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IDENTIFICATION OF SOURCES OF TOLERANCE TO SALINITY STRESS IN PEAS (*PISUM SATIVUM* L.)

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Abstract

Salinity stress has become an important limiting factor for plant growth and productivity. To evaluate the effect of salinity on plant and root development three pea genotypes were grown under different concentrations of NaCl (50, 100 and 200 mM NaCl) in an *in vitro* and *in vivo* conditions. After two weeks of cultivation in culture medium the seedling emergence rate was not significantly affected by 50 mM NaCl. In culture medium containing 200 mM NaCl only in variety Prometei over 50% of the seeds developed to the plants. The data indicated that increasing NaCl level significantly reduced shoot and root lengths, and plant fresh weight. Six weeks (42 days) after sowing *in vivo*, a reduction in plant height and weight was observed at 200 mM NaCl concentration. A proven differences of the root traits were established only in variety Prometei. On the bases of the obtained results it can be concluded that variety Prometei was the most tolerant to applied salt stress.

Keywords: garden pea, abiotic stress, NaCl, seedling emergence, *in vitro* and *in vivo* culture.

INTRODUCTION

Salinity is one of the major abiotic stress that massively restricts plant growth and productivity (Joshi et al., 2009). In Bulgaria about 33 000 ha are affected by salinity mainly in areas with intensive agriculture (Stoeva and Kaymakanova, 2015).

Pea is one of the most tolerant to salinity leguminous seed crop (Subbarao and Johansen, 1994). Nevertheless degree of salinity level affects the yield decreases especially during vegetative and reproductive phases (Najafi et al., 2007). Salinity has devastating impacts on plants by affecting germination and growth, reproductive stage and the ability of biological nitrogen fixation in grain legumes (Van Hoorn et al., 2001). Some changes in photosynthetic activities are also a reason to reduced plant growth and yield (Jamil et al., 2007). Plants developed a number of mechanisms to overcome the impact of salt stress, which ultimately leads to their tolerance. An important role in this process is the choice of a variety that gives a stable yield under unfavorable conditions. There is an immense need to develop new legume varieties suitable for grown at the high soil salinity (Nadeem et al., 2019).

In vitro screening has been used as an effective method for rapid evaluation of a large number of genotypes to salt stress in different stages of plant growth (Queiros et al., 2007, Piwowarczyk et al., 2016). There are data on using of *in vitro* technique for selection of salt tolerant pea lines and genotypes (El Sayed and El Sayd, 2011, Miljuš-Djukić et al., 2013) but also for studying the effect of salinity on seed germination, growth and development of garden pea seedlings and plants (Piwowarczyk et al., 2016, Grozeva et al., 2019).

The aim of this study was to investigate changes of plant growth and development of three pea genotypes in different salt levels in *in vitro* and *in vivo* conditions.

MATERIAL AND METHODS

The experiment included three garden pea genotypes from the collection of the Maritsa Vegetable Crops Research Institute, Plovdiv. These are:

- variety Prometei - old local cultivar from the late maturity group with vegetation period 49-50 days. Grains are dark green, average number 6-7 per pod. Approximately weight per 1000 seeds is 142 g.

- variety Marsi - new Maritsa VCRI cultivar from the mid-late maturity group with vegetation period 69-70 days. Grains are dark green, average number 8-10 per pod. Approximately weight per 1000 seeds is 190 g.

- breeding line 1/17 created at Maritsa VCRI from the mid-late maturity group with vegetation period 67-69 days. Grains are green, average number 7-8 per pod. Approximately weight per 1000 seeds is 135 g.

In vitro treatment

Dry, healthy seeds with uniform size of the three studied pea genotypes, were surface sterilized by soaked in 5% Calcium hypochlorite for one hour, rinsed three times in sterile distilled water and sown on basal medium containing macro and microsalts by Murashige and Skoog (1962), 20 gL^{-1} Sucrose and 7 gL^{-1} Agar. Salinity stress was induced by adding of Sodium chloride (NaCl) in basal medium at three concentrations 50, 100 and 200 mM and control (without NaCl). The culture vessels with seeds were incubated in a growth chamber at 25ºC ± 1ºC temperature, a photosynthetic proton flux density (PPFD) of 200 μmol m^{-2} s^{-1} and 16/8 h photoperiod. The experiment was repeated two-times in three replications each, with 5 seeds per replication for each genotype and treatment. Germination rate was determined after 7 days of seeds cultivation while seedling emergency was recorded after 14 days. The shoot and root length (mm) and shoot weight (g) of 14-days-old seedling were also measured.

***In vivo* treatment**

The study was conducted during the spring season under glasshouse conditions at the Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria. Ten seeds per genotype were sown in equal distance at 2 cm depth in pots contained 4 L mixture peat moss and perlite in the ratio 1:1 (v/v). Initially pots were irrigated with tap water. After seeds germination a modified Hoagland's nutrient solution was used for irrigation (1 mM $Ca(NO_3)_2$; 1 mM KNO_3; 0.4 mM KH_2PO_4; 0.4 mM $MgSO_4$; 17.9 μM FeEDTA; 4.6 μM H_3BO_3; 0.9 μM $MnCl_2$; 0.08 μM $ZnCl_2$; 0.03 μM $CuCl_2$). Modification was made to induce salinity stress by supplementing the nutrient solution with 0 (control), 50, 100 and 200 mM NaCl. First and second treatments were made with ½ strengths solutions. During the vegetation period a total of eight treatments with 200 mL saline solutions per pot were made. Electrical conductivity (EC) of freshly prepared solutions were determined (Table 1). The experiment was conducted in three replications with 10 plants per replication. At plant maturity stage the following characteristics were measured: plant length (cm), plant fresh weight (g), root length (cm) and root fresh weight (g).

The results were presented as means ± standard deviation (SD). Duncan's multiple range test was used (SPSS software) to compare means. The percentage of characteristics decrease compared to non-treated control (T-C%) was also calculated.

Table 1. Salt concentration EC, mS cm^{-1}

Nutrient solution, NaCl, mM	Number of treatment, EC, mS cm^{-1}							
	1^{st} tr	2^{nd} tr	3^{rd} tr	4^{th} tr	5^{th} tr	6^{th} tr	7^{th} tr	8^{th} tr
0	1.45	1.45	2.35	2.38	2.21	2.21	2.33	2.33
50	4.09	4.09	7.45	7.56	7.37	7.37	7.47	7.47
100	6.45	6.45	12.08	12.40	12.26	12.26	12.36	12.36
200	11.64	11.64	21.70	21.60	21.60	21.60	21.70	21.70

RESULTS AND DISCISSION

In vitro conditions

In *in vitro* conditions the germination and emergency rates were not remarkably affected by lower salt concentrations especially for varieties Prometei and Marsi (Table 2). The highest decrease of seed germination was observed in culture medium containing 200 mM NaCl. In this salt concentration the percentage of germination seeds varied from 13.3% to 70.0% depending on the genotypes. Seedling emergency rate was significantly inhibited at the highest level of salt in three tested genotypes. In culture medium containing 200 mM NaCl over 50% of the seeds developed to plants only in variety Prometei while on the line 1/17 this percentage was 10%. The results of many studies of grain legumes indicated that the stage of seedling emergence was more sensitive to salinity than seed germination (Kaya et al., 2008; Piwowarczyk et al., 2016). The same results were obtained by other authors who emphasize that the level of seedlings emergence on salinity caused by NaCl concentration more than 150 mM rarely exceed 50% and in some genotypes fell to 0 (Mahdavi and Sanavy, 2007; Piwowarczyk et al., 2016). The negative impact of salinity on germination and seedling development is a result of low osmotic potential of the soil solution and toxic effect of Na^+ and Cl^- ions (Taiz and Zeiger, 2010).

Table 2. *In vitro* seed germination and development of three garden pea genotypes under salt stress

Genotype	NaCl, mM	Seed			
		germination %	±SD	emergency %	±SD
Prometei	0	93.3^{ab}	10.3	93.3^{ab}	10.3
	50	96.7^{a}	8.2	93.3^{ab}	10.3
	100	86.7^{abc}	10.3	83.3^{abc}	8.2
	200	$70.0c^{d}$	16.7	53.3^{ef}	16.3
Marsi	0	90.0^{ab}	11.0	83.3^{abc}	8.2
	50	86.7^{abc}	10.3	66.7^{cde}	20.7
	100	76.7^{bc}	23.4	73.3^{bcd}	24.2
	200	40.0^{e}	0.0	30.0^{g}	11.0
Line 1/17	0	100.0^{a}	0.0	96.7^{a}	8.2
	50	70.0^{cd}	21.0	56.7^{de}	23.4
	100	53.3^{de}	24.2	36.7^{fg}	19.7
	200	13.3^{f}	20.7	10.0^{h}	16.7

The most typical symptom of salinity impact on plant is growth reduction through inhibition of cell elongation due to turgor decrease (Manchanda and Garg, 2008). The data reported in Table 3, clearly indicated that with increasing NaCl concentration the plants growth was inhibited.

Table 3. Morphology evaluation of 14 day-old pea seedlings under salinity stress

Genotype	NaCl (mM)	Shoot length (mm)	±SD	Root length (mm)	±SD	Shoot weight (mg)	±SD
Prometei	0	63.3^{a}	5.3	137.8^{a}	15.7	892.1^{a}	91.4
	50	49.2^{ab}	4.9	116.7^{ab}	8.6	793.4^{a}	166.3
	100	36.7^{c}	4.8	83.0^{c}	18.4	524.0^{b}	109.5
	200	11.8^{ef}	2.5	9.3^{fg}	3.7	103.1^{c}	23.5
Marsi	0	42.3^{bc}	12.0	60.8d	16.4	459.6^{b}	166.1
	50	24.5^{d}	7.5	41.8^{de}	14.5	206.7^{c}	96.2
	100	13.5^{e}	6.3	19.3^{efg}	8.5	122.6^{c}	59.9
	200	6.0^{ef}	0.6	12.0^{fg}	3.5	93.9^{c}	12.4
Line 1/17	0	59.0^{a}	10.3	97.8^{bc}	34.7	777.5^{a}	265.6
	50	49.0^{b}	9.9	88.7^{c}	37.0	602.3^{b}	227.7
	100	23.7^{d}	13.4	36.2^{ef}	11.8	191.4^{c}	120.8
	200	3.0^{f}	5.0	4.0^{g}	6.2	34.0^{c}	55.1

Increasing NaCl concentration has led to reduction of shoot length: between 22 and 81% in variety Prometei, between 42 and 86% in variety Marsi and 17 and 96% in line1/17 with (Fig.1 a). The length of roots was reduced by 9-33%, 39-68% and approximately 80-96% on the media supplemented with 50, 100 and 200 mM NaCl, respectively (Fig. 1 b). After 14 days of cultivation, salinity induced by 50 mM

NaCl reduced the shoot weight by more than 50% in variety Marsi. In variety Prometei and line 1/17 the lowest NaCl concentration did not inhibit significantly shoot growth. The highest percentage of decrease in a shoot weight was registered in culture medium with 200 mM NaCl (from 79% to 95%) (Fig. 1 c).

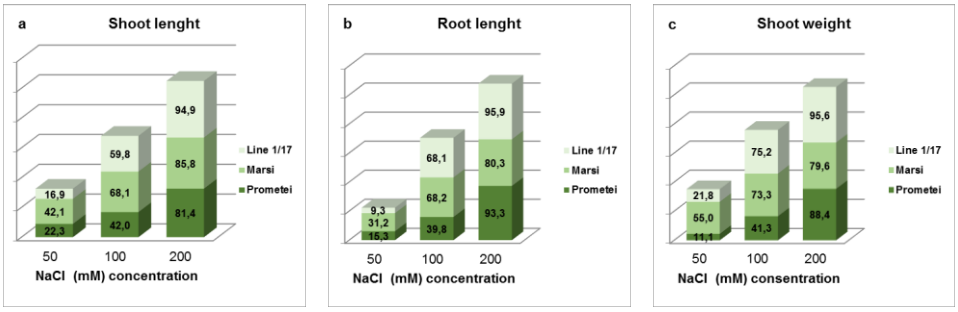

Fig. 1. Reduction of shoot length, root length, and shoot weigh of 14 day-old garden pea seedlings under salinity stress, %

***In vivo* conditions**

Data presented in Fig. 2 showed, that *in vivo* germination response is affected by the applied salt concentrations in the nutrient solution by different degrees, depending on the genotype. In variety Prometei and line 1/17 increasing the salt concentration has led to decreased seed germination rate from 76.67% (50 mM) to 50% (200 mM). In variety Marsi with increasing salt concentration, the percentage of germinated seeds increases from 33.33% (50 mM) to 60% (200 mM). Our results confirm the findings of Okcu et al. (2005), who observed that NaCl had a lesser impact on the seed germination and in some genotypes had a stimulation effect.

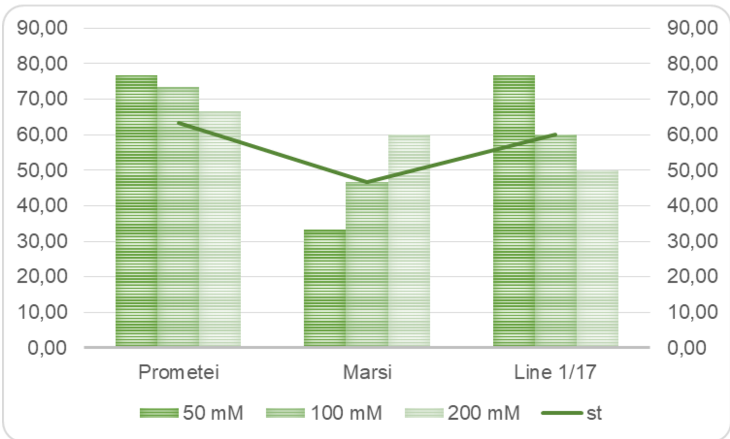

Fig. 2. Germinated (sprouted) seeds on the 21st day after sowing, %

The growing number of reports showed that salt stress has an adverse effect on some plant growth parameters. The decrease is greater at higher NaCl concentrations (Hammad et al., 2017; Nadeem et al., 2019). The results of present study indicated a stimulating effect on plant length and fresh weight at low salt concentration (50 mM), except for variety Marsi (Fig. 3). Root length and weight values increased at high doses of salinity (200 mM). The root length varied from 30.9 cm to 42.4 cm and the root weight was between 1.1 and 1.3 g depending on the genotype. This non-linear dose dependence of root parameters is associated with increased tissue hydration under salinity conditions (Topalova, 2020). Salt stress is first perceived by root system and impairs plant growth by inducing osmotic stress caused by reduced water availability leading to a reduction growth in plants at the expense of root growth (Huez-Lopez et al., 2011, Topalova, 2020).

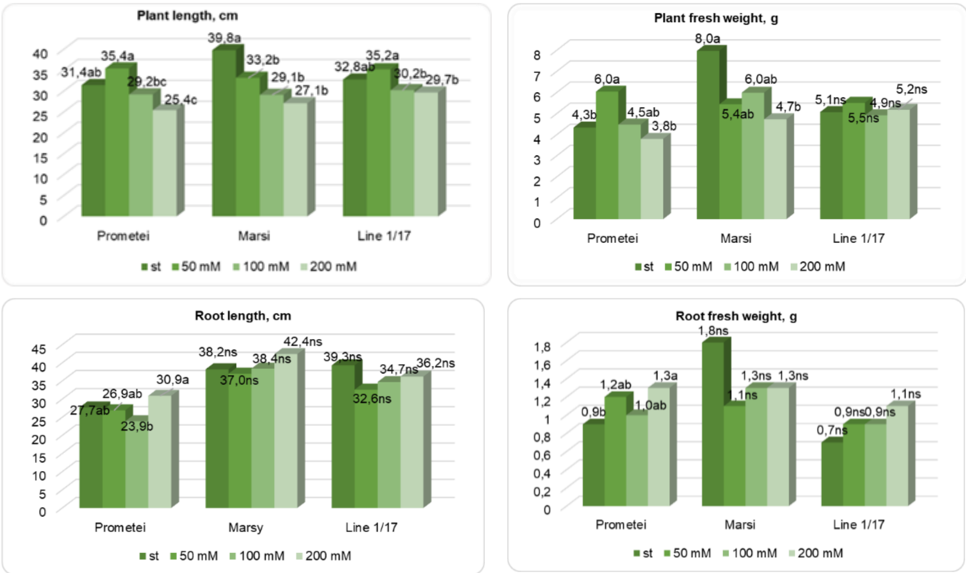

Fig. 3. Morphology evaluation of *in vivo* grown plants of three garden pea cultivars under salinity stress, 2020

CONCLUSIONS

In *in vitro* conditions seedling growth stages were more sensitive to salinity than seed germination. The 100 mM NaCl concentration can be used as a suitable for evaluation of salt stress tolerance in pea.

Variety Prometei was distinguished as tolerant to the applied stress and could be used as a base for the development of new varieties less sensitive to the soil salinity.

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