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ORIGINAL ARTICLE

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EFFECTS OF DROUGHT STRESS ON GERMINATION AND SEEDLING GROWTH OF DIFFERENT FIELD PEA VARIETIES

ABSTRACT: Germination and seedling growth of seven genotypes of field pea (*Pisum sativum* L.) were studied in PEG-6000 solution having osmotic potentials -0.1 and -0.2 MPa. A study was undertaken to evaluate the influence of different osmotic potentials (MPa) on seed germination percentage (GP) and mean germination time (MGT). Results show that the percentage of germination decreased with a decrease in osmotic potential, while mean germination time increased. Variety Javor is much more sensitive than the other varieties at all levels of osmotic stress. By contrast, the lowest sensitivity of germination and MGT was found in varieties Mraz and Trezor. Seed germination tests at -0.1 to -0.2 MPa have the potential to be used as tests in field pea. Osmotic stress exposure and its duration significantly affected the growth of seedlings (shoot and root) and the accumulation of biomass, while its effect was more prominent on the growth of the shoot than on root growth, which was also confirmed by the root tolerance index.
KEYWORDS: *Pisum sativum*, seed, shoot, varieties, tolerance index

INTRODUCTION

Pea (*Pisum sativum*) is the second most important food legume worldwide after common beans. The increasing demand for protein-rich raw materials for animal feed or intermediary products for human nutrition has led to a greater interest in this crop as a protein source. Pea seeds are rich in protein (23–25%),

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slowly digestible starch (50%), soluble sugars (5%), fibre, minerals, and vitamins (Bastianelli et al., 1998; Červenski et al., 2017).

Nowadays, legumes provide one-third of the entire amount of protein for human consumption, representing an important source of fodder and forage for animals, as well as in the production of edible and industrial oils (Smýkal et al., 2012). Due to their high protein content, they can partially replace soybean in the meal of ruminants and non-ruminants (Gružauskas et al., 2016). One of the most important attributes of legumes is their capacity for symbiotic nitrogen fixation, underscoring their importance as sources of nitrogen in both natural and agricultural ecosystems (Heldt and Piechulla, 2011; Smýkal et al., 2012).

During their growth, crop plants are usually exposed to different environmental stresses that can limit their growth and productivity (Fleury et al., 2010; Vujaković et al., 2011). Salinity and drought are major environmental factors limiting plant growth and productivity, causing great economic losses (Jovičić et al., 2014; Petrović et al., 2016). Field pea, like other pulses, is moderately sensitive to a number of abiotic stress factors, particularly involving water deficit, soil nutrition such as salinity and alkaline-induced boron toxicity, reproductive frost damage, and heat stress (Dita et al., 2006; Petrović et al., 2016).

Seed germination is the first critical and the most sensitive stage in the life cycle of plants and it can compromise the seedlings' establishment (Kolb and Barsch, 2010; Karagić et al., 2010). Seeds exposed to unfavorable environmental conditions, like water stress, germinate with difficulties (Jovičić et al., 2014). Water stress limits plant survival and early seedling growth by delaying its beginning or decreasing the final germinability (Okçu et al., 2005; Kaydan and Yagmur, 2008; Silva et al., 2010). Considering that pea, like other legumes, requires a relatively large amount of water during the germination process, it is suggested that this is the most critical phase of development stages.

Drought tolerance testing in the initial stages of plant development is of vital importance because the seed with more rapid germination under water deficit conditions can be expected to achieve a rapid seedling establishment, resulting in higher yields (Jovičić et al., 2014). Polyethylene glycol (PEG 6000) is commonly used to simulate water-stress conditions in seed germination studies. PEG 6000 is an inert osmotic agent whose molecules are too large to penetrate the seed, thus preventing any toxic effects. Because PEG 6000 does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall (Verslues et al., 2006). PEG-based in vitro screening for drought tolerance has proven to be an effective screening method for large sets of germplasm (Okçu et al., 2005; Generozova et al., 2009).

Measurement of germination can provide valuable information about the start, rate, uniformity, and final percentage of germination. However, two seed lots can have the same germination percentage but differ in speed or uniformity. Therefore, a total germination percentage after a specific period does not give a full explanation of the dynamics of germination (Joosen et al., 2010). It is also useful to calculate MGT. MGT is defined as a measure of the rate and time-spread of germination (Soltani et al., 2016).

Since researchers have paid little attention to improve the locally cultivated pea in this area, the present study contributes to understanding the responses under osmotic and drought stress conditions and the further improvement of our field pea cultivars.

The aim of this study was to determine whether the pea seed germination and seedling growth of different field pea cultivars were inhibited by the osmotic effect of water deficit, as well as to establish a simple and fast screening method for drought tolerance in pea genotypes.

MATERIALS AND METHODS

The study was conducted using seven selected genotypes of the genus *Pisum* from the collection of the Institute of Field and Vegetable Crops in Novi Sad. Seeds were sterilized with 0.5% sodium hypochlorite solution for 1 min and after that washed with distilled water. Pea seeds were germinated in 15 x 24 cm plastic boxes filled with sand. For each cultivar, 4 replicates of 100 seeds were sown for each treatment.

Drought stress was stimulated by two different osmotic potential levels (-0.1 MPa and -0.2 MPa) using PEG 6000. Solutions of PEG 6000 were prepared according to Michel and Kaufmann (1973). Distilled water served as a control.

Plastic boxes were placed in a germination chamber at 20 °C under conditions of a 12h light/dark cycle.The sand was moistened daily with distilled water for control and the solution of PEG for treatments. Seed germination was recorded every 24h for 10 days by evaluating normal seedlings. A seed was considered to have germinated when the radicle was 2 mm long (Kaya et al., 2006; Kim et al., 2006).

The germination percentage was determined by counting the number of germinated seeds as follows: (GP) = Number of germinated seed / Total Number of seed tested x 100.

Mean germination time (MGT) was determined according to Kandil et al. (2012): $MGT= \sum dn/ \sum n$, where (n) is the number of seeds that were germinated on the day (d), and (dn) is the number of days counted from the beginning of germination.

The radicle and hypocotyl length (mm) and the seedling fresh and dry weights (g) were measured on the 10th day after sowing. Dry weights of hypocotyl and radicle were measured after drying samples in the oven at 80 °C for 24h, to an accuracy of 0.1 mg using an analytical balance, until a constant weight was achieved. The results were expressed as the total mass of ten shoots (g).

The shoot/root ratio of dry weight was calculated from the obtained results. Tolerance index (TI) was calculated for the shoot and the root according to Maiti et al. (1994):

Tolerance index (TI) = Dry weight of plant material in the control group (g) / Dry weight of plant material in the treated group (g).

Data given in percentages were subjected to arcsine transformation before statistical analysis. For all investigated parameters, the analysis of variance was performed using the Statistical software (Sigmaplot 10.0., Systat Software Inc., San Jose, CA, U.S.A.). Significant differences among the mean values were compared by Student's t-test (*p < 0.05, **p < 0.01 and ***p < 0.001). Figures were drawn using Sigmaplot.

RESULTS AND DISCUSSION

Germination of seeds is a complex physiological process triggered by the imbibition of water after possible dormancy mechanisms have been released by appropriate triggers. Under favorable conditions, a rapid expansion growth of the embryo culminates in rupture of the covering layers and emergence of the radicle. Radicle emergence is considered the completion of germination. Water stress can affect germination by decreasing the percentage of germination.

The results of this study show that different concentrations of PEG in germination media significantly affect the seed germination of field pea. An increase in osmotic stress significantly decreased germination percentage (GP) in all the tested varieties. Increased Mean Germination Time (MGT) and decreased total germination time occurred already at the lowest level of stress (-0.1 MPa).

Germination in controls ranged between 96.25% (Partner) and 99.75% (Mraz), except for variety Javor where it amounted to 83.75%. In all varieties, a decrease in germination depending on the level of stress was observed, while this reduction differs among varieties (Figure 1a).

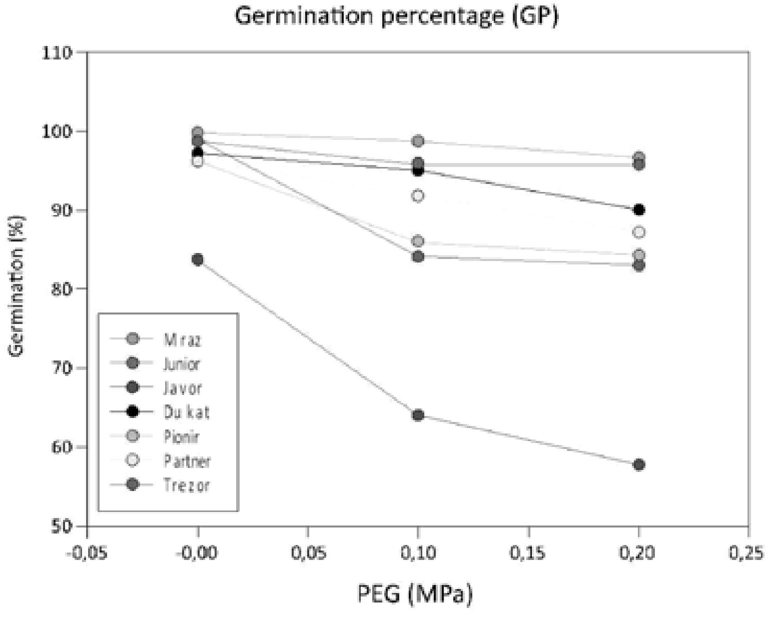
Variety Mraz showed the least reduction of GP (control 100%; -0.1 MPa 99%; -0.2 MPa 97%), while the largest decrease was in Javor variety (control 84%; -0.1 MPa 64; -0.2 MPa 58%). GP decreased with the increased PEG 6000 concentration, which is due to the reduction in water potential gradient between the seed and surrounding media (Okçu et al., 2005). Obtained results are in accordance with the results of other similar studies (Almansouri et al., 2001; Kaydan and Yagmur, 2008; Jovičić et al., 2013; Petrović et al., 2016).

Mavi et al. (2010) suggest that differences in MGT or the rate of germination between seed lots are crucial for determining the emergence performance both in terms of the rate of emergence, final count, and seedling size and variation. It is useful to calculate MGT because, according to Demir et al. (2008), slower germinating lots, as indicated by MGT, with a greater spread of germination over time, produced smaller and more variable seedlings in the laboratory.

MGT increased progressively as osmotic potential decreased (Figure 1B). Okçu et al. (2005) also found that mean germination time was delayed by decreasing water potential. MGT in controls ranged from 5.04 (Mraz) and 5.58 (Javor) days, at -0.1 MPa between 5.14 (Pionir) and 6.91 (Javor) days, while in the solution of -0.2 MPa PEG ranged between 5.17 (Trezor) and 7.24 (Javor) days.

The trend of increasing MGT, or slowing germination, with increasing levels of stress was observed in all varieties, except for varieties Mraz and Trezor in the solution of -0.2 MPa when they shorten the germination compared

A.



B.

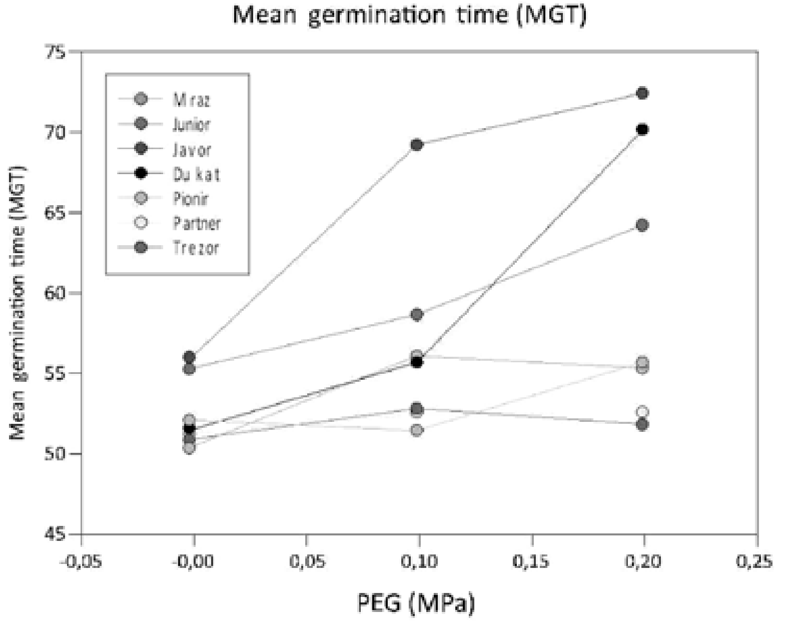


Figure 1. A. The effect of different concentrations of osmoticum PEG 6000 on seed germination in field pea genotypes; B. The effect of different concentrations of osmoticum PEG 6000 on mean germination time of field pea genotypes.

to -0.1 MPa. The increase of MGT in conditions of osmotic stress was particularly noticeable in variety Javor in which germination occurred later compared to other varieties.

Taking into account the results of measurements of the germination percentage and mean germination time, it is observed that variety Javor is much more sensitive than the other varieties at all levels of osmotic stress. On the contrary, the lowest sensitivity of germination and MGT was found in varieties Mraz and Trezor.

In order to examine the influence of different levels of osmotic stress on morphological changes of seedlings, growth parameters were determined.

An increase in osmotic stress resulted in decreased shoot and root length, but there were differences between the examined varieties. The Student's t-test showed that the decrease in shoot length compared to the control was very significant ($p < 0.001$), already at the lowest level of osmotic stress (-0.1 MPa) in all tested varieties. The lowest rate of reduction in the shoot length, relative to the control, was in the variety Dukat (73.4%, from 149.52 mm in control to 39.75 mm). On the contrary, the highest reduction (83.7%) was measured in Junior (from 215 mm in control to 35 mm in -0.1 MPa PEG solution, respectively) (Table 1).

A significant decrease in root length compared to the control was also observed. The smallest reduction in the root length relative to the control was in the variety Trezor (43.9%, from 200.48 mm to 112.48), while the highest reduction (70.7%) was in the variety Javor (211.50 mm to 112.48 mm) at the lowest level of osmotic stress (Table 1). The analysis of results showed differences between the examined varieties.

Water stress depressed the shoot growth of the cultivars rather than their root growth. The roots of the seedlings were longer indicating that the PEG significantly influenced the reduction of cell division and cell elongation. The same conclusion was made by Okçu et al. (2005) examining the effects of salt and drought stresses at the water potentials of -2, -4, -6, and -8 bars, induced by NaCl and PEG 6000 each, on germination and early seedling growth for three pea cultivars. PEG-induced osmotic stress caused more growth inhibition as compared to NaCl-induced osmotic stress (Petrović et al., 2016). The obtained results are in accordance with the results of Dobranszki et al. (2006) who analyzed the effect of PEG on 8 pea genotypes and found that osmotic stress caused by the solution of PEG 6000 affected the development of shoots more than the roots of the seedlings and that these changes were statistically significant at a solution of 5%, which would correspond to a solution of the osmotic potential of -0.1 MPa.

The average dry mass of the shoot in control ranged from 0.24 g (Pionir) to 0.47 g (Dukat). Varieties Junior, Pionir, and Trezor had the smallest dry mass of the shoots in the PEG solution of -0.1 MPa (0.07 g), while Dukat had a maximum of 0.12 g. At -0.2 MPa, Javor and Pionir had the lowest dry mass (0.05 g), while Dukat had 0.09 g. It is noticeable that the Dukat variety had the highest value of the dry mass of the shoot in all the examined groups (Table 1). The decrease of the shoot dry mass in the control was the largest in the Mraz variety

Table 1. Seedlings growth at a different level of osmotic stress

Variety	Treatment (PEG)	Shoot length (mm)	Root length (mm)	Shoot dry weight (g)	Root dry weight (g)	Shoot dry weight/ Root dry weight
Mraz	Control	119.38±1.23	212.38±1.48	0.290±0.000	0.168±0.000	1.72±0.004
	-0.1MPa	31.38^{***}±1.16	105.62^{***}±1.16	0.100^{***}±0.001	0.080^{***}±0.000	1.26^{***}±0.02
	-0.2MPa	20.50^{***}±0.79	83.00^{***}±0.54	0.700^{***}±0.000	0.080^{***}±0.002	0.88^{***}±0.03
Junior	Control	215.00±0.04	165.12±0.43	0.394±0.001	0.160±0.002	2.18±0.04
	-0.1MPa	35.00^{***}±0.16	107.00^{***}±0.12	0.069^{***}±0.001	0.078^{***}±0.002	0.88^{***}±0.00
	-0.2MPa	29.05^{***}±0.06	64.00^{***}±0.04	0.070^{***}±0.001	0.060^{***}±0.001	1.16^{***}±0.04
Javor	Control	126.50±0.18	211.50±0.74	0.350±0.002	0.208±0.002	1.69±0.02
	-0.1MPa	27.50^{***}±0.08	62.00^{***}±0.20	0.080^{***}±0.000	0.080^{*}±0.001	0.10^{***}±0.01
	-0.2MPa	11.00^{***}±0.04	53.50^{***}±0.31	0.050±0.001	0.070^{**}±0.001	0.71^{***}±0.01
Dukat	Control	149.52±0.70	210.05±1.03	0.470±0.001	0.310±0.001	1.52±0.00
	-0.1MPa	39.75^{***}±0.53	88.50^{***}±0.11	0.120^{***}±0.004	0.160^{***}±0.000	0.75^{***}±0.00
	-0.2MPa	23.05^{***}±0.18	77.50±0.04	0.091^{***}±0.001	0.131^{***}±0.000	0.69^{***}±0.01
Pionir	Control	210.00±0.63	161.5±1.39	0.240±0.001	0.070±0.000	3.44±0.00
	-0.1MPa	52.50^{***}±0.28	77.02^{***}±0.16	0.070^{***}±0.001	0.040^{***}±0.000	1.60^{*}±0.01
	-0.2MPa	29.50^{***}±0.04	63.00±0.63	0.050^{***}±0.001	0.040^{***}±0.001	1.26^{**}±0.01
Partner	Control	163.50±0.18	183.50±0.12	0.370±0.001	0.330±0.001	1.12±0.00
	-0.1MPa	38.52^{***}±0.02	85.00^{***}±0.07	0.100^{***}±0.001	0.180^{***}±0.001	0.56^{***}±0.00
	-0.2MPa	29.95^{***}±0.73	58.000±0.04	0.080^{***}±0.001	0.010^{***}±0.000	0.81^{***}±0.11
Trezor	Control	153.52±0.06	200.48±0.20	0.360±0.001	0.230±0.000	1.56±0.00
	-0.1MPa	24.92^{***}±0.05	112.48^{***}±0.06	0.070^{***}±0.000	0.130^{***}±0.000	0.54^{***}±0.00
	-0.2MPa	22.98^{***}±0.19	103.48^{***}±0.19	0.070^{***}0.000	0.120^{***}±0.000	0.56^{***}±0.00

*** – $p<0.001$, **- $p<0.01$, * $p<0.05$

(65.1%), and the smallest in the Dukat variety (35.8%). Results of the stress of -0.2 MPa showed that the largest decrease in the shoot dry mass was in the Javor variety (81.7%), and the smallest in the Dukat variety (44.3%).

The average value of the root dry mass in control was from 0.07 g (Pionir) to 0.33 g (Partner), in -0.1 MPa solution from 0.04 g (Pioneer) to 0.18 g (Partner), and in -0.2 MPa solution from 0.04 g (Pionir) to 0.13 g (Dukat). The decrease in control was the largest in the Javor variety (61.6%) and the smallest in the Trevor variety (43.5%). At the stress of -0.2 MPa, the largest decrease in relation to control was in the Partner (69.8%), while the smallest reduction was in the Pionir variety (57%). Comparing to other varieties, the duration of osmotic stress had no large effect on the change in dry weight in the Pionir variety.

In the Dukat variety, only the ratio of dry matter of the shoot and root dropped below 1.00 already at -0.1 MPa, which indicates that this variety, in the first stages of plant exposure to stress, assimilated referring to the root.

Jaleel et al. (2009) pointed out that dry mass accumulation is a desirable characteristic in the case of water deficiency, while Sakthivelu et al. (2008) claim that the dry mass is in correlation with tolerance to drought.

Table 2. Tolerance index (TI) of the shoot and root of the seedlings of field pea at different levels of osmotic stress.

Variety	Treatment (PEG)	Shoot TI	Root TI
Mraz	Control	1.00	1.00
	-0.1 MPa	0.35*	0.47*
	-0.2 MPa	0.24*	0.47*
Junior	Control	1.00	1.00
	-0.1 MPa	0.20*	0.49*
	-0.2 MPa	0.20*	0.38*
Javor	Control	1.00	1.00
	-0.1 MPa	0.23*	0.38*
	-0.2 MPa	0.14*	0.33*
Dukat	Control	1.00	1.00
	-0.1 MPa	0.26*	0.52*
	-0.2 MPa	0.19*	0.42*
Pionir	Control	1.00	1.00
	-0.1 MPa	0.29*	0.57*
	-0.2 MPa	0.21*	0.57*
Partner	Control	1.00	1.00
	-0.1 MPa	0.27*	0.41*
	-0.2 MPa	0.22*	0.26*
Trezor	Control	1.00	1.00
	-0.1 MPa	0.19*	0.43*
	-0.2 MPa	0.19*	0.32*

* $p<0.05$

Based on dry mass data of shoot and root of the seedlings in the control and treated group, values of tolerance index on drought, as well as other stress factors, could be calculated (Maiti et al., 1994). The tolerance index (TI) in this study is calculated for shoot and root separately, for each level of stress (Table 2).

There was a statistically significant downward trend in the value of TI of the shoot, which points to the reduced tolerance of the tested varieties to enhanced and prolonged osmotic stress (Table 2). TI at the lowest level of osmotic stress was from 0.56 (Trezor) to 0.47 (Mraz). At -0.2 MPa TI was between 0.14 (Javor) and 0.24 (Mraz). The largest decrease in TI of the shoot at osmotic stress of -0.1 MPa was in the Mraz variety (65%) and the smallest in the Dukat variety (35.5%). In stress conditions of -0.2 MPa, the largest decrease was found in the Javor variety (82%) and the smallest in the Dukat variety (44%).

The values of TI of the seedling roots (Table 2) also showed a downward trend like in the shoot. In the -0.1 MP solution, the TI was between 0.38 (Javor) and 0.56 (Trezor), while in osmotic stress of -0.2 MPa it was between 0.30 (Partner) and 0.57 (Pionir). The largest decrease in the TI at the osmotic stress of -0.1 MPa was in the Javor variety (62%) and the smallest in the Trezor variety (43.8%). In the case of the stress of -0.2 MPa, the largest decrease was observed in the varieties Pionir and Partner (70%) and the smallest in the Trezor variety (47.8%).

Maiti et al. (1994) concluded that the stress conditions have a greater impact on the IT of the shoot of the plant than on the roots. The decrease in tolerance index, under the influence of osmotic stress caused by NaCl, was determined by Dang et al. (2011).

Comparison of the values of the tolerance index of the shoot and root indicates that the values of the root tolerance index are significantly higher than the values for the shoot, at all levels of stress. The values obtained for the root under the influence of stress are closer to the control values compared to the values for the shoot, which indicates a lesser impact of osmotic stress on the dry mass accumulation in the root comparing to the shoot. Taking into account the obtained values of the index of tolerance of the shoot and root at all levels of stress and mutual comparison of varieties, it can be said that the Mraz variety is in the group of tolerant varieties, along with the Dukat and Pionir varieties. Trezor and Partner belong to a group of medium tolerant varieties, while Junior and Javor are the least tolerant varieties.

CONCLUSION

The obtained results show that the reaction to osmotic stress at low osmotic potential can be tested in different pea plants, based on simple methods such as germination percentage and seedling growth rate. Seed germination at -0.1 to -0.2 MPa appears to be a good selection criterion for field pea varieties to be planted in arid or semi-arid areas.

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УТИЦАЈ СТРЕСА СУШЕ НА КЛИЈАЊЕ И ПОРАСТ ПОНИКА РАЗЛИЧИТИХ ГЕНОТИПОВА СТОЧНОГ ГРАШКА

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РЕЗИМЕ: У раду је проучавано клијање и пораст поника седам генотипова сточног грашка (*Pisum sativum* L.) у раствору PEG-6000 са вредностима осмотских потенцијала од -0,1 и -0,2 MPa. Испитивање је извршено како би се утврдио утицај различитих осмотских потенцијала (MPa) на проценат клијавости семена (GP) и просечно време клијања (MGT). Резултати показују да се проценат клијавости смањивао упоредо са смањењем осмотског потенцијала, док се просечно време клијања повећавало. Сорта „Јавор” много је осетљивија од осталих сорти при свим нивоима осмотског стреса. Супротно томе, најмањи утицај на клијање и MGT утврђен је код сорти „Мраз” и „Трезор”. Одређивање клијавости семена у растворима PEG од -0,1 до -0,2 MPa може се користити за тестирање сточног грашка. Изложеност осмотском стресу и његово трајање значајно су утицали на раст поника (изданак и корен) и акумулацију биомасе, при чему је његов утицај био израженији на раст изданка него на раст корена, што је потврдио и индекс толерантности корена.

КЉУЧНЕ РЕЧИ: *Pisum sativum*, семе, клијанац, сорте, индекс толерантности