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Tanja MAKSIMOVIĆ, Nina JANJIĆ, Biljana LUBARDA [1]

EFFECT OF DIFFERENT CONCENTRATIONS OF MANNITOL ON GERMINATION OF PEA SEEDS (*Pisum sativum* L.)

SUMMARY

During the life cycle, plants are exposed to various abiotic and biotic stress factors that adversely affect their growth, development and productivity. Drought is one of the stress factors affecting the decline in plant growth and productivity worldwide. In this study, the effect of water deficiency caused by different concentrations of mannitol (5%, 10%, and 20%) on the germination of seeds of two varieties of peas (Petit Provencal and Joff) was studied. The results of the study showed that the germination percentage and germination potential of both pea varieties decreased with increasing mannitol concentration compared to the control. The Petit Provencal variety had a higher percentage of germination, germination potential and vigor index of drought resistance and this variety was considered more tolerant to drought stress.

Keywords: drought, mannitol, seed, germination, *Pisum sativum* L.

INTRODUCTION

In the past few years, drought has been one of the most common factors damaging plant growth and development, and is becoming an increasingly serious problem in many regions around the world. Extreme water shortages cause considerable physiological, metabolic and morphological changes in plants which in turn reduce the yield and quality of the crop (Al-Jebory, 2012; Duan et al., 2017; Chaves and Oliveira, 2004).

Pea (*Pisum sativum*) is a very important agricultural crop from the legume (Fabaceae) family that plays a large role in human nutrition. It is of particular importance primarily because of its high nutritional value, intensive production and its short cultivation period (Kumar and Choundhary, 2014). Since peas are grown in climatically different regions under different production conditions, pea seeds are inevitably susceptible to various stress conditions. Stressful conditions inhibit seed germination, resulting in poor crops, reduced biomass, and ultimately, reduced yield and quality (Machado Neto et al., 2004;

[1]Tanja Maksimović, (corresponding author: tanja.maksimovic@pmf.unibl.org), Nina Janjić, Biljana Lubarda, Department of Biology, Faculty of Natural Sciences and Mathematics, University of Banja Luka, Mladena Stojanovića 2, 78 000 Banja Luka, Republic of Srpska, BOSNIA AND HERZEGOVINA

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Khodarahmpour, 2011; Al-Jebory, 2012; Duan et al., 2017). Water stress acts by reducing the percentage, germination rate and seed growth. The adverse impact of water scarcity has been well studied in different crops such as maize (Radić et al., 2007; Khodarahmpour, 2011; Jain et al., 2013; Liu et al., 2015), soybean (Machado Neto et al., 2004), peas (Al-Jebory, 2012), wheat (Koka et al., 2015; Duan et al., 2017), and sunflower (Luan et al., 2014).

The most popular technique for causing drought is by using high molecular weight substances such as mannitol and polyethylene glycol. Mannitol is a white, crystalline solid of the chemical formula $C_6H_8(OH)_6$ which does not pass through the cell wall, has low toxicity so it is an ideal material used to simulate arid soil (Al-Jebory, 2012; Liu et al., 2015).

The aim of this paper is to study the differences in germination and seed growth of drought-exposed seeds in two varieties of peas to determine which variety is more tolerant and suitable for cultivation in water scarcity conditions.

MATERIAL AND METHODS

In this paper, the effect of water deficit on seed germination and seedling growth in two varieties of peas, Petit Provencal and Joff, was monitored. The pea seeds were first sterilized with 70% ethanol and then washed with distilled water. Each variety was germinated in sterile petri dishes containing 30 seeds in 3 repetitions. The seeds were treated with different concentrations of mannitol from the beginning: 5%, 10% and 20%, and distilled water was used in the control. After setting up the experiment, seed germination was performed at a temperature of 26°C in a thermostat. The percentage of germination was monitored on the third, fifth, and seventh days, and after seven days, the germination potential, vigor index of drought resistance, and seedlings growth were calculated.

Germination percentage

Germination percentage was calculated as follows:

Germination percentage= (germinated seed number/total seed number) x 100% (Liu et al., 2015).

Germination potential

Germination potential is an index that shows the relationship between the percentage of germination and the uniformity of germination, and it is calculated as follows:

Germination potential= (germinated seed number at germination peak/total seed number) x 100% (Liu et al., 2015).

Germination and vigor index of drought resistance

Germination and vigor index of drought resistance were calculated as follows: drought resistance index of germinated seed = (seed promptness index under water deficiency- PIS)/ seed germination promptness index -PIC (control); Promptness index PI= $(nd_2 \cdot 1.00) + (nd_4 \cdot 0.75) + (nd_6 \cdot 0.50) + (nd_8 \cdot 0.25)$; where nd_x= number of germinated seeds by the xth day of measurement (Grzesiak et al., 2013).

After seven days, the growth of seedlings and roots was measured in control and treated seeds.

All results are presented as the average value of the three replicas ± standard deviation (SD). The statistical significance of the analyzed parameters was tested by ANOVA test ($p<0.05$) two-way analysis of variance.

RESULTS AND DISCUSSION

The seeds of the Petit Provencal variety germinated better than the Joff variety under different mannitol treatments. The percentage of germination in both pea varieties tested decreased with increasing mannitol concentration compated to the control (Figure 1).

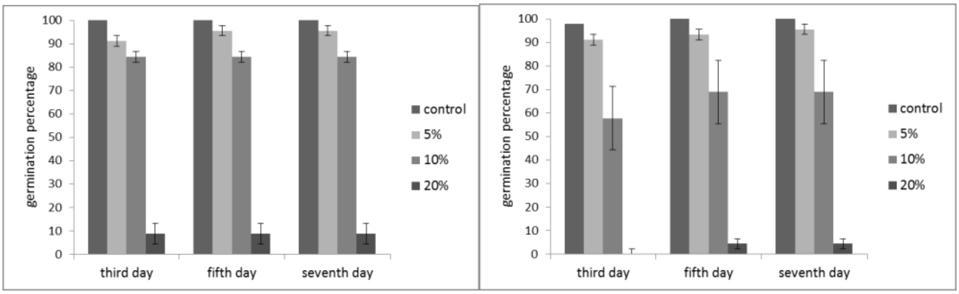

Figure 1. Germination percentage of seeds of two studied pea varieties (Petit Provencal - left, Joff- right) under different concentrations of mannitol

Under the influence of 5% mannitol solution, the percentage of seed germination of the Petit Provencal variety decreased by 4.5%; under the influence of 10% mannitol by 15.6%, and under the influence of 20% mannitol by 91.2% compared to the control. Under the influence of 5% mannitol, the germination percentage of the Joff variety was lower by 4.5% compared to the control, while under the influence of 10% it decreased by 31.1% and under the influence of 20% mannitol by 95.6%. Based on the results of the ANOVA test, it was concluded that there are no statistically significant differences between the same treatment depending on the day for both investigated varieties. Also, a statistically significant difference was obtained between different groups depending on the concentration of mannitol, both for the variety Petit Provencal and for Joff (Figure 1).

The germination potential of the two pea varieties decreased significantly due to mannitol treatment with significant differences between them. Under the influence of 5% mannitol, the potential of the Petit Provencal variety decreased by 33.3% and in the Joff variety by 46.7%. Under the influence of 10% mannitol, the germination potential of the Petit Provencal variety decreased by 66.6%, while in the Joff variety it decreased by 80%. At the highest concentration of mannitol, no germination of seeds of any variety occurred. The results of the ANOVA test for the germination potential parameter show that there are statistically significant differences between different varieties at a mannitol concentration of 5% and 10%. Statistically significant differences also exist between different concentrations of mannitol in the same pea variety (Figure 2).

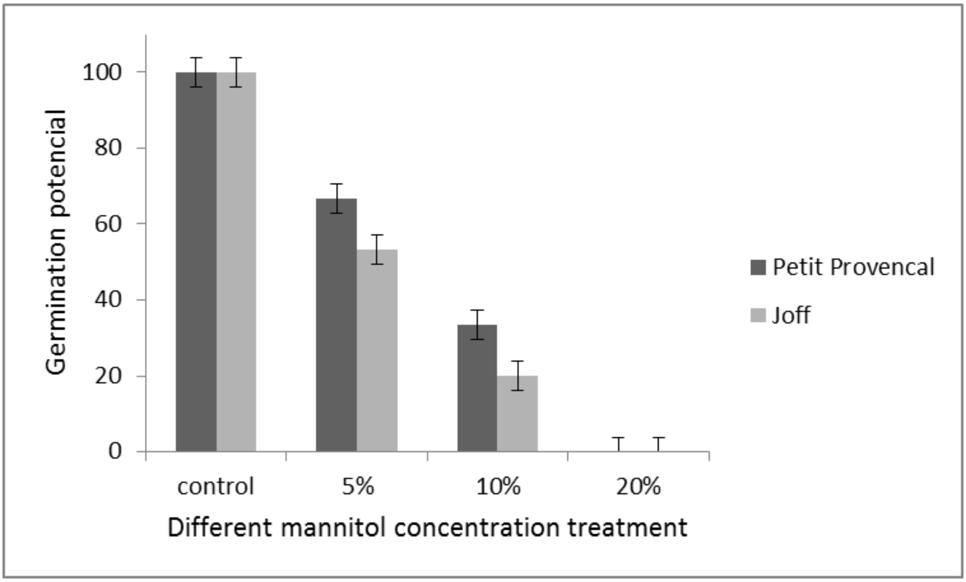

Figure 2: Germination potential of two studied pea varieties under different concentrations of mannitol

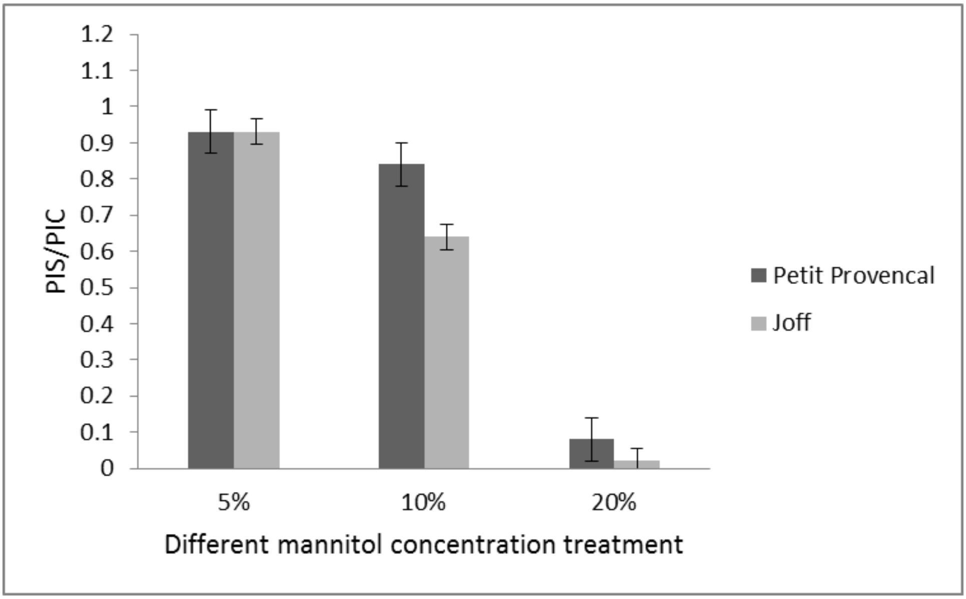

Figure 3: Vigor index of drought resistance in studied pea varieties

Vigor index of drought resistance (PIS/PIC) of the two pea varieties decreased with increasing mannitol concentration. In the case of the Joff variety

PIS/PIC with the treatment of 10% and 20% of mannitol decreased more than in the Petit Provencal variety, while under the influence of 5% of mannitol the value of this parameter was uniform in both varieties. As for the germination potential parameter, the test shows that for the vigor index there are statistically significant differences between different varieties at a mannitol concentration of 10% and 20%. As for the germination potential parameter, the test shows that for the vigor index there are statistically significant differences between different varieties at a mannitol concentration of 10% and 20% (Figure 3).

The average root length of the Petit Provencal variety was greater than the Joff variety and decreased on average by 2-4 times under the influence of mannitol compared to the control (Figure 4). The highest root length was recorded in control. The mean value of the root length of the Petit Provencal variety under the influence of 5% mannitol decreased by 75.7%, and under the influence of 10% mannitol by 77.3%. In the Joff variety under the influence of 5% mannitol, the root length decreased by 50.9%, and under treatment with 10% mannitol by 60.4% compared to the control. These values were measured on the seventh day. There is a statistically significant difference in root length depending on the concentration of mannitol, and also between the fifth and seventh day for the same variety. In addition, there is a statistically significant difference between different varieties in the same treatments especially for a concentration of 10%. No root was present in the treatment with 20% mannitol in both varieties of peas.

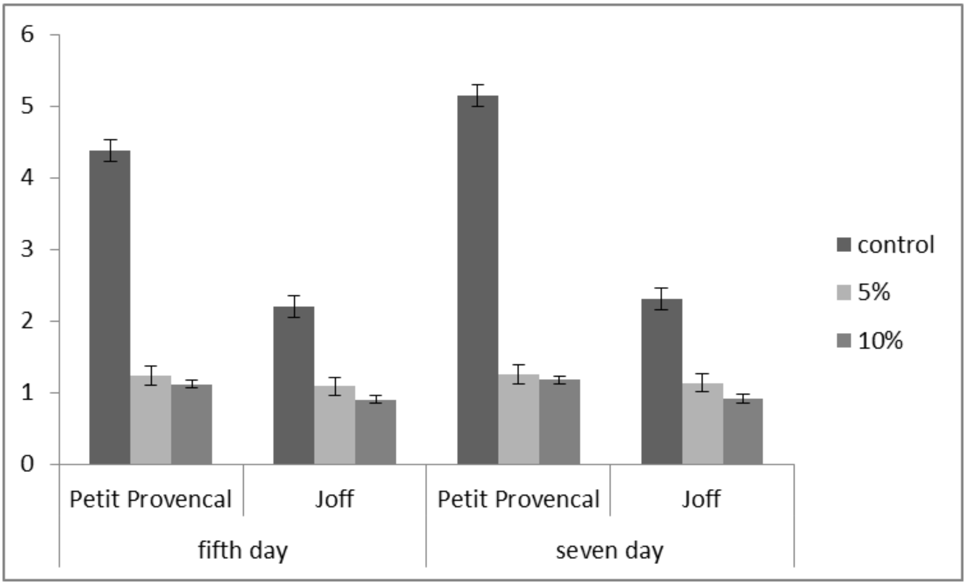

Figure 4: Average root length [cm] in Petit Provencal and Joff variety

No seedling was present in both pea varieties, indicating that administration of mannitol concentration (5%, 10%, and 20%) completely inhibited plant growth.

Plants are exposed to various stress factors throughout their life cycle that greatly affect their growth, development and productivity. Drought is among the first abiotic stressors, which limit the normal functioning of the plant (Machado Neto et al., 2004; Radić et al., 2007; Khodarahmpour, 2011; Al-Jebory, 2012; Jain et al., 2013; Luan et al. al., 2014; Liu et al., 2015; Koka et al., 2015; Duan et al., 2017). The effect of drought, on the level of the whole pea plant is multiple, and is reflected in the reduced ability to germinate and sprout seeds, poorer root development and above-ground part of the plant, reduced ability to create and accumulate dry matter, as well as a negative impact on flower formation, pollen formation, pollination, grain formation and quality (Al-Jebory, 2012; Elkoca, 2014).

In this paper, drought resistance of two varieties of peas under simulated mannitol-induced conditions was monitored. The percentage of germination of both varieties of peas declined under the influence of drought. By comparison of the tested varieties it was found that the percentage of germination was higher in the Petit Provencal variety compared to Joff. Treatment with the highest concentration of mannitol (20%) caused a 91.2% decrease in germination rates for the Petit Provencal variety and 96.5% for the Joff variety, indicating that the Petit Provencal variety exhibited higher drought resistance (Figures 1). These results agree with those of Machado Neto et al. (2004) for soybean and with Koka et al. (2015) for the oat.

According to Elkoca (2014) water uptake is directly related to the osmotic potential of the applied polyethylene glycol and mannitol in the experiment. More specifically, when treating seeds with these substances, the promptness and percentage of seed germination are reduced.

Similar studies were carried out by Machado Neto et al. (2004) on certain soybean varieties, whereby they found that the percentage of germination decreased depending on the concentrations of mannitol applied, which may be related to the results of our studies.

Germination potential is a parameter that shows the degree of germination and evenness of seed germination (Liu et al., 2015). In this paper, it was shown that the germination potential of both pea varieties declined under the influence of drought, but that it decreased more in the Joff variety than in the Petit Provencal variety (Figure 2), indicating that the Petit Provencal seed germinated faster than the Joff variety seed, showing at the same time stronger drought resistance. Water stress not only affects seed germination but also extends the period required for germination (Khodarahmpour, 2011; Liu et al., 2015), as we have noted in this paper.

The drought resistance index is a parameter that better and more accurately indicates to us the drought resistance and germination characteristics (Grzesiak et al., 2012; 2013). The resistance index for both tested pea varieties decreased with increasing mannitol concentration, but for the Petit Provencal variety decreased less than for Joff, which tells us that the Little Provencal variety showed greater resistance to drought (Figure 3). Liu et al. (2015) in their study on two maize varieties also found a decrease in drought resistance index with increasing mannitol concentration.

From the obtained results, it was found that the Petit Provencal variety had longer roots under the influence of drought (Figure 4), implying that this variety had a more developed root system and therefore showed higher resistance than the Joff variety. Reduced root growth in 4 mannitol-induced soybean cultivars was reported by Machado Neto et al. (2004) as well as Koka et al. (2015) on the oat. Duan et al. (2017) reported in their study on wheat that inhibitions of root growth under the influence of polyethylene glycol-induced drought occur, and this may be related to the results obtained in this work. Khodarahmpour (2011) by studying 7 maize hybrids under drought stress conditions showed that root length decreased by up to 60% compared to the control.

The Petit Provencal cultivar had a higher percentage of germination and higher promptness, root length, indicating that this variety was more drought tolerant than the Joff variety.

CONCLUSIONS

The growth of plants in arid and semi-arid conditions depends on the susceptibility of the plants to drought and the ability of the seed to achieve optimal germination in adverse conditions. It is therefore of great importance to determine the tolerance of varieties to drought in the initial stages of growth. Taking all the characteristics into account, in this paper the Petit Provencal variety germinated faster and had a higher percentage and potential of germination than the Joff variety. Also, the cultivar Little Provencal had a better root system, which indicates a better ability for osmotic adaptation, which allows cultivation of the tested variety in dry conditions. This type of research shows that monitoring seed germination index can be used to determine the tolerance and sensitivity of a variety to the stress caused by drought in the initial stages of growth.

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