



## Priming seeds-method for increasing the germination of soybean seeds under drought stress conditions

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### ABSTRACT

Drought is one of the most important factors limiting the successful production of cultivated plants. One of the most sensitive stage to the water deficit in plants is seed germination. There are various methods of pre-sowing treatments that aim to reduce the negative impact of drought stress and improve seed germination. One of them is priming seeds. The aim of this experiment was to examine the effect of priming seeds in solutions - KNO<sub>3</sub> (1%), ascorbic acid - AsA (100 mg l<sup>-1</sup>) and potassium chloride KCl (1%) on the reduction of the negative effect of drought stress. The effect of drought stress was simulated using different concentrations of PEG 6000 (0 (control), -0.30, -0.51, -0.80 MPa). The results of the research showed that with the increase of water deficiency, the effect of priming seeds is greater. At the water potential of the solution of -0.30 MPa, germination energy and seed germination increased on average by 6.77% and 5.08%, while at the water potential of the solution of -0.80 MPa, the increase was 19.28% and 16.75%, respectively. Also, priming seeds significantly reduced the intensity of lipid peroxidation and the content of free proline.

From all the above, it can be concluded that priming of seeds is a method that can serve to improve the germination of soybean seeds in conditions of drought stress.

*Keywords: drought stress, Glycine max., priming seed, seed germination.*

### ИЗВОД

Suša je jedan od najvažnijih faktora koji ograničava uspešnu proizvodnju kulturnih biljaka. Jedna od najosetljivijih faza na nedostatak vode kod biljaka je klijanje semena. Postoje razne metode odnosno predsetveni tretmani koj imaju cilj da smanje negativan uticaj nedostatka vode i poboljšaju klijanje semena. Jedan od njih je potapaње semena. Cilj ovog eksperimenta bio je da se ispita uticaj potapaња semena u rastvore - KNO<sub>3</sub> (1%), askorbinsku kiselinu - AsA (100mg l<sup>-1</sup>) i kalijum hlorid KCl (1%) na smanjeње negativnog uticaja suše. Dejstvo suše simulirano je primenom razlicitih koncentracija PEG-a 6000 (0 (kontrola), -0,30, -0,51, -0,80 MPa). Rezultati istraživanja su pokazali da je sa poveћanjem nedostatka vode efekat potapaња semena veći. Pri vodnom potencijalu rastvora od -0,30 MPa enerija klijanja i klijavost semena poveћani su, u proseku, za 6,77% odnosno 5,08% dok je pri vodnom potencijalu rastvora od -0,80 MPa poveћanje iznosilo 19,28% odnosno 16,75%. Takođe, potapaње semena značajno je smanjilo intenzitet lipidne peroksidacije i sadržaj slobodnog proлина.

Iz svega navedenog može se zaključiti da je potapaње semena metod koji može da poboljša klijanje semena soje u uslovima suše.

*Кључне речи: суша, Glycine max., потапање семена, клијавост семена.*

### 1. Introduction

Seed priming was an age-old technique practiced by the Greek farmers. Theophrastus (372–287 BC) primed cucumber seeds before sowing in milk or water to germinate earlier and have greater germination energy (Evenari, 1984). Pre-hydration of legume seeds before sowing was performed by Roman farmers in order to increase the germination rate and synchronize germination as reported by the Roman naturalist Gaius Plinius Secundus. Several centuries later, these techniques were still used for a wide range of species according to the French agronomist Olivier de Serres (1539–1619) (Evenari, 1984). The word seed priming

was coined by Heydecker in 1973 and he successfully adopted seed priming to improved seed germination and emergence under stressful conditions (Sivasubramaniam et al., 2011). In the beginning of the XXI century, priming seed is a economic and simple pre-sowing treatment that is usually used in countries in transition (Harris et al., 2001) that is, applied by farmers who cannot use other expensive and complex methods to stimulate germination (Ozdemir et al., 2012). Several methods of seed priming have been developed in order to invigorate seeds and alleviate the environmental stresses. A common feature of water-based priming techniques, which distinguishes them from other pre-sowing treatments, is partial seed pre-hydration and the activation of early

germination events in seed. The benefits of priming seed on all crops are faster and greater germination and sprouting, greater vigor of the plant, earlier flowering, and thus earlier harvest and higher yield.

A major limitation in agricultural production worldwide is the deficit water during germination, leading to a reduction or even complete absence of germination (Kaya et al., 2006). In many countries in Asia and Africa, seed priming is the best method to mitigate the negative effects of drought stress in the germination and sprouting (Harris, 2001). Certain solutions can be used to induce drought under controlled conditions. One of them is polyethylene glycol - PEG (H(OCH<sub>2</sub>CH<sub>2</sub>)<sub>n</sub>OH). PEG is a long-chain, chemically inert polymer, used in water stress simulation experiments (Murillo-Amador et al., 2002). Back in 1961, PEG was introduced as a compound that can be used to alter the osmotic potential of a solution and cause a water deficit. PEG 6000 binds water, but due to the size of its molecules, it cannot be absorbed by the plant. Thus, it causes a state of physiological drought, that is, prevents the plant from accepting water (Alexieva et al., 2001). PEG has no toxic effect (Khajeh-Hosseini et al., 2003).

Therefore, the objective of this experiment was to compare the effect of drought stress on two soybean varieties and seed priming with potassium nitrate (KNO<sub>3</sub>), ascorbic acid (AsA) and potassium chloride (KCl), with the hypothesis that seed priming can mitigate the adverse effect of drought stress.

## 2. Material 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. 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 PEG, seeds were primed into different solutions: potassium nitrate - KNO<sub>3</sub> (1 %), ascorbic acid AsA - (100mg/l) 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%. The effect of drought was examined by germinating seeds at different concentrations of water-soluble PEG 6000. These are the concentrations that provide the osmotic potential of solution 0 (control), -0.30, -0.51, and -0.80 MPa, respectively. 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. 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).

After 8 days, 4 replicates x 10 average seedlings were taken from each treatment and used to determine the intensity of lipid peroxidation and free proline

content. Intensity of lipid peroxidation was carried out using the method of Placer (1966), free proline concentration was determined by the method of Bates (1973). According to the method of Placer (1966), extraction of malondialdehyde (MDA) from soybean seedling was done by using solution of thiobarbituric acid (TBA), trichloroacetic acid (CCl<sub>3</sub>COOH) and perchloric acid (HClO<sub>4</sub>) and concentration was determined spectro photometrically at 532 nm. Soybean seedling (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<sup>-1</sup> of fresh weight. 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 molg<sup>-1</sup> DW against standard proline.

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. The standard error of the arithmetic mean was used to calculate the significance of the difference using the Tuckey's test. The results presented in the graphs were obtained on the basis of oneway analysis of variance. The interval bars in the figures represent the difference between the standard error of the arithmetic mean.

## 3. Results and discussion

Studies have shown that increasing the intensity of drought from -0.30 MPa to -0.80 MPa reduced seed germination and increases the intensity of lipid peroxidation and free proline content. Priming seeds in solutions of KNO<sub>3</sub>, AsA and KCl reduced the negative impact of drought stress.

All analyzed traits differed significantly at different levels of drought, selected solution, while no difference was found between varieties. A significant difference was also found in the interaction drought stress and solutions, as well as varieties and solution. No interaction was found between cultivar and drought, ie in the case of triple cultivar interaction, drought and solution (Table 1).

### *Germination energy and germination*

A major limitation in agricultural production worldwide is the drought stress during germination, leading to a decrease or even complete absence of germination (Kaya et al., 2006). Depending on the degree of water deficit, even seed death may occur (Silva et al., 2010). One of the conditions for high yield in conditions of deficit water is to provide fast germination seed and sprouting, and thus help the plants to get out of that phase as soon as possible and develop a root system that will go into deeper layers of soil which have enough water (Gupta et al., 2008). The results of the study showed that drought stress on the germination of soybean seeds was more pronounced at lower water potential of the solution. The results are in line with research conducted on seeds: lentils (Muscolo

et al., 2014), oats (Mut et al., 2010), sunflower (Ahmad et al., 2009), maize (Mei and Song, 2008) in which it was found that lack of water significantly reduces seed germination. With the increase of drought stress, especially at the lowest value of the water potential of

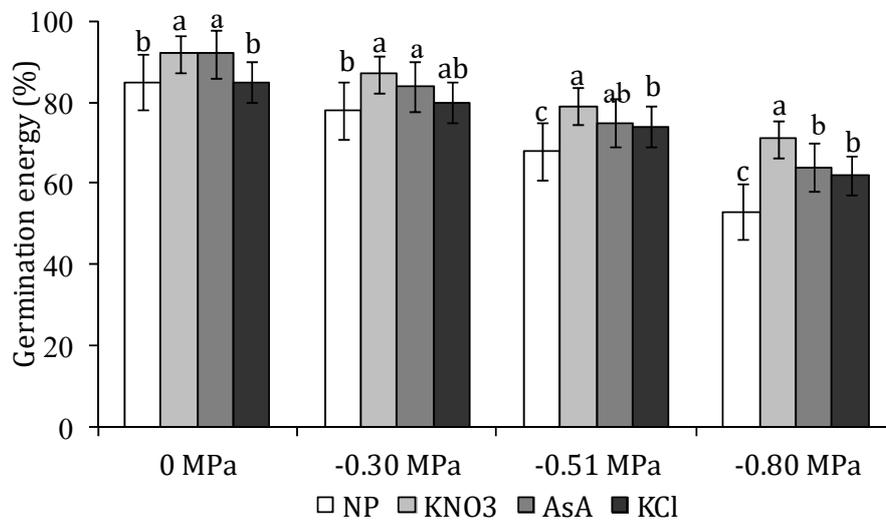
the solution (-0.80 MPa), the time necessary for germination of soybean seeds has been extended (Figure 1,2).

**Table 1.**

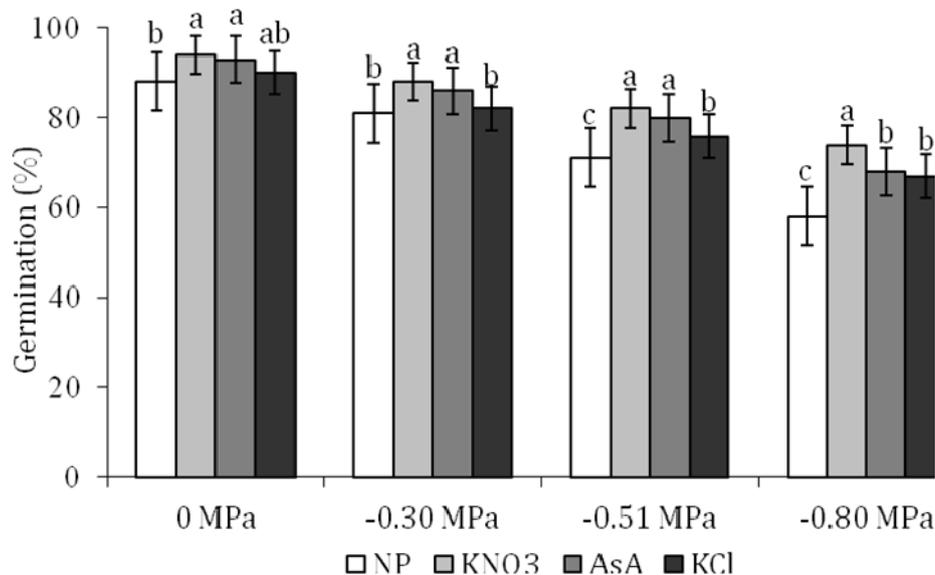
Analysis of the variance of the influence of priming soybean seeds in  $\text{KNO}_3$ , AsA and KCl solutions on and seedlings in drought conditions.

Source of variation	Germination energy	Germination	Lipid peroxidation	Free proline
	Soybean seed		Soybean seedling	
Variety (A)	0.47	0.92	1.45	2.78
Drought (B)	11.78**	13.32**	6.21**	5.71**
Treatment (C)	5.12**	5.19**	4.62*	6.12**
A x B	0.36	1.18	2.07	2.21
A x C	7.71**	8.14**	5.19**	5.49**
B x C	8.18**	9.17**	5.92**	6.41**
A x B x C	0.29	0.34	3.29	2.19

The significance of the F test at the level of 5% \* and at the level of 1% \*\*



**Figure 1.** Effect of seed priming in  $\text{KNO}_3$ , AsA and KCl solutions on germination energy in drought stress ( $p < 0.05$ )  
The different letters above the bars indicate a significant difference of 5%. The vertical lines at the top of the columns represent the standard errors according to the treatments shown on the abscissa.



**Figure 2.** Effect of seed priming in KNO<sub>3</sub>, AsA and KCl solutions on germination in drought stress ( $p < 0.05$ )  
The different letters above the bars indicate a significant difference of 5%. The vertical lines at the top of the columns represent the standard errors according to the treatments shown on the abscissa.

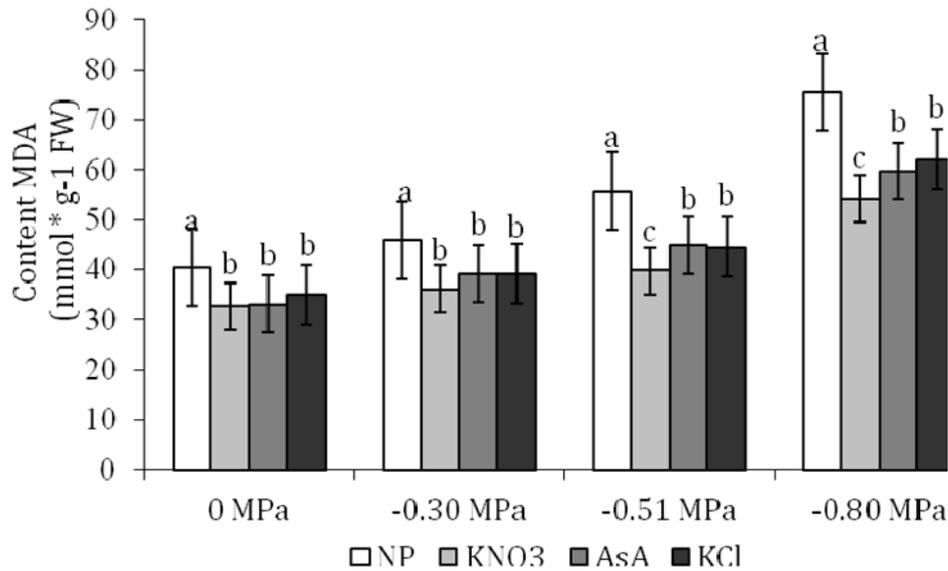
Gholami et al. (2010) found that drought stress reduced the activity of hydrolytic enzymes, which are responsible for the decomposition of reserve substances in cotyledons and the provision of energy that is necessary in the initial stages of seed plant development, resulting in inhibition of seed germination. Priming seeds of soybeans in KNO<sub>3</sub>, AsA and KCl solutions improved germination energy and seed germination. At the water potential of the solution of -0.30 MPa, the germination energy and germination of primed seeds increased, on average 6.77% and 5.08%, respectively, while at the water potential of the solution -0.80 MPa, the increase was 19.28% or 16.75%, respectively. The positive effect of this method due to water deficit can be explained by the fact that priming stimulates germination, accelerates the formation and growth of a root system that will absorb water from deeper layers of soil, resulting in a reduction in the negative impact of deficit water (Zhang et al., 2015). The same authors point out that priming significantly increases the stability of the cell membrane, ie partly eliminates the negative impact of water deficiency on cell membrane degradation. The KNO<sub>3</sub> solution had the greatest effect on the seed germination energy. If seed germination is analyzed, it can be seen that KNO<sub>3</sub> and AsA have a similar effect. Potassium is a cofactor in the action of some enzymes that play a role in cell division, nucleic acid metabolism and protein synthesis (Cakmak and Horst, 1991). The positive effect of KNO<sub>3</sub> is also attributed to elevated nitrate levels (Benech-Arnold et al., 2000). Nitrates stimulate seed germination because they act as a signaling molecule (Alboresi et al., 2005). A good result was also achieved with the use of AsA. Exogenous application of AsA solution can affect various processes in plants, including seed germination. Ascorbic acid contributes to the regulation of ethylene biosynthesis (Dong et al., 2016), gibberellin (Shu et al., 2013), abscisic acid, and cytokinin (Huang et al., 2017). Gibberellins and cytokinins have a predominantly stimulating effect on the germination process, while abscisic acid has an inhibitory effect.

### Lipid peroxidation

As the intensity of drought stress, the intensity of lipid peroxidation increases, and the result is lower germination (Figure 3). Cai et al. (2011) point out that the decrease in seed germination is due to damage to the cell membrane and uncontrolled accumulation of ROS. An increased amount of ROS has the potential to oxidize cellular proteins, lipids, and DNA resulting in a change in structure and function, cell damage and death (Valko et al., 2004). Increased intensity of lipid peroxidation in conditions of drought stress was found in seed rapeseed (Rezayian et al., 2018), *Xanthoceras sorbifolium* (Cai et al., 2011), *Cercidiphyllum japonicum* (Mai et al., 2009) and *Picea asperata* (Yang et al., 2010). Use of priming, intensity of lipid peroxidation is reduced, and the effect is greater at lower water potential of the solution, ie at higher drought intensity. At higher intensity of drought, the effect of priming is greater. Thus, at the water potential of -0.30 MPa, the intensity of lipid peroxidation decreased, on average, by 16.85%, while at the water potential of -0.80 MPa, the decrease was 22.4%. Similar results were received on the seeds of sorghum (Zhang et al., 2015). Chiu et al., (2006) point out that the decrease in lipid peroxidation due to seed priming is a consequence of increased antioxidant activity, and the result of all this is improved seed germination. Bailly et al., (2000) point out that the decrease in the intensity of lipid peroxidation is a consequence of increased enzyme activity. Observing the influence of solutions in these studies, it can be seen that with increasing drought intensity, there is a greater difference in the efficiency of the applied solutions. At higher drought intensities, the effect of KNO<sub>3</sub> solution is most effective, while AsA and KCl solutions have a similar effect. The positive effect of KNO<sub>3</sub> on the reduction of the negative effect of drought was determined not only on the example of soybeans (Langeroodi and Noora, 2017) but also on rapeseed (Mohammadi and Amiri, 2010) and Chinese cabbage (Yan, 2015). Potassium from KNO<sub>3</sub> plays a key role in reducing the negative impact of

drought, as it maintains cell turgor due to deficit water and helps maintain the balance of the osmotic potential of the cell and its environment (Umar and Moinuddin 2002). Also, enough provision of plants with

this element encourages the growth of the root system, which results in increased water intake from the substrate (Mujtaba et al., 2007).

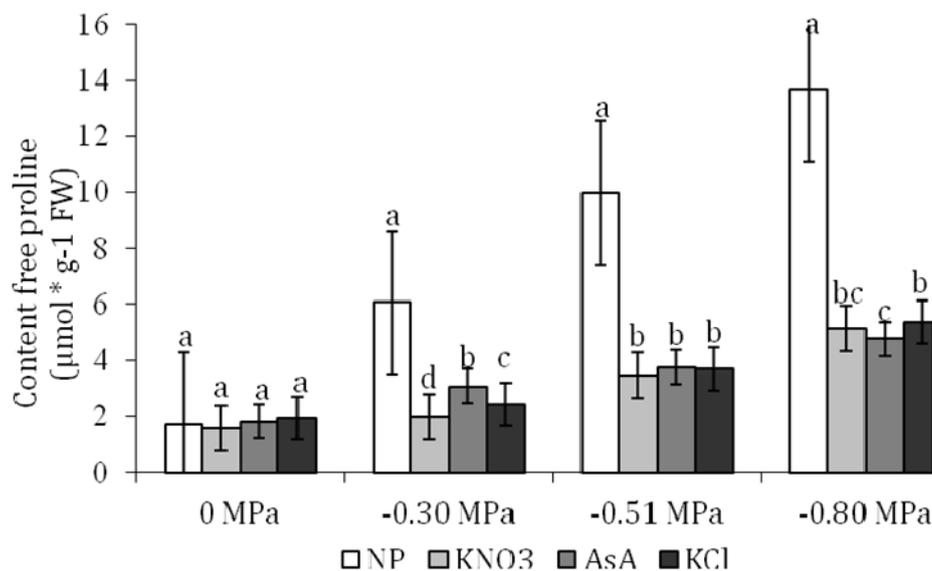


**Figure 3.** Effect of seed priming in KNO<sub>3</sub>, AsA and KCl solutions on content MDA in drought stress ( $p < 0.05$ ) The different letters above the bars indicate a significant difference of 5%. The vertical lines at the top of the columns represent the standard errors according to the treatments shown on the abscissa.

### Free proline

In addition to increasing the intensity of lipid peroxidation, the content of free proline also increases

(Figure 4). The influence of drought stress on its content in soybean seedlings was more pronounced at lower concentrations of water potential.



**Figure 4.** Effect of seed priming in KNO<sub>3</sub>, AsA and KCl solutions on content free proline in drought stress ( $p < 0.05$ ) The different letters above the bars indicate a significant difference of 5%. The vertical lines at the top of the columns represent the standard errors according to the treatments shown on the abscissa.

Ashraf and Foolad (2007) point out that one of the mechanisms of protection against osmotic stress, in our research it is drought stress, accumulation of compatible osmolytes (free proline, soluble sugars, glycine betaine and organic acids) in the cytoplasm to keep the cell water potential lower than water potential of the external environment. When exposed

to drought, plants accumulate large amounts of free proline, so in some cases its concentration is several times higher than the total concentration of all other amino acids. Increased content of free proline in conditions of drought stress during germination was found in lentils (Muscoli et al., 2013) and millet (Sneha et al., 2014). Priming seeds in solutions of KNO<sub>3</sub>, AsA

and KCl, the content of free proline in soybean seedlings was significantly reduced in conditions of lower water potential. Regardless of the water potential, the content of free proline was reduced by more than two times. In addition to the beneficial effect, free proline can also have a negative effect, if it accumulates in a higher concentration. The increased content of free proline results in a disturbance in the relationships between inorganic ions, and the consequence is a decrease in seed germination (Heuer, 2003).

#### 4. Conclusions

Quality of soybean seed is reduced in drought stress. As the water potential decreases, germination energy and seed germination decrease significantly, while the intensity of lipid peroxidation and the content of free proline increased. Priming of soybean seed with potassium nitrate, ascorbic acid and potassium chloride solutions resulted in the higher seed quality regardless of the soybean variety or water potential. Application of pre-sowing treatment might be used for improving germination in the case of crop cultivation under drought stress conditions. Improved germination would also affect crop canopy and ensure crop stability, ultimately resulting in higher and stable yields.

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