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Original scientific paper

COMPARATIVE STUDY OF DROUGHT AND SALT STRESS EFFECTS ON GERMINATION AND SEEDLING GROWTH OF PEA

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Seed germination is first critical and the most sensitive stage in the life cycle of plants compromise the seedlings establishment. Salt and drought tolerance testing in initial stages of plant development is of vital importance, because the seed with more rapid germination under salt or water deficit conditions may be expected to achieve a rapid seedling establishment, resulting in higher yields. The aim of this study was to determine whether the pea seed germination and seedling growth were inhibited by the salt toxicity and osmotic effect during the seedling development, and also identification of the sensitive seedling growth parameters in response to those stresses. Based on the obtained results, pea has been presented to be more tolerant to salt than water stress during germination and early embryo growth. Investigated cultivars showed greater susceptibility to both abiotic stresses when it comes growth parameters compared to seed germination.

Key words: abiotic stress, *Pisum sativum*, NaCl, PEG

INTRODUCTION

Pea belongs to the Leguminosae family which represent the second most important family of crop plants after Poaceae, accounting for approximately 27% of the world's crop production (GRAHAM and VANCE, 2003). Nowadays, legumes provide one-third of the entire amount of protein for human consumption, representing an important source of fodder and forage for animals and in the production of edible and industrial oils (SMYKAL *et al*., 2012). Besides

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nutritional role of fiels peas, they are also useful as rotation crops that improve soil fertility through nitrogen fixation. This is a relationship between field pea plants and nitrogen-fixing bacteria (Rhizobium) in which atmospheric nitrogen (N_2) is reduced to plant available ammonium (NH_3) (HELDT and PIECHULLA, 2011). Field pea, like other pulses, is comparatively sensitive to a number of abiotic stress factors, particularly involving soil nutrition such as salinity and alkaline-induced boron toxicity, reproductive frost damage, heat stress and water deficit (DITA *et al*., 2006).

During their growth crop plants are usually exposed to different environmental stresses which may limit their growth and productivity (MOUD and MAGSHOUDI, 2008; VUJAKOVIĆ *et al*., 2011). Salinity and drought are major environmental factors limiting plant growth and productivity, causing great economic losses (JOVIČIĆ *et al.*, 2014). Those two abiotic factors affect plants in a similar manner, primarily due to reduced water potential. In order to overcome changes in environmental conditions and ensure survival, plants have evolved a number of complex protective mechanisms (MAHAJAN and TUTEJA, 2005).

It has long been known that seeds exposed to unfavorable environmental conditions, like salinity or water stress, germinate difficulty. Seed germination is first critical and the most sensitive stage in the life cycle of plants compromise the seedlings establishment (KOLB and BARSCH, 2010; KARAGIĆ *et al.*, 2010). Drought and salinity play an important role both in determining the germination rate and the development of seedlings (KALEFETOGLU MACAR *et al*., 2009). Salt and drought tolerance testing in initial stages of plant development is of vital importance, because the seed with more rapid germination under salt or water deficit conditions may be expected to achieve a rapid seedling establishment, resulting in higher yields. Considering that pea as other legumes, requires relatively large amounts of water during the germination process, it is suggested that this is the most critical phase of development stages. Furthermore, uptake of such large quantities of water implies/leads to the enhanced absorption of salt if their concentration in the soil solution is increased. Polyethylene glycol (PEG 6000) has been used to simulate water-stress conditions. PEG is an inert osmotic agent whose molecules are too large to penetrate into seed, thus preventing any toxic effects. Because PEG does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of low–Mr osmotica, which infiltrate the cell wall with (VERSLUES *et al*., 2006).

It has not fully been clarified to which extent seed germination is affected by salinity through osmotic effect and to which extent through ion toxicity. Although each of these stresses differ according to the mode of action and provoke different responses of plants, they can cause a common response in plants which leads to stress tolerance of affected plants (TUTEJA, 2007).

The aim of this study was to determine whether the pea seed germination and seedling growth were inhibited by the salt toxicity and osmotic effect during the seedling development, and also identification of the sensitive seedling growth parameters in response to those stresses.

MATERIAL AND METHODS

Seeds of two pea cultivar (Dukat, Partner), produced in Institute of Field and Vegetables Crops, Serbia, were used in this investigation. Seeds were sterilized with 0.5% sodium hypochlorite solution for 1 min and after that they were washed with distilled water. Seeds of pea were germinated in 15 x 24 cm plastic boxes fullfilled with sand. For each cultivar 4 replicates by 100 seeds for each treatment was sown.

Salinity stress was stimulated by 3 different concentrations of NaCl solution (50 mmol/l, 100 mmol/l, 150 mmol/l) and drought stress was stimulated by two different osmotic potential levels (-0.2 MPa, -0.3 MPa) using Polyethylene Glycol 6000 – PEG 6000.

Plastic boxes were placed in germination chamber at 20°C under conditions of a 12-h light/dark cycle. The sand was moistened daily with destilled water for control and the solution of NaCl and PEG for treatments.

Seed germination was determined after 8 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. Typical or normal seedlings have good following structures: primary root, straight and intact hypocotyl, healthy and whole cotyledons and the beginnings of the first true leaves (ISTA, 2014). The radicle and hypocotyl length (mm) and the seedling fresh and dry weights (g) were measured on 10th and 15th day after sowing. Dry weights of hypocotyl and radicle were measured after drying samples in oven at 80°C for 24h, to accuracy of 0.1 mg using an analytical balance, until a constant weight was achieved. The results were expressed as total mass of ten shoots (g).

Differences among treatments and genotypes, as well as interaction between these variables, were tested using Statistica softer, Version 12. Averages of the main effects and their interactions were compared using the Duncan's multiple range at 5% level of probability.

RESULTS AND DISCUSSION

The present research studied the changes in germination characteristics and early embryo growth of two cultivars of pea exposed to a reduced water potential in PEG or NaCl solution in laboratory conditions. A similar approach was used to study the comparative effects of water and salt stress on seed germination in cowpea (MURILLO-AMADOR *et al*., 2002), sunflower (KAYA *et al*., 2006), sweet sorghum (PATANE *et al*., 2013) and pea (JOVIČIĆ *et al.*, 2013).

MUSCOLO *et al*. (2014) used 10, 15, 18 and 21% of PEG (MW 6000) concentration corresponding to final osmotic potentials of -0.30, -0.51, -0.58, and -0.80 MPa, respectively. We used PEG corresponding to final osmotic potentials of -0.20 and -0.30 MPa in order to obtain osmotic potentials comparable to that of NaCl at the concentrations of 50, 100 and 150 mM, as SIDARI *et al*. (2008).

The results show the impact of water and salt stress as a significant reduction in the percentage of germination in both cultivars. It was reported that PEG and NaCl decreased germination in pea, but is more affected with PEG than NaCl (OKCU *et al*., 2005; JOVIČIĆ *et al*., 2013). A significant reduction in germination rate was observed at higher concentrations of NaCl (100 mM), while in the experiments with the decrease of water stress reduction is observed even at the lowest concentrations of PEG (-0.2 MPa). The observed reduction in the percentage of germination under stress conditions in both cultivars may be due to a reduction in osmotic potential substrates for germination, which reduces the amount of water available for absorption as a consequence of the mechanism of action of both the above mentioned abiotic factors (OKCU *et al*., 2005). PEG molecules affect on the germination and seedling growth, preventing the entry of water molecules in plant tissues, causing physiological drought (KALAFETOGLU MACAR *et al*., 2009). In some studies it has been shown that osmotic stress induced by PEG can cause storage compounds hydrolysis which results in a further decreasing of the internal osmotic potentials of the seed (AFZALI *et al*., 2006). KAYA *et al*. (2006) showed that PEG did not have a toxic effect in sunflower seeds. As the stress was removed, all seeds germinated. At the same level of water potential, the results shows that the pea seeds more efficiently adapts to salinity stress. The reason for this may be an assumption that the absorbed Na^+ ions lower the osmotic potential of the seed and thereby facilitate the absorption of water.

On the other hand, adverse effect of salt stress on seed germination can be attributed to osmotic stress and specific ion toxicity (FAHEED *et al*., 2005; JOVIČIĆ *et al*., 2014). NaCl can directly affect the growth of the embryo which may adversely reflect on the process of seed germination (AL-TAISAN, 2010; JOVIČIĆ *et al*., 2012). The ions Na^+ and Cl^- infiltrate into plant cells wherein cell of tolerant plants or cultivar the ability to compartmentalize these ions in the vacuole or in the cytoplasm for sensitive cultivars (KEFU *et al*., 2003). Also, NaCl may cause inhibitons of the activities of some enzymes that may play critical roles in seed germination (AL-TAISAN, 2010). The second but not less important adverse effect of NaCl is its ability to increase the osmotic potential of the growing media aggravating normal seed imbibition.

Although, NaCl causes osmotic stress by reducing the amount of available water, and at the same time the toxicity of Na and Cl ions, in this study saline stress had a lesser effect on the germination of seeds than hydric stress simulated with PEG at the same osmotic potentials. Also, Na+ ions accumulated in the seed embryo create the difference in water potential between the substrate and the seed, allowing water uptake during germination (SHITOLE and DHUMAL, 2012). Same results were found in *Vigna unguiculata* (MURILLO-AMADOR *et al*., 2002) and in *Solanum melongena* seeds (DEMIR *et al*., 2003).

Table 1. Mean comparisons of seed germination (Duncan test, P <0.05)

		After 10 days	
Cultivars		**Dukat**	**Partner**
Control		97^a	87^b
PEG	**0.2 PEG**	83^{bc}	83^{bc}
	0.3 PEG	79^c	82^c
NaCl	**50 NaCl**	94^a	82^c
	100 NaCl	83^{bc}	71^d
	150 NaCl	81^c	69^d

[#]Means followed by the same letter are not significantly different. Means are comnpared by Duncan test

Results of this study show that the salinity and drought, in addition to germination, had a negative impact on early seedling growth in *P. sativum*. The negative effects of water and salt stress on the shoot and root growth of early seedling was much more than on the germination of pea seeds. In addition, a significant reduction in the increase in stress conditions was observed after 10 days compared to 15 days. In contrast to the germination, in the length of shoots and roots a significant reduction was observed at the lowest level of stress, as well in PEG as in NaCl, indicating serious damage to the plant cells and plant suffering. In cultivar Partner, the percentage of reduction in both types of stress was lower after day 15 compared to day 10, indicating a certain degree of adaptation to stressful conditions, which was not observed in cultivar Dukat. MUNNS and TESTER (2008) considered that a reduction of growth in terms of increased salt content in the initial stage of development is the consequences of an osmotic potential induced by salt, while in the later stages the toxic effect of Na^+ ions, which accumulate in leaves, is manifesting and reduced plant growth. This observation may explain the lack of difference between the impact of salinity and drought in pea during germination and initial stages of growth after 10 days.

The inhibiting effect on seed germination and following embryo growth was stronger in PEG than in NaCl solution at the equivalent water potential. Indeed, both cultivars of pea were able to well germinate even at the highest level of NaCl stress (150mM) (Table 1), although root and shoot growth was slowed down (Table 2). These results indicate a stronger effect of stress in PEG than in salt. Similar effects were observed in seed germination and shoot and root growth of sunflower by KAYA *et al*. (2006), who suggested an osmotic effect of PEG rather than an ion accumulation, because when PEG stress was removed, the seeds were able to germinate.

Table 2. Mean comparisons of shoot and root lenght (Duncan test, P <0.05)

		Shoot fresh lenght				**Root fresh lenght**			
		After 10 days		**After 15 days**		**After 10 days**		**After 15 days**	
Cultivars		**Dukat**	**Partner**	**Dukat**	**Partner**	**Dukat**	**Partner**	**Dukat**	**Partner**
Control		149,53$^{b\#}$	162,75^{a}	248,00^{a}	252,50^{a}	210,05^{a}	183,50^{b}	219,00^{a}	204,23^{b}
PEG	**0.2 PEG**	23,05^{h}	29,95^{g}	43,03^{e}	64,03^{d}	77,50^{f}	58,00^{g}	62,98^{g}	80,53^{f}
	0.3 PEG	19,48^{h}	19,03^{h}	37,00^{f}	23,48^{g}	55,00^{g}	52,93^{g}	64,50^{g}	76,53^{fg}
NaCl	**50 NaCl**	96,63^{c}	96,00^{d}	171,25^{b}	163,38^{b}	183,38^{b}	173,88^{c}	191,75^{b}	196,25^{b}
	100 NaCl	64,00^{e}	73,38^{d}	142,13^{c}	136,38^{c}	146,88^{d}	142,13^{d}	165,50^{c}	158,7^{c}
	150 NaCl	33,63^{g}	34,00^{g}	62,88^{d}	70,25^{d}	122,13^{e}	81,13^{f}	139,75^{d}	112,25^{e}

[#]Means followed by the same letter are not significantly different. Means are comnpared by Duncan test

The root of the seedling was greater after 10 days and after 15 days of salinity stress conditions, indicating that the PEG significantly influenced the reduction of cell division and cell elongation. It can also be concluded that seed size and quality contributes vital role to cope with drought stress. The same conclusion was made by KUMAR *et al*. (2011) examining the seeds of *Cajanus cajan.* Anyway, PEG induced osmotic stress caused more growth inhibition as compared to NaCl induced osmotic stress (Table 2). When it comes to osmotic stress induced by PEG, the reduction in shoot and root growth is important due to PEG affects root volume and root length.

In our experiment, root dry weight was reduced by osmotic stress; however, the decreased values of ratio between root dimensions (length to dry weight) in PEG (Table 4) indicate that the osmotic stress affects root elongation more than dry mass accumuation, and root becomes progressively thicker as an adaptive response to water stress. Moreover, root water content progressively declined with the decrease in water potential (Table 3), suggesting that, although smaller, root tissue became more concentrated. This phenomenon did not occur in NaCl solution.

Examining wheat seed, MOUD and MAGHSOUDI (2008) indicate that more respiration rate occure in more vigorous seedlings under salt stress condition, which may increase the degree of crop establishment.

Table 3. Mean comparisons of shoot and root fresh weight (Duncan test, P <0.05)

		Shoot fresh weight				**Root fresh weight**			
		After 10 days		**After 15 days**		**After 10 days**		**After 15 days**	
Cultivars		**Dukat**	**Partner**	**Dukat**	**Partner**	**Dukat**	**Partner**	**Dukat**	**Partner**
Control		$6,71^{a}$	$5,49^{c}$	$5,73^{c}$	$10,22^{a}$	$6,35^{a}$	$4,65^{c}$	$5,73^{b}$	$6,26^{a}$
PEG	**0.2 PEG**	$0,52^{j}$	$0,73^{j}$	$1,11^{g}$	$1,49^{g}$	$1,15^{h}$	$0,85^{i}$	$2,76^{e}$	$1,60^{h}$
	0.3 PEG	$0,69^{i}$	$0,44^{i}$	$1,36^{g}$	$0,64^{h}$	$0,63^{i}$	$0,66^{i}$	$1,11^{i}$	$1,24^{i}$
NaCl	**50 NaCl**	$6,41^{b}$	$4,81^{d}$	$5,61^{c}$	$6,28^{b}$	$5,84^{b}$	$3,81^{d}$	$5,16^{c}$	$5,13^{c}$
	100 NaCl	$3,80^{e}$	$2,91^{f}$	$3,48^{e}$	$4,42^{d}$	$3,67^{d}$	$2,88^{e}$	$3,77^{d}$	$3,71^{d}$
	150 NaCl	$2,78^{g}$	$1,65^{h}$	$3,05^{f}$	$3,04^{f}$	$2,58^{f}$	$2,21^{g}$	$2,14^{g}$	$2,45^{ef}$

[#]Means followed by the same letter are not significantly different. Means are comnpared by Duncan test

Table 4. Mean comparisons of shoot and root dry weight (Duncan test, P <0.05)

		Shoot dry weight				**Root dry weight**			
		After 10 days		**After 15 days**		**After 10 days**		**After 15 days**	
Cultivars		**Dukat**	**Partner**	**Dukat**	**Partner**	**Dukat**	**Partner**	**Dukat**	**Partner**
Control		$0,470^{a}$	$0,387^{b}$	$1,060^{a}$	$0,640^{b}$	$0,310^{b}$	$0,330^{a}$	$0,740^{a}$	$0,320^{d}$
PEG	**0.2 PEG**	$0,091^{g}$	$0,080^{gh}$	$0,590^{c}$	$0,170^{g}$	$0,131^{d}$	$0,100^{e}$	$0,570^{b}$	$0,211^{g}$
	0.3 PEG	$0,080^{gh}$	$0,060^{h}$	$0,101^{h}$	$0,080^{h}$	$0,080^{f}$	$0,080^{f}$	$0,120^{g}$	$0,160^{g}$
NaCl	**50 NaCl**	$0,226^{c}$	$0,201^{c}$	$0,572^{c}$	$0,568^{c}$	$0,158^{c}$	$0,166^{c}$	$0,419^{c}$	$0,299^{d}$
	100 NaCl	$0,201^{c}$	$0,172^{d}$	$0,524^{d}$	$0,375^{e}$	$0,141^{d}$	$0,154^{c}$	$0,308^{d}$	$0,197^{e}$
	150 NaCl	$0,182^{cd}$	$0,149^{e}$	$0,404^{e}$	$0,283^{f}$	$0,103^{e}$	$0,104^{e}$	$0,217^{e}$	$0,220^{e}$

[#]Means followed by the same letter are not significantly different. Means are comnpared by Duncan test

At equivalent level of stress, NaCl proved more harmful to germination, seedling growth, vigour index, as well as initial mobilizing efficiency of food material from seed to the growing seedling, while PEG-6000 was more harmful to imbibition rate and mobilization efficiency in further days.

CONCLUSION

Based on the obtained results, pea has been presented to be more tolerant to salt than water stress during germination and early embryo growth. Investigated cultivars showed greater susceptibility to both abiotic stresses when it comes to growth parameters compared to seed germination.

Knowing the difference in tolerance between species and genotypes of special importance to ecological and economic aspects, allowing proper agricultural production in regions that are facing this problem. Since breeders are still searching for characteristics that are appropriate for screening pea germplasm for traits that are responsible for the survival of plants under stress conditions of salinity and drought, this research is of great importance for obtaining additional information.

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UPOREDNO ISPITIVANJE EFEKATA SUŠE I ZASLANJENOSTI NA KLIJANJE I RAST PONIKA GRAŠKA

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Izvod

Klijanje semena je najkritičniji i najosetljiviji stadijum životnog ciklusa biljaka koji može ugroziti razvoj biljaka. Ispitivanje tolerantnosti na zaslanjenost i sušu u početnim stadijumima razvoja biljaka je od velikog značaja zbog toga što se od semena koja brže klijaju u uslovima zaslanjenosti i vodenog deficita može očekivati da postignu brzi porast ponika, rezultujući većim prinosima. Cilj ovog rada je bio da se utvrdi da li je klijanje semena i porast ponika graška inhibiran uticajem soli i efektom osmotskog stresa za vreme razvoja ponika, kao i identifikacija parametara osetljivosti porasta ponika kao odgovor na te stresne uticaje. Na osnovu dobijenih rezultata, utvrđeno je da je grašak tolerantniji na soni stres nego na stres suše za vreme klijanja i ranog porasta embriona. Ispitivane sorte su pokazale veću osetljivost na oba abiotička stresa kada su u pitanju parametri porasta ponika u poređenju sa klijanjem semena.

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