RESEARCH ARTICLE
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Priming seed mitigates the effects of saline stress in soybean seedlings

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ABSTRACT

Seed germination and seedling growth are the most sensitive plant stages towards salinity. Various techniques can improve emergence and stand formation under salt conditions. Seeds priming is one of the most frequently used technique. Seed priming could develop different defence mechanisms of seeds against salinity stress. This experiment aims to examine the seed priming into potassium nitrate - KNO_3 (1%), ascorbic acid – ASA (100mgl^{-1}) and potassium chloride - KCl (1%) solutions, that may reduce harmful effects of salinity stress. Salinity was imposed by treatment of seeds with different concentrations of NaCl [0 (control), 50, 100, 200m MNaCl]. All data were analysed statistically by three-way ANOVA. Individual testing of probability was carried out using Tukey's method $p \leq 0.05$ and $p \leq 0.01$. Correlation dependence was determined using the Pearson correlation coefficient. Test results demonstrated positive effect of seed priming. This was showed by improved soybean seed quality and increased seed germination rate. The intensity of lipid peroxidation, free proline concentration and Na^+ was decreased, while K^+ in seedlings increased due to priming treatment. Priming of seeds into ASA solution resulted in increased vitamin C concentration, while priming into KNO_3 and KCl solutions reduced the content of vitamin C in soybean seedlings.

Key words: Germination, Priming, Salinity, Soybean.

INTRODUCTION

Soil salinity is recognized as one of the factors that highly slow down the successful crop development. Higher level of salt concentration in solution can lead to oxidative stress, toxic ion effects, nutritional disorders, changes in metabolic processes, etc. Also, compromised plant growth and development on saline soils is caused by the inhibition of different enzymes, due to accumulation of salt in different cell compartments.

Salinity has impact on all stages of plant development, where the most sensitive stages are seed germination and initial plant growth (Sadeghipour, 2017). Salinity effects germination by creating osmotic potential which effects water uptake, salt surrounding make changes in mobilization of nutrients during germination, resulting in reduced seed germination and ultimately, lower crop yields (Viradiya *et al.,* 2008). The achievement or failure of emergence and seedling establishment in saline soils can have significant economic implications in areas where soil salinity is a potential problem for soybean.

Seed germination can be improved by seed priming into different solutions. This pre-sowing treatment was introduced with the aim to overcome unfavourable environmental conditions such as low temperature (Li *et al.,* 2017), salinity (Miladinov *et al.,* 2015) and drought (Arun *et al.,* 2017). Therefore, the objective of this experiment was to compare the effect of salt stress on two soybean varieties and seed priming with potassium nitrate (KNO_3), ascorbic acid (ASA) and potassium chloride (KCl), with the hypothesis that seed priming can mitigate the adverse effect of salt stress.

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MATERIALS AND METHODS

Trial was set and carried out in the Institute of Field and Vegetable Crops Novi Sad. The tests included two soybean varieties, NS Zita and Victoria. During 2016, selected varieties were produced at the experimental field at Rimski Šanèevi. Initial germination value of both varieties was 88%, while moisture content was 10-11%. Before priming, seeds were sterilized with 3% solution of sodium hypochlorite (NaOCl) for two minutes and rinsed with distilled water. In order to determine the impact of seed priming on germination in the presence of NaCl, seeds were immersed into different solutions: potassium nitrate - KNO_3 (1 %), ascorbic acid ASA - (100m g l^{-1}) and potassium chloride - KCl (1 %). The ratio among seed mass and solution volume was 1:5 (v/v). After

6 h (Miladinov *et al.,* 2015) seeds were washed out with a jet of distilled water and dried at 25°C up to moisture content of 10-11%. Salinity was imposed by treatment of NaCl to the distilled water which was added during germination, in the following concentrations: 0 (control), 50, 100, 200 mMNaCl. Seeds were distributed on a sterilized filter paper, in Petri dishes 9 cm in diameter. Then, 4 x 50 seeds were treated with 10 ml of distilled water and set as a randomized block design. Petri pots were closed using paraffin tape in order to prevent moisture loss and avoid contamination. Petri pots were placed in chambers to germinate at 25°C and 95% relative humidity for 8 days. Seed germination results were collected daily and seed germination was defined by protrusion of the radical by at least 2 mm. Germination energy was recorded after 4 days and germination after 8 days (ISTA, 2008).

For each treatment it was provided 4 x 10 average seedlings and biochemical analyses of fresh samples were carried out. Intensity of lipid peroxidation was carried out using the method of Placer (1966), free proline concentration was determined by the method of Bates (1973) and vitamin C content by the method of Benderitter (1998).

According to the method of Placer (1966), extraction of malondialdehyde (MDA) from soybean seed was done by using solution of thiobarbituric acid (TBA), trichloroacetic acid (CCl_3COOH) and perchloric acid ($HClO_4$) and concentration was determined spectro photometrically at 532nm. Soybean seed (0.5 g) were homogenized in mortar with 4.5 ml extraction solution and incubated in water bath at 90°C for 20 minutes. After incubation, solutions were cooled to stop the reaction and centrifuged for 10 min at 5500 r/min. MDA concentration i.e. intensity of lipid peroxidation was expressed as nmol of MDA g^{-1} of fresh mass. Extraction and determination of proline was done according to Bates (1973). Seedlings (1 g) were extracted with 3% sulphosalicylic acid. Extracts (2 ml) were held for 1h in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene (4 ml) was added. Proline content was measured by a spectrophotometer at 520 nm and calculated as mol g-1 DW against standard proline. According to Benderitter (1998) 75 µl DNPH solution (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg copper sulphate ($CuSO_4$. $5H_2O$) in 100 ml of 5 ml/L H_2SO_4) were added to 500 µl extract mixture (300 µl of an appropriate dilution of the extract with 100 µl 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 h at 37°C, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm in a UV spectrophotometer. The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard. Potassium and sodium concentrations were assessed by the inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

Data analysis was performed using the statistical software ’Statistica’ (StatSoft, Inc., Tulsa, Oklahoma, SAD). The obtained data were processed using three-factor analysis of variance. Individual testing of probability was carried out using Tukey’s method $p \leq 0.05$ and $p \leq 0.01$. Correlation dependence was determined using the Pearson correlation coefficient.

RESULTS AND DISCUSION

Germination energy and seed germination

The best results were on control, which indicated that the higher concentration of salt affects negatively on the basic metabolic processes. Priming seed into the solution of KNO_3, KCl and ASA improves seed quality in conditions of higher salt concentration (Fig 1 and 2). This positive effect has been determined on soybean in earlier experiments (Miladinov *et. al.,* 2015), etc. KNO_3 solution acted positively on reducing the harmful effects of salt, which is in accordance with other results that has been taken on different crops. Nitrates act positively on germination as signal molecules and potassium nitrate is also correlated to higher levels of nitrate. The use of ASA solution result positively. Ascorbic acid is a small antioxidant molecule which works as primary substrate in a cyclic path of hydrogen peroxide enzymatic detoxification, and beside of that it directly neutralizes superoxide radicals (Noctor and Foyer, 1998).

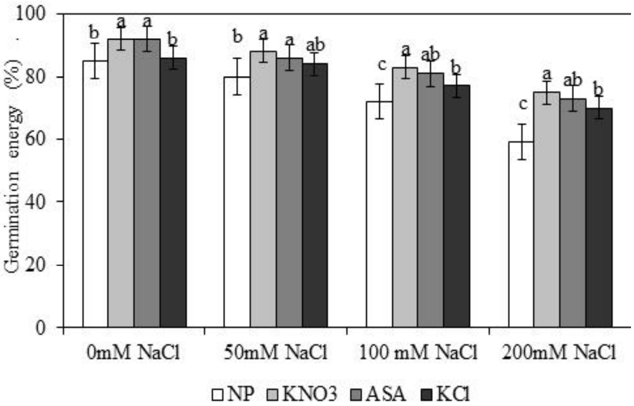

Fig 1: The effect of seed priming on germination energy of two soybean varieties (p<0.05).

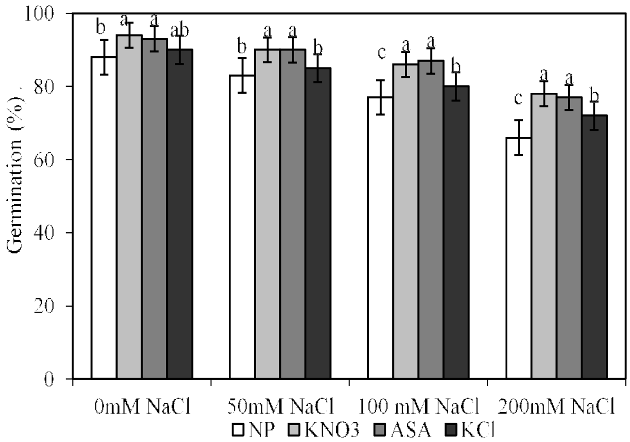

Fig 2: The effect of seed priming on germination of two soybean varieties (p<0.05).

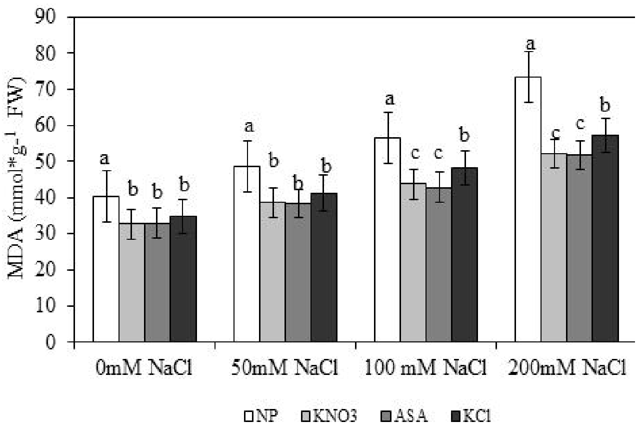

Fig 3: The effect of seed priming on MDA concentration (mmol*g^{-1} FW) of two soybean varieties ($p<0.05$).

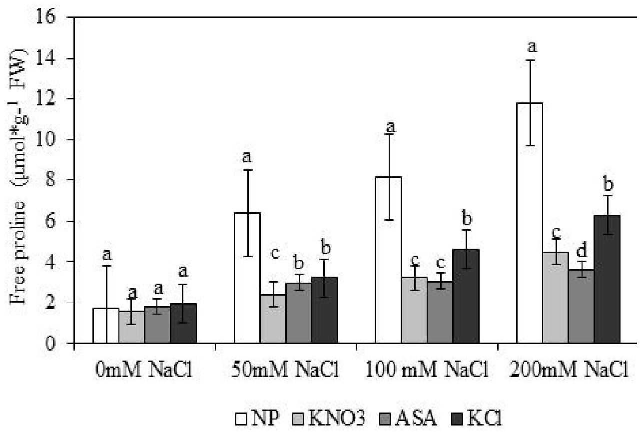

Fig 4: The effect of seed priming on free proline concentration (mmol*g^{-1} FW) of two soybean varieties ($p<0.05$).

Intensity lipid peroxidation

When NaCl concentration rises, the lipid peroxidation intensifies as well, and that proves that higher concentration of salt damage the cell membrane and reduces seed quality (Fig 3). When concentration of NaCl is increased, concentration of MDA is decreased. The application of the KNO_3 solution had positive impact on germination, reducing the activity of the lipid peroxidation and also enhancing the work of antioxidants. This can be explained by fact that exogenous application of ascorbic acid increases resistance to higher salt concentration, reducing oxidative stress by activating particular enzymes.

Free proline concentration

With increasing lipid peroxidation, the concentration of free proline rises also (Fig 4). Intensive accumulation of free proline is a typical response to increased salt concentration. In organisms, from bacteria to plants, there is a strong correlation between increased content of free proline in cell and their abilities to survive water deficit and high salt concentration in different production conditions.

K^+ and N^+ concentration

This indicates the fact that the Na^+ is very mobile and easy to accumulate in the seed (Fig 5, 6). Due to higher salt concentration, K^+ is replaced with Na^+ and a result is the disorder of biochemical reactions in plants. Maintaining a greater K^+/ Na^+ ratio works favourably on metabolic processes. The use of this measure reduced the concentration of Na^+ and did not increase K^+ content. Priming of seed induces greater tolerance on high salt concentration in soil, and improves seedlings vitality, increases K^+ content and reduces Na^+ accumulation.

Vitamin C concentration

By seed priming with solution of KNO_3 and KCl the vitamin C content is reduced, but when the seed is primed in the solution of ASA, content of vitamin C is rising. The reason for this is because vitamin C absorbs from the solution, where he is mobile and easy to absorb. Higher absorption

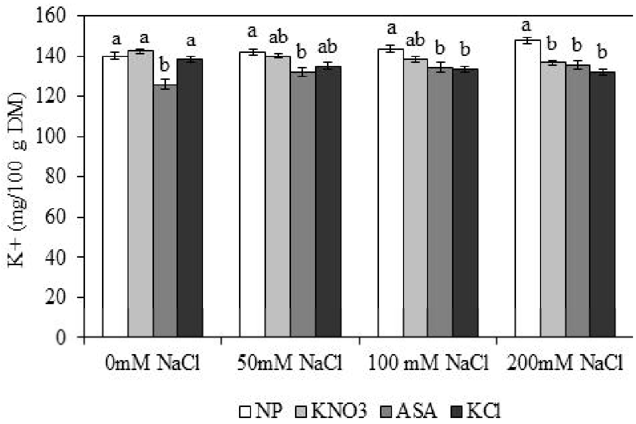

Fig 5: The effect of seed priming on the K^+ concentration (mg*g^{-1} DW) of two soybean varieties ($p<0.05$).

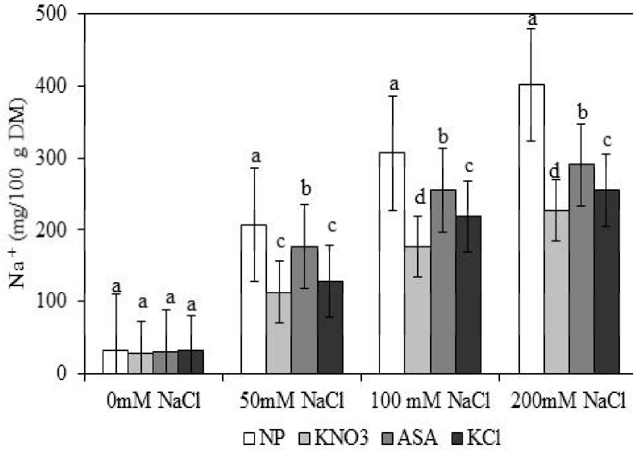

Fig 6: The effect of seed priming on Na^+ concentration (mg*g^{-1} DW) of two soybean varieties ($p<0.05$).

was determined in seeds that have not been exposed to salt, 6mg/100g green matter. Regardless of salt concentration in the solution the content of vitamin C in the seeds was the same, 6mg/100g of green matter (Fig 7). Exogenous application of ascorbic acid can affect many of different processes in plants including seeding, because it

Table 1: Correlation among examined traits using the Pearson's coefficient.

Source of variation		Germination	Lipid peroxidation	Free proline	Vitamin C	K+	Na+
Germination energy	NP	0.664**	-0.550**	-0.646**	-0.113^{ns}	-0.127^{ns}	-0.847**
	P	0.818**	-0.428*	-0.308*	0.425*	0.096^{ns}	-0.766**
Germination	NP	1	-0.485*	-0.612**	-0.107^{ns}	-0.112^{ns}	-0.793**
	P	1	-0.317*	-0.510**	0.421*	0.087^{ns}	-0.757**
Lipid per oxidation	NP		1	0.596**	0.123^{ns}	0.105^{ns}	0.779**
	P		1	0.394*	0.313*	-0.081^{ns}	0.396*
Free proline	NP			1	0.089^{ns}	0.116^{ns}	0.846**
	P			1	0.083^{ns}	-0.187^{ns}	0.379*
Vitamine C	NP				1	0.085^{ns}	-0.286
	P				1	-0.037^{ns}	0.081^{ns}
K^+	NP					1	0.077^{ns}
	P					1	-0.046^{ns}

NP - non primed seeds; P - primed seeds; ns-not significant; *p<0.05, **p<0.01.

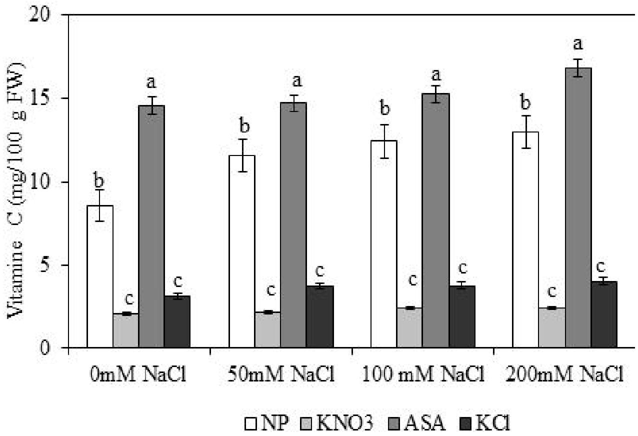

Fig 7: Effect of the seed priming on vitamin C content(mg/100gFW) of two soybean varieties (p<0.05).

increases its adsorption in different tissues and participates in hormone biosynthesis, such as gyberellic acid and ethylene, which are necessary in the process of germination.

Correlations among the analysed traits

The results showed that the germination energy and the germination of seed are reduced with an increase in Na^+ content and the intensity of lipid peroxidation. Priming seed negative impact was reduced or accumulations Na^+ and MDA in soybean seedlings (Table 1). Also, the content of the free proline increases, but significantly more than non-primed. In the priming seed vitamin C content significantly increased. Salinity can affect the germination by affecting the osmotic component, which the ionic component, i.e., Na and Cl accumulation. Proline, as an important osmo-protectant, contributes to osmotic adjustment, protecting enzymes from oxidative damage under saline condition.

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CONCLUSION

Quality of soybean seed is reduced in salt stress. Within experiment every measured variable showed to be significantly decreased. Priming of soybean seed with potassium nitrate, ascorbic acid and potassium chloride solutions resulted in the higher seed quality and shorter germination period, regardless of the soybean variety or NaCl content. Application of pre-sowing treatment might be used for improving germination in the case of crop cultivation under salt stress conditions. Improved germination would also effect crop canopy and ensure crop stability, ultimately resulting in higher and stable yields.

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