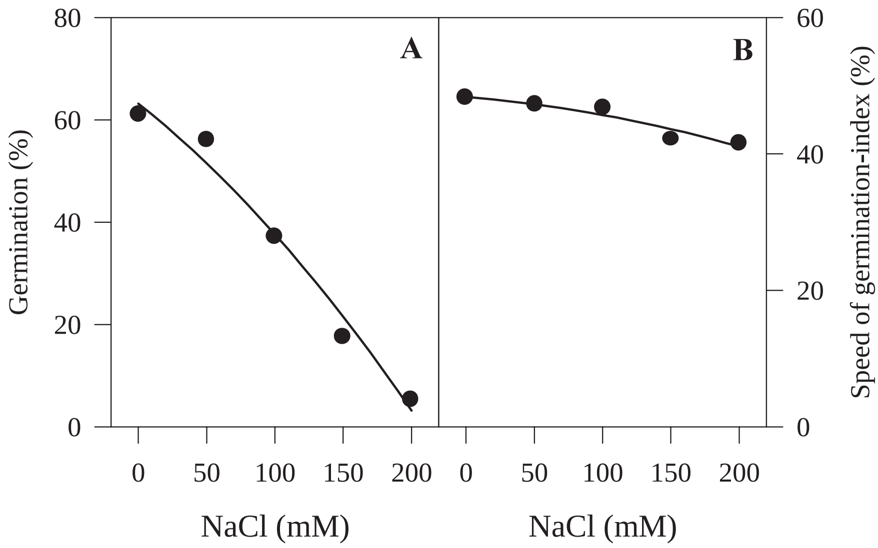

Figure 1. Accumulated seed germination (**A**) and speed of germination-index (**B**) of soybean treated with different concentrations of NaCl after eight days. The continuous lines are the regressions curves calculated as $\hat{Y} = 62.2 - 0.21X - 4\cdot10^{-4}X^2$, $R^2 = 0.98$ (in **A**) and $\hat{Y} = 48.3 - 0.02X - 1.1\cdot10^{-4}X^2$, $R^2 = 0.90$ (in **B**).

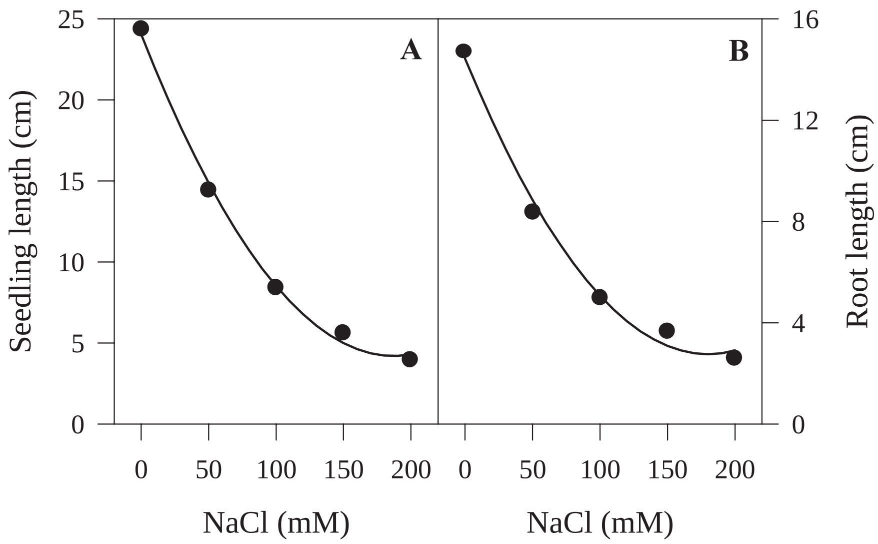

Figure 2. Seedling (**A**) and root length (**B**) of soybean treated with different concentrations of NaCl after eight days. The continuous lines are the regressions curves calculated as $\hat{Y} = 24.1 - 0.21X + 5.7\cdot10^{-4}X^2$, $R^2 = 0.99$ (in **A**) and $\hat{Y} = 14.4 - 0.13X + 3.6\cdot10^{-4}X^2$, $R^2 = 0.99$ (in **B**).

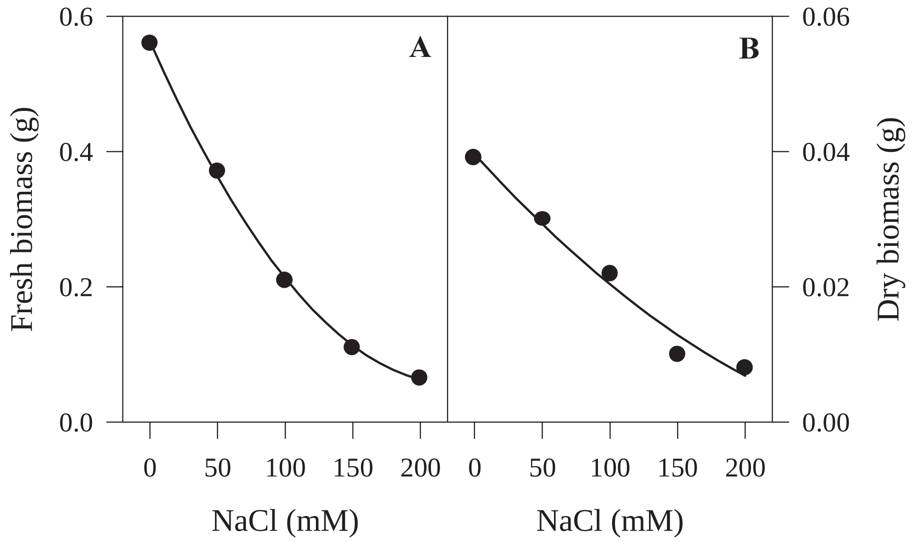

Figure 3. Seedling fresh biomass (**A**) dry biomass (**B**) of soybean treated with different concentrations of NaCl after eight days. The continuous lines are the regressions curves calculated as $\hat{Y} = 0.56 - 4.5 \cdot 10^{-3}X + 1.5 \cdot 10^{-5}X^2$, $R^2 = 0.99$ (in **A**) and $\hat{Y} = 0.04 - 2.2 \cdot 10^{-4}X + 2.6 \cdot 10^{-7}X^2$, $R^2 = 0.99$ (in **B**).

Taken together, the results obtained here show that NaCl stress has significant effects on soybean seed germination and seedling growth. As reported earlier, saline stress interfere to some degree with many vital plant processes. Reported studies using NaCl have shown inhibitory effects on seed germination, seedling growth and biomass of rice (*Oryza sativa*), maize (*Zea mays*), bean (*Phaseolus vulgaris*), alfalfa (*Medicago sativa*) and sorghum (*Sorghum bicolor*) as cited by different authors (ALLA et al., 2002; AMORIM et al., 2002; CAMPOS; ASSUNÇÃO, 1990; HILAL et al., 1998; LACERDA et al., 2001; LIN; KAO, 2001a,b;). With respect to soybean, Essa (2002) reported that germination percentages were significantly reduced with increasing salinity levels. Significant reduction in plant height was found in three soybean cultivars. Plants of the Lee cultivar were larger than plants of the others two cultivars (Coquiit and Clark63). Also recently, Hosseini, Powell, Binghan (2002) related that seed germination of the cultivar Williams decreased at NaCl concentrations of 330 mM and above. Seedling growth rate decreased with increasing salinity (almost zero at 330 mM). Hence, the data in the present work agree with results obtained by other investigations.

Although salt stress reduces significantly seed germination and seedling growth of different plant species the mechanisms involved remains an enigma. In general, results reported to date have been analyzed from the physiological and metabolic aspects. Salinization is manifested basically as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell. The presence of sodium ions may cause membrane depolarization, which may lead to the disruption not only of ion selectivity but also of mechanisms of ion uptake (WANG; VINOCUR; ALTMAN, 2003). Then, the influence of NaCl on the fluidity and permeability of cellular membranes may be expected. In fact, high rate of lipid peroxidation has been shown in a salt-susceptible cotton (*Gossypium hirsutum*) cultivar (MELONI et al., 2003), sunflower (*Helianthus annus*) cells (DAVENPORT et al., 2003), rice (*Oryza sativa*) roots (KHAN; PHANDA, 2002) and barley (*Hordeum vulgare*) seedlings (FEDINA; GRIGIROVA; GEORGIEVA, 2003). Furthermore, translocations of salt into roots and to shoots is an outcome of the transpirational flux required to maintain the water status of the plant and unregulated transpiration may cause toxic levels of ion accumulation in the shoot (YEO, 1998). For Braccini et al. (1996) the effects of the salt stress is relatively similar to water stress, which reduces the physiological and biochemical processes affecting the seedling growth.

In addition, oxidative stress may cause denaturation of functional and structural proteins and, as consequence, often activates cellular responses (WANG; VINOCUR; ALTMAN, 2003). The activity of antioxidant enzymes was reported to increase under saline conditions in the case of shoot cultures of rice (*Oryza sativa*), cucumber (*Cucumis sativus*), wheat (*Triticum aestivum*) shoot and pea (*Pisum sativum*) as cited by Rios-Gonzales; Erdei; Lips (2002). More recently, Meloni et al. (2003)

related the effects of NaCl stress on the activity of antioxidant enzymes. Salinity led to a significant increase in superoxide dismutase, peroxidase and glutathione reductase in salt-tolerant cotton (*Gossypium hirsutum*) cultivar. Similar results were obtained in maize (*Zea mays*) and sunflower (*Helianthus annuus*) seedlings (RIOS-GONZALES; ERDEI; LIPS, 2002). The authors suggested that the increase of the activities of antioxidant enzymes protects the plant against reactive oxygen species and, therefore, against salt stress.

Finally, another aspect have been considered, i.e, metabolic events associated to lignification. Hilal et al. (1998) related that saline stress retards primary xylem differentiation and induces acceleration of the development of secondary xylem in soybean roots. Additionally, Cachorro et al. (1993) reported the lignin deposition in vascular tissues of bean (*Phaseolus vulgaris*) roots in response to salt stress and suggested that lignification is a factor that inhibits root growth and which contributes to the structural integrity of xylem. Contrarily, Lin and Kao (2001a) showed that lignin levels reduced in NaCl-reduced root of rice (*Oryza sativa*) seedlings. In agreement with Cachorro et al. (1993), preliminary results with NaCl-stressed soybean roots showed an increase in the lignin contents. It is possible, at least for soybean, that lignification plays a regulatory role in the root growth reduction caused by NaCl. Additional experiments are in progress to confirm this idea.

CONCLUSION

Observations of the present study showed that salt stress affected both seed germination and seedlings growth of soybean. Seed germination, seedling and root growth and biomass were reduced significantly against NaCl concentration. Consequently, these results strengthen the role of NaCl as a stress-inducing factor, and also the susceptibility of soybean to salinity.

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RESUMO: Germinação de sementes e crescimento de plântulas de soja (*Glycine max* (L.) Merr.) sob estresse salino. A germinação das sementes e o crescimento das plântulas de soja (*cv.* BRS-133) foram examinados em papel pré-umedecido com NaCl (0, 50, 100, 150 e 200 mM). As sementes foram avaliadas pelo teste padrão de germinação e pelo índice de velocidade de germinação, enquanto o crescimento das plântulas foi avaliado pelos comprimentos das plântulas e raízes e as biomassas frescas e secas. Os resultados mostraram que o estresse salino reduziu a germinação das sementes e o crescimento das plântulas. A porcentagem de germinação sem NaCl foi de 61% em média, enquanto que na mais alta concentração (200 mM) foi reduzida a 5%. Com o aumento da concentração do sal, os comprimentos das plântulas e das raízes e as biomassas frescas e secas diminuíram significativamente. O aumento da concentração do sal também reduziu o desenvolvimento de raízes secundárias. Os resultados são discutidos com base nos efeitos do estresse salino na germinação e crescimento de plântulas de soja.

UNITERMOS: Crescimento; Germinação de sementes, Salinidade, Soja.

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