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Effect of sodium nitroprusside (SNP) on the germination of *Senna macranthera* seeds (DC. ex Collad.) H. S. Irwin & Baneby under salt stress[1]

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ABSTRACT – Nitric oxide (NO) has been used as stimulating of the germination process for many species. However, there are few studies evaluating the effect of nitric oxide donor in the regulation of seed germination under salt stress, especially for native forest species. The objective was to evaluate the effects of SNP, an NO donor substance, on germination of *Senna macranthera* seeds under salt stress. The seeds were germinated at different osmotic potentials induced by NaCl solution (0.0, -0.1, -0.2, -0.3, -0.4 and -0.5 MPa). To evaluate the effect of the SNP, potentials -0.3 and -0,4 MPa were selected, applying SNP at different concentrations: 100, 200, 300 and 400 µM. Germination tests were conducted at 25 °C, with photoperiod of 8 hours. Percentage of radicle protrusion, radicle protrusion speed index, percentage of normal seedlings, shoots and roots length and dry matter were evaluated. Salt stress with NaCl is harmful to germination of *S. macranthera* seeds. SNP has the potential to recover germination under salt stress, especially in the concentration of 100 µM.

Index terms: "fedegoso", NaCl, nitric oxide, native species.

Efeito do nitroprussiato de sódio (SNP) na germinação de sementes de *Senna macranthera* (DC. ex Collad.) H. S. Irwin & Baneby sob estresse salino

RESUMO – O óxido nítrico (ON) vem se destacando como estimulador do processo de germinação para muitas espécies. Há poucos estudos que avaliam o efeito do doador de óxido nítrico na regulação da germinação de sementes sob estresse salino, principalmente na germinação de espécies florestais nativas. Objetivou-se avaliar os efeitos do nitroprussiato de sódio (SNP), uma substância doadora de ON, na germinação de sementes de *Senna macranthera* sob estresse salino. As sementes foram colocadas para germinar em diferentes potenciais osmóticos, induzidos por solução de NaCl (0,0; -0,1; -0,2; -0,3; -0,4 e -0,5 MPa). Para testar o efeito do SNP, selecionou-se os potenciais de -0,3 e -0,4 MPa, com aplicação de SNP em diferentes concentrações: 100, 200, 300 e 400 µM. Os ensaios germinativos foram conduzidos em câmara de germinação a 25 °C, com fotoperíodo de 8 horas. Foram avaliados a protrusão radicular, o índice de velocidade de protrusão radicular, a porcentagem de plântulas normais, o comprimento da parte aérea e do sistema radicular, massa seca da parte aérea e do sistema radicular. O estresse salino com NaCl é prejudicial a germinação de sementes de *S. macranthera*. O SNP tem potencial para promover a recuperação da germinação das sementes sob estresse salino, sendo a concentração de 100 µM a mais eficaz.

Termos para indexação: fedegoso, NaCl, óxido nítrico, espécie nativa.

Introduction

Senna macranthera is an arboreal species, belonging to the Fabaceae – Caesalpinioideae family, popularly known as "Fedegoso" (Lorenzi, 2000). The species is widely used in landscaping due to its ornamental characteristics and small size, which is ideal for urban trees. It is a pioneering, fast-growing species, recommended for use in plantations in degraded areas (Lorenzi, 2000).

Demand for seedlings of native forest species has been growing due to the need of reforestation. Most of these species are propagated by seeds; success in the formation of

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seedlings depends on the knowledge about the regulation of seeds germination and the quality thereof, for each species (Rego et al., 2009).

The seeds are often exposed to various environmental stresses that negatively interfere in germination, vegetative development, plant productivity and, in severe cases, can lead to seedlings death. According to Braga et al. (2009), the time period required for germination is important for the survival of forest species, especially where water availability is limited during certain periods of the year.

Germination is a physiological event that depends on seed quality and environmental conditions such as water supply and oxygen, and temperature and light suitability (Maekawa et al., 2010). Seed hydration is the most important event, because water is the matrix where most biochemical and physiological processes occur and result in primary root protrusion (Moraes et al., 2005). Salt stress affects one of the main processes of the plant life cycle, namely, seeds germination (Cesur and Tabur, 2011; Zheng et al., 2009), leading to a reduced and delayed germination rate (Singh et al., 2012). One of the most widespread methods to determine plants tolerance to salt stress is the observation of germination in saline substrates (Lima and Torres, 2009).

Salinity affects germination, not only due to hindering water absorption, but also to facilitating the entry of toxic amounts of ions in the seeds during imbibition (Simaei et al., 2012). Moreover, salt stress causes changes in seeds physiology due to ionic toxicity, osmotic stress and increased reactive oxygen species (ROS) (Mittler, 2002), leading to gradual lipid peroxidation and antioxidant enzyme inactivation (Tanou et al., 2009). Fan et al. (2013) emphasize the importance of further studies to understand the physiological mechanisms involved on seed germination under salt stress and develop appropriate measures to alleviate the negative effects of salinity on germination.

There are several reports in the literature on the harmful effect of salt stress on seed germination. In seeds of forest species, this effect for species *Schizolobium amazonicum* (Braga et al., 2008), *Enterolobium schomburgkii* (Braga et al., 2009), *Chorisia speciosa* (Fanti and Perez, 2004), *Dimorphandra mollis* (Masetto et al., 2014), *Gliricidia sepium* (Farias et al., 2009) and *Zizyphus joazeiro* (Lima and Torres, 2009) have already been observed.

Among the factors that favor germination under stress conditions, nitric oxide (NO) has stood out as a stimulator of the process in many species. NO is a free radical produced from L-arginine, toxic, inorganic, and colorless gas, with seven nitrogen atoms and eight oxygen atoms, being one of the most important mediators of cellular signaling (Dusse et al., 2003). One of the limiting factors in the studies on the possible actions of NO in plants is the absence of mutants with a differential production of NO, in addition to the lack of mechanisms to perceive and translate the signals induced by this compound (Ederli et al., 2009). Therefore, ON donor and sequestering substances have been widely used in studies to elucidate their functions. The most used reagents as ON donors are sodium nitroprusside (SNP) and S-Nitroso-N-acetylpenicillamine (SNAP).

Several studies have shown a positive effect of NO on the recovery of seed germination under different types of stress. However, there are few studies that evaluate the effect of NO on seeds germination in salt stress conditions, particularly in native species. It was found in *Lupinus luteu* seeds that SNP was effective in reversing the negative impact of NaCl on germination (Kopyra and Gwóźdź´, 2003)*;* this reversal was also observed in *Cucumis sativus* (Fan et al., 2013) and *Ocimum basilicum* (Saeidnejad et al., 2013) seeds.

The positive effect of NO on the seed germination process is observed in some studies, but there are no reports of the effect of SNP on seed germination of *S. macranthera* under saline conditions. Given the above, this study has aimed to evaluate the effects of SNP on seed germination of *S. macranthera* under salt stress induced by NaCl.

Material and Methods

Senna macranthera seeds were collected in 2012 in the Brazilian city of Viçosa, Minas Gerais. The seeds were stored in cold room at 5 ºC and relative humidity of 60%. Before establishing the tests, all seeds were mechanically scarified with sandpaper number 100 on the opposite side to the hilum and then they were treated with fungicide Captan at 0.2%.

Experiment I – Evaluation of root protrusion and seed germination of S. macranthera under salt stress conditions

The seeds were germinated in saline stress conditions, obtained by using saline solution of sodium chloride (NaCl, P.M. 58.44). The following osmotic potentials were tested: -0.1; -0.2; -0.3; -0.4 and -0.5 MPa. The salt concentrations for each osmotic potential were obtained based on the equation by J. H. van't Hoff, cited by Salisbury and Ross (1992): $\psi_{os} = - RTC$, where:

ψ_{os} = osmotic potential (atm);
R = general ideal gas constant (0.082 atm**.** 1**.** mol^{-10}. k^{-1});
T = temperature (ºK); and
C = concentration (mol/1) (No. of moles/l).

For the germination test, four replications of 50 seeds

were placed on paper towel rolls moistened with water (control) or saline solution at a ratio of 2.5 times the dry paper weight. Subsequently, the rolls were placed in plastic bags. The germination test was conducted in a germinator at 25 °C, with a photoperiod of 8 hours.

The numbers of seeds with protruded primary radicle and germination percentage (normal seedlings) were daily assessed. Seeds whose primary root had at least 2 mm in length were considered protruded. The percentage of normal seedlings was detemined following the criteria established by Rules for Seed Testing (Brasil, 2009). On the seventh day after sowing, the percentage of root protrusion and germination was calculated. With daily data, radicle protrusion speed index (RPSI) was calculated.

At the end of the germination test, shoot length (CPA) and root system length (RSL) of seedlings were evaluated, with the aid of a digital caliper. The results were expressed in millimeters per seedling. Dry matter of shoot (DMS) and root dry matter (RDM) were determined by the oven method with forced air circulation, set at 70 °C, where the seedlings remained until constant weight.

Experiment II – Nitric oxide effect on root protrusion and germination of S. macranthera seeds under salt stress conditions

The seeds were germinated under salt stress conditions in osmotic potentials -0.3 and -0.4 MPa of NaCl (defined according to the results of the previous experiment). For each potential, sodium nitroprusside solution (SNP) were applied in concentrations of 100, 200, 300 and 400 μM.

For the germination test, four replications of 50 seeds were germinated in accordance with the procedures adopted in the previous experiment. The substrate was moistened with a mixture of NaCl and SNP solutions, in the proportion of 2.5 times the weight of the dry paper. Percentage of primary root protrusion, RPSI and germination percentage were determined.

Statistical analysis

Experiment I was conducted in a completely randomized design with four replications. The data were submitted to analysis of variance (ANOVA) and expressed as mean ± standard deviation. Experiment II was conducted in a completely randomized design with four replications in a 2 x 4 factorial arrangement, i.e., two osmotic potentials (-0.3 and -0.4 MPa) and four concentrations of SNP (100, 200, 300 and 400 μM). Root protrusion data were transformed by the arcsine function and then they were subjected to analysis of variance. The results obtained for the salt concentrations were expressed as mean ± standard deviation.

Results and Discussion

S. macranthera seeds present integument hardness, which corresponds to the characteristic primary dormancy mechanism of the species (Lorenzi, 2000). In this study, the control treatment seeds (water) presented high root protrusion (87%), showing the efficiency of the mechanical scarification procedure indicated by Lemos Filho et al. (1997) in overcoming the species dormancy.

There was a gradual decrease in root protrusion and seed vigor by reducing the osmotic potential (Figure 1), where the seeds protrusion percentage under potential 0.0 MPa was 87% to 36% in potential -0.5 MPa. A more drastic effect of salinity was observed, with a significant reduction in radicle protrusion speed index, germination, shoot length and root system length with increased salt stress (Figures 1B, 1C, 1D and 1E). Although some seeds still emit radicle, there was no formation of seedling in saline potentials -0.4 and -0.5 MPa. There was a slight increase in the aerial part dry matter in potentials -0.3 and -0.4 MPa and root remained stable at concentrations of -0.1; -0.2 and -0.3 MPa in relation to control (Figures 1F and 1G).

In seeds of other forest species, harmful effect of salt stress has also been found, such as for *Schizolobium amazonicum* (Braga et al., 2008) and *Enterolobium schomburgkii* (Braga et al., 2009), especially in reducing germination percentage, with significant decreases from potentials at -0.2 MPa in the solution of NaCl. However, in studies conducted by Fanti and Perez (2004) significant decreases in germination percentage to the osmotic potential of -0.4 MPa in *Chorisia speciosa* seeds were not observed, but from -0.6 MPa significant reductions in viability were recorded. For *Gliricidia sepium* (Farias et al., 2009) germination was significantly affected only in osmotic potentials above -1.0 MPa. In *Zizyphus joazeiro* all salt concentrations used (-0.3; -0.6 and -0.9 MPa) significantly decreased germination (Lima and Torres, 2009).

According to the results found in this study, *S. macranthera* seeds showed greater decrease in root protrusion from osmotic potential -0.4 MPa; for other species, this potential also proved harmful, such as for *Senna occidentalis* (Norsworthy and Oliveira, 2005), *Senna obtusifolia* (Pereira et al., 2014) and *Dimorphandra mollis* (Masetto et al., 2014).

It is noticed that the seeds of different species support different osmotic salt stresses on germination, and the seedlings are the most sensitive. One of the factors responsible for the reduction in germination may have been an excess of ions Na^+ and Cl^-, since they cause decreased protoplasmic swelling (Ferreira and Borghetti, 2004). Another factor may have been the excess of soluble salts, resulting in reduced

water potential, i.e., the water absorption capacity of the seeds is reduced. This reduction of water potential and the salts toxic effects initially interfere with the process of water uptake by seeds, influencing vigor, affecting speed and thus the germination time of these seeds in non-toxic levels of salinity (Cavalcante and Perez, 1995).

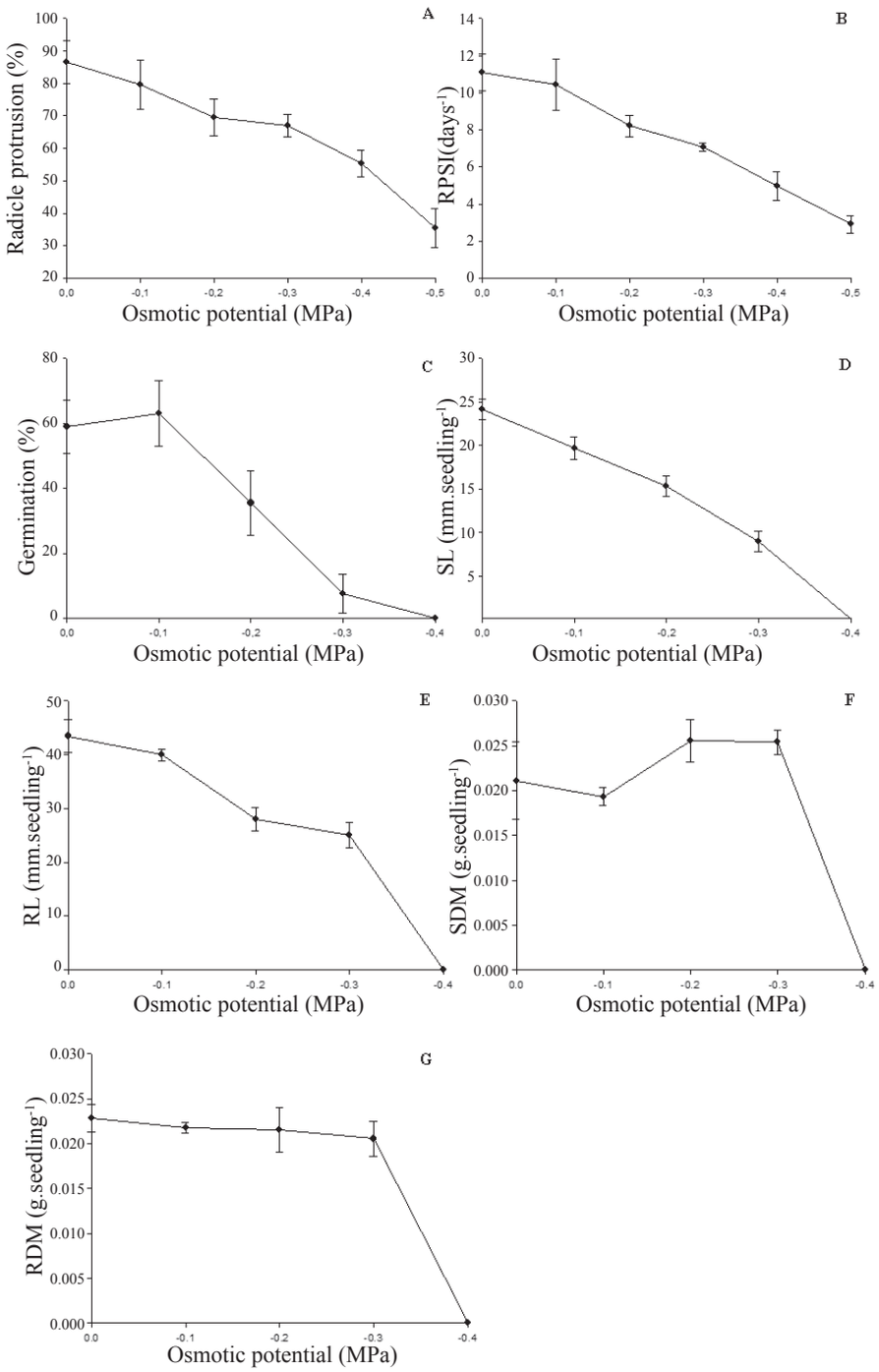

Figure 1. A: Percentage of root protrusion, B: root protrusion speed index (RPSI), C: percentage of germination, D: shoot length (SL), E: root length (RL), F: dry matter of shoot (DMS) and G: root dry matter (RDM), of *Senna macranthera* seeds, scarified and germinated in different osmotic potentials with NaCl (0.0; -0.1; -0.2; -0.3; -0.4 and -0.5 MPa) at 25 °C.

Bewley et al. (2013) have pointed out that the seeds moderate resistance to salt stress is useful when trying to use saline soils in dry regions, since 6% of terrestrial continents are made up of saline soils. Knowledge of species able to withstand these conditions can aid in the proper recommendation of species for planting, especially for native species, for which there is limited information (Rego et al., 2011).

Species tolerant to salt stress are classified as halophytes or glycophytes due to their tolerance to salt stress, both respond similarly to salt stress, and the percentage and germination rate are inversely proportional to increased salinity, varying only the salt tolerance limit (Jeller and Perez, 2001). Halophyte species are highly tolerant, germinating in the environment with up to 8% of NaCl (Ungar, 1978), and the ones that are little tolerant have their germination inhibited at 1.5% of NaCl. Most glycophytes do not germinate in medium with concentrations higher than 1.5% of NaCl. In this study, *S. macranthera* may be included among the little salt-tolerant glycophytes because it does not have a high tolerance limit (up to -0.4 MPa). Similar results were found for *Senna spectabilis,* also included among the little salt-tolerant glycophytes, with a limit of -1.6 MPa (Jeller and Perez, 2001).

From the results of radicle protrusion percentage obtained in the first assay (Figure 1A), two salt concentrations were selected, which caused moderate stress, with root protrusion around 50% to evaluate SNP effect on the recovery of the seeds physiological potential. The selected concentrations were -0.3 and -0.4 MPa, due to promoting moderate stress levels in radicle protrusion of *S. macranthera* seeds (67 and 55%, respectively).

Thus, there was an increase in root protrusion and shoot length of the seedlings, and decrease in radicle protrusion speed index for all concentrations of SNP tested (100, 200, 300 and 400 μM) in the potential of -0.3 MPa (Figures 2 A and B). There was an increase of 18% in root protrusion and of 225% in germination, compared to the control (without application of SNP) at a concentration of 100 μM of SNP (Figure 2). Although there is a small reduction in dry matter of shoot and root system compared to the control, this was not significant (Figures 2 F and G).

There was an increase in seeds root protrusion under salt stress -0.4 MPa with the application of SNP at concentrations of 100 (15%), 200 (10%) and 300 μM (17%), compared to pure salt stress (Figure 3). The radicle protrusion speed index decreased in all concentrations (100, 200, 300 and 400 μM) of SNP, compared to the control (-0.4 MPa). Thus, it is possible to observe the limitation of SNP effect in reversing the inhibitory effect of salt stress in seedling production.

There are no reports in the literature about the germination behavior of native forest species with nitric oxide donor application in the reversal of salt stress. However, in *Lupinus luteus* seeds from the Fabaceae family, SNP also proved effective in reversing the decline of germination caused by NaCl (Kopyra and Gwóźdź´, 2003). For *Cucumis sativus*, the recovery of salt stresses was more effective at the concentration of 50 μM of SNP; however, there was a decrease in germination at the highest concentration of SNP (400 μM) (Fan et al., 2013). In *Triticum aestivum* seeds there was a significant increase in germination after seven days of seed germination with SNP under salt stress with NaCl (Zheng et al., 2009).

However, in *Ocimum basilicum* seeds there was no increase in seed germination with nitric oxide donor, probably because it relieves salt stress through changes in physiological properties and the antioxidant system (Saeidnejad et al., 2013).

According to Correa-Aragunde et al. (2004) and Lombardo et al. (2006), nitric oxide is involved in the regulation of root morphology. Correa-Aragunde et al. (2004) have found that nitric oxide affects the growth of *Lycopersicon esculentum* roots in a dose-dependent manner, wherein SNP at low concentrations stimulates root growth, while high concentrations have an inhibitory effect on growth.

In general, *S. macranthera* seeds are sensitive to salt stress induced by NaCl, with reduction of root protrusion, germination and seed vigor, especially in osmotic potential below -0.4 MPa. SNP was efficient in the recovery of the physiological potential of *S. macranthera* seeds at the concentration of 100 μM.

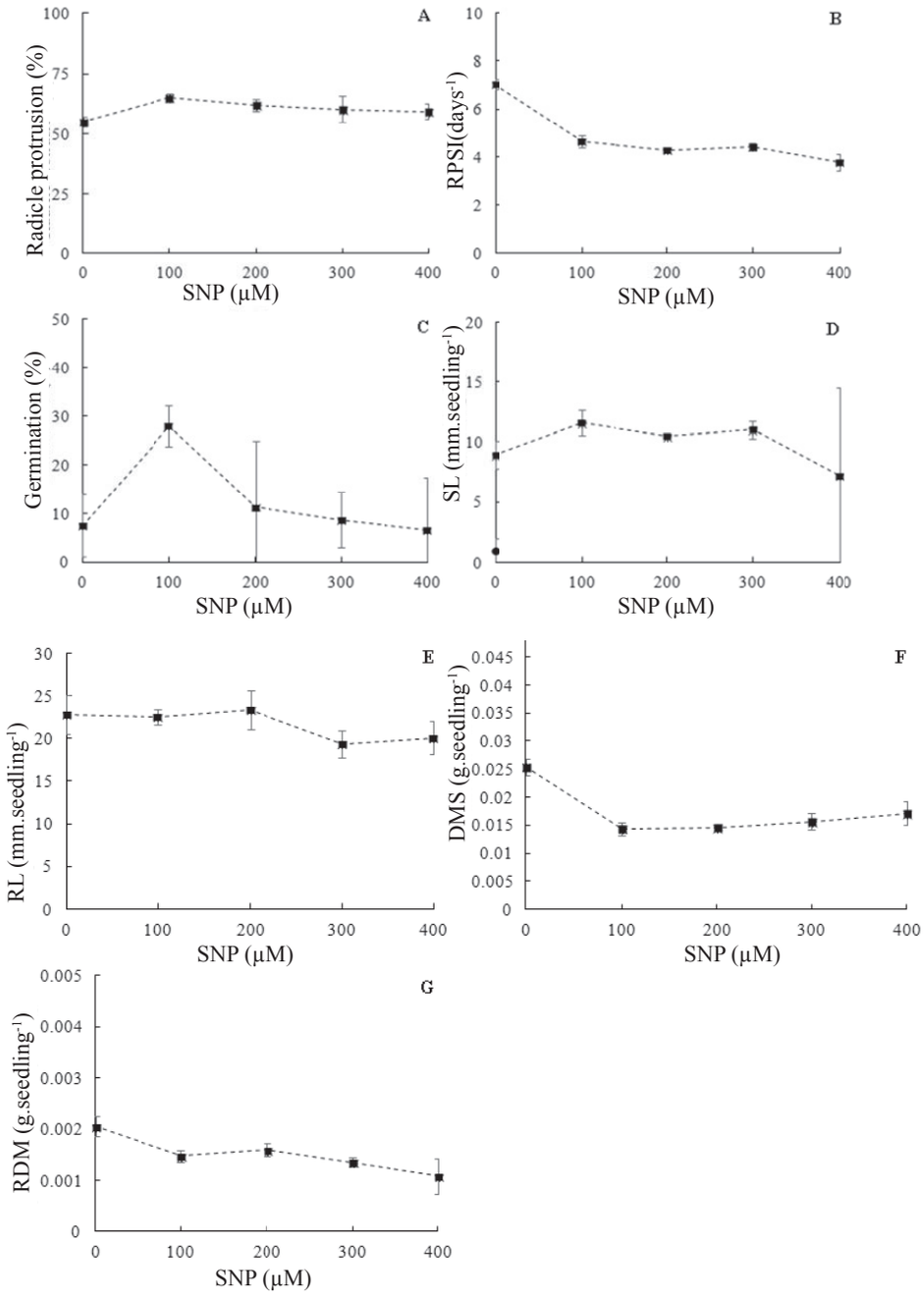

Figure 2. A: Percentage of root protrusion, B: radicle protrusion speed index (RPSI), C: percentage of germination, D: shoot length (SL), E: root length (RL), F: dry matter of shoot (DMS) and G: root dry matter (RDM), of *Senna macranthera* seeds, scarified and germinated in osmotic potential with NaCl -0.3 MPa together with different concentrations of SNP (100, 200, 300 and 400 µM) at 25 °C.

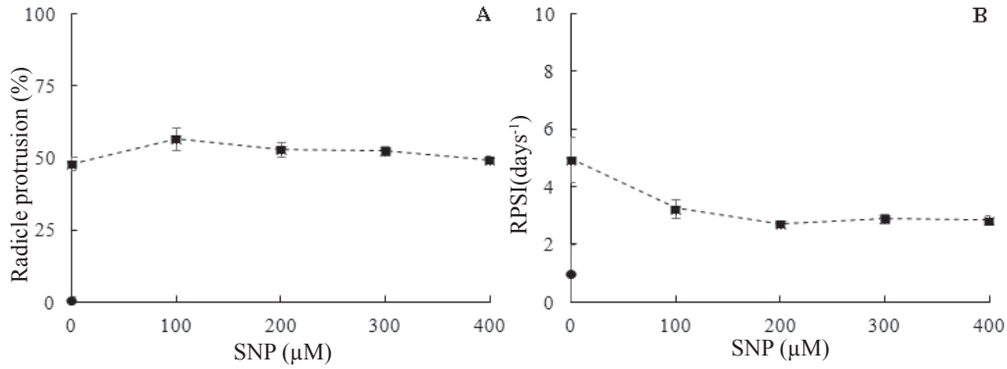

Figure 3. A: Percentage of root protrusion, B: radicle protrusion speed index (RPSI) of *Senna macranthera* seeds, scarified and germinated in osmotic potential with NaCl -0.4 MPa together with concentrations of SNP (100, 200, 300 and 400 μM) at 25 ºC.

Conclusions

Salt stress is harmful to germination of *S. macranthera* seeds.

SNP has the potential to promote germination recovery under salt stress more effectively when applied at a concentration of 100 μM.

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