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Salt and drought stress effects on germination and seedling growth on field pea (*Pisum sativum* L.)

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Abstract- Salinity and water deficit can be major environmental constraints, which affect seed germination and seedling growth of many forage crops in semi-arid and arid regions. Pea (*Pisum sativum* L.) is one of the most important grain legume crops around the world, is famous for its high biomass yield and nutritional value for livestock. In the present study, we tested under controlled conditions two separate factorial experiments were conducted based on a randomized complete block design under polyethylene glycol (PEG) 6000 simulated drought stress and NaCl salinity stress conditions. The used saline concentrations were: 0 (distilled water); 25; 50; 75 and 150 mmol.l^{-1} of NaCl, whereas for the water deficit, simulated with PEG 6000, the following osmotic potentials were used: 0 (distilled water);-0.03;-0.1;-0.7 and -1.0 MPa. Germination percentage, root and shoot length, seedling fresh and dry weight and water content were measured in the study.
Higher concentrations of NaCl (150 mmol.l^{-1}) were more inhibitory to seedling fresh and dry weight and water content than osmotic PEG solutions. The influence of PEG (-1.0 MPa) was more inhibitory to germination percentage and seedling root and shoot length.
The germination percentage was higher (100%) but mean germination time were lower in PEG than NaCl conditions. Seeds of *P. sativum* were able to germinate at all concentrations of NaCl after 3 days but mean germination time of 5 days was observed at -1.0 MPa of PEG treatments. PEG had less inhibitor effect on seedling growth than the germination. It was concluded that inhibition of seedling growth at the same water potential of NaCl and PEG resulted from osmotic effect rather than salt toxicity. The percentage germination of seeds incubated is 100%, incubating seeds of *P. sativum* with a saline solution of NaCl and PEG solution for 7 days had no adverse effect on their germinability.

Key words: *Pisum sativum L.*; Germination; Drought stress; Salt stress; Osmotic potential; Seedling growth

1. Introduction

The Mediterranean basin has been identified as one of the most prominent climate change hotspots due to ongoing and projected changes in both means and variability of temperature and precipitation (Diffenbaugh and Giorgi, 2012). Observed climate showed a trend towards increasing temperature and declining rainfall over recent decades (Hartmann *et al*., 2013).
Salinity and drought are the major abiotic stresses that affect crop production in arid and semiarid areas. Seed germination and seedling growth are the stages most sensitive to salinity and water deficit. Salt stress causes adverse physiological and biochemical changes in germinating seeds. It can affect the seed germination and stand establishment through osmotic stress, ion-specific effects and oxidative stress.
In plant production, the most important factors limiting crop productivity are environmental stresses. Disruption of plant water management caused by drought, salinity or low temperature is a major yield-decreasing factor (Jaleel *et al*., 2009). Salinity and drought are the main osmotic stresses affecting seed germination, seedling establishment in the field and crop productivity. Environments with water restriction and salt excess produce harmful effects on seeds, such as a significant reduction in the germination percentage and rate in the root length and fresh mass and in the shoot of seedlings (Yan, 2015); they may even cause a complete inhibition of the germination process of seeds (Atia *et al*., 2009). 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 very important, 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. Speed of germination and emergence is an important determinant of successful crop establishment. In these areas, the use of plants

that are more resistant to environmental stresses, particularly in the germination and early seedling growth stage, is more important (Fallahi *et al*., 2015).

Drought, a worldwide problem currently is expected to elaborate in the near future (Dai, 2013). Water shortages negatively influence plant growth and development, consequently heavily limit plant productivity. A search for plant species that are tolerant to drought and elucidating the mechanisms of their responses to osmotic stress is of significant importance.

Soil salinity may influence the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake, or the toxic effects of Na^+ and Cl^- ions on the germinating seeds (Khajeh-Hosseini *et al*., 2003).

Seed germination and seedling growth are the two critical stages for the establishment of crops (Hubbard *et al*., 2012). These stages are the most sensitive to abiotic stress (Patade *et al*., 2011). Germination of high-quality seeds may be delayed or prevented by various abiotic stresses (Jamil *et al*., 2005; Fazlali *et al*., 2013).

Plants growing under natural conditions are exposed to a variety of stresses, which can lead to undesirable changes in the physiological processes and yielding. The development of plants is influenced by environmental stresses which determine the acreage and yields of various plants, including agricultural crops (Etesami and Gwyn, 2017). The cultivation of species able to withstand abiotic stresses while maintaining high productivity could be a solution to this problem.

Pea is among economically viable crops (Gepts *et al*., 2005). It has an important ecological advantage because it contributes to the development of low-input farming systems by fixing atmospheric nitrogen and it serves as a break crop which further minimizes the need for external inputs (Singh *et al*., 2007). Field pea (*Pisum sativum* L.) is one of the most important grain legumes in the world. Its grain is a major source of plant-based dietary protein for animals. Field pea can provide protein-rich feed and improve the sustainability of organic systems. It ranks third in the world production amongst the food legumes (Ghafoor & Arshad 2008). Field pea continues to be an important crop worldwide both for food and feed and as a rotational crop with other cultures. Pea seeds are rich in protein (23–25%), slowly digestible starch (50%), soluble sugars (5%), fiber, minerals and vitamins (Janko *et al*., 2017).

Despite the advances in studies about plant responses to environmental stresses, there is a lack of investigations on the use seeds, in order to increase their tolerance to unfavorable conditions. The objective of the study was to determine factors responsible for germination and early seedling growth of *P. sativum* due to salt toxicity or osmotic effect and to optimize the best priming treatment for these stress conditions. Understanding how pea manages salinity and water stress is important for the reclamation of drought and salinity-prone soils and crop production.

This work reports two experiments involving the germination of seeds of *P. sativum* in Petri dishes, performed to study the effect of water deficit and salinity on the seedling growth. The objective is to determine factors (salt toxicity or osmotic stress due to PEG) inhibiting seed germination and to verify how these abiotic stresses may limit the crop growth during the very early stages of growing season.

2. Materials and methods

2.1. Design of simulated drought and salinity conditions

Drought and salinity conditions were simulated by polyethylene glycol-6000 (PEG) and NaCl at one of four concentrations. Table 1 results shows how osmotic potential decreases with increasing PEG-6000 and NaCl concentration.

Table 1. The amounts of polyethylene glycol and sodium chloride used for producing of different levels of osmotic and salinity stresses

Water stress (MPa)	**0**	**-0.03**	**-0.1**	**-0.7**	**-1.0**
***Amount of PEG (g PEG/100ml H_2O)**	**0**	**0.53**	**1.53**	**6.39**	**8.03**
Salinity level (mM)	**0**	**25**	**50**	**75**	**150**
Amount of NaCl (gNaCl/100ml H_2O)	**0**	**0.15**	**0.29**	**0.44**	**0.87**

*According to Michel and Kaufmann 1973 at temperature of 25 ºC
(OP= $(-1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C + (2.67 \times 10^{-4}) \times CxT + (8.39 \times 10^{-7}) \times C^2T$, where C=PEG concentration, T=Temperature)

2.2. Plant materials and growing conditions

To determine the tolerance of the germination to the salt or the water stress, the *P. sativum* seeds were imbibed in the distilled water, solutions of Polyethylene Glycol (PEG-6000) or NaCl. For each experiment, 20 seeds were soaked in NaCl solutions (25, 50, 75 and 150 mM NaCl) or PEG 6000 solution of different water potentials (-1.0, -0.7, -0.1 and -0.03 MPa) were prepared. The number of seeds germinated was counted daily for 7 days. PEG solutions were prepared dissolving different concentrations of PEG 6000 in deionized water according to the water potential to induce, as described by Michel and Kaufmann (1973).

Seeds of pea (*Pisum sativum* L.) were disinfected with 2% of sodium hypochlorite for 10 min and then rinsed thoroughly and soaked in distilled water. Seeds were germinated at 25 °C in the dark for 7 days over two sheets of filter paper moistened with 10 ml distilled water, aqueous solution of chloride salt or PEG solution. The papers were replaced every 2 days to prevent accumulation of salts or PEG (Rehman *et al*., 1996). Seeds were considered germinated when the radicle emerged 1 mm from the seed. Distilled water was used as a control (0 MPa).

2.3. Measurement of germination parameters

The seedling fresh, dry weight expressed as mg/plant and length of root and shoot (mm) were determined after 7th day of germination test in all the germinated seedlings. The seedling dry weight was determined after drying at 80 ºC for 24 h (Almekinders and Louwaars, 1999)

The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated (ISTA, 2003). The germination process was evaluated during 7 days; germinated seeds were counted every day.

- The final germination percentage (GP) was calculated in the seventh day by using the following equation:

GP = (total number of germinated seeds/total seed) × 100.

- The vigor index was calculated according to following formula,

Vigor index (VI) = [seedling length (cm) × germination percentage] (Dezfuli *et al*., 2008, Memon *et al*. (2013)

- Germinated seeds fresh and dry weight.
- Root length and shoot length of seedling
- Water content at different osmotic potentials

2.4. Statistical analysis

The experimental design was two factors factorial (2 × 5) arranged in a completely randomized design; with 4 replications and 20 seeds per replicate. The first factor was solutions (NaCl or PEG) and the second was osmotic potential levels (0, -0.03, -0.1, -0.7 and -1.0 MPa) and salinity solutions (25, 50, 75 and 150 mM NaCl). Data for germination were subjected to analysis of variance using SATISTICA. The differences between the means were compared using LSD values ($P < 0.05$).

3. Results

3.1. Germination percentages at different osmotic potentials of PEG and NaCl

The present research studied the changes in germination characteristics and early embryo growth of field pea exposed to a reduced water potential in PEG or NaCl solution in laboratory conditions. Seed germination in PEG and NaCl solution was unaffected by decreasing water potential reaching 100% in *P. sativum*. Germination percentage was not significantly decreased by NaCl and PEG solutions, contrary it increased considerably with increasing in PEG and NaCl solutions (100%). The maximum of germination (100%) was obtained after 3 days in all NaCl treatments; this same rate was obtained after 5 days in -1.0 MPa treatment. Mean germination time was delayed by decreasing water potential thus PEG affected it more adversely than did NaCl (Figure1). Increasing salt or PEG in solution had no depressive effects upon seed germination percentage, which approached a final value 100% at all treatments.

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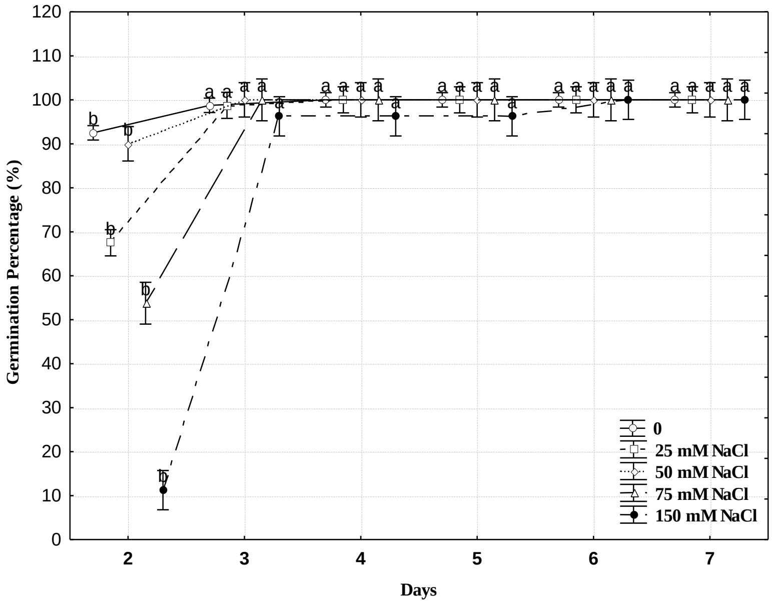


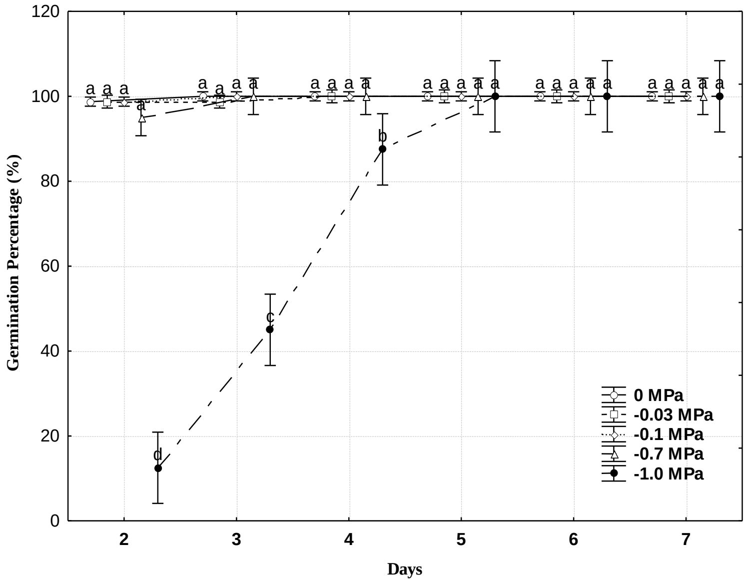
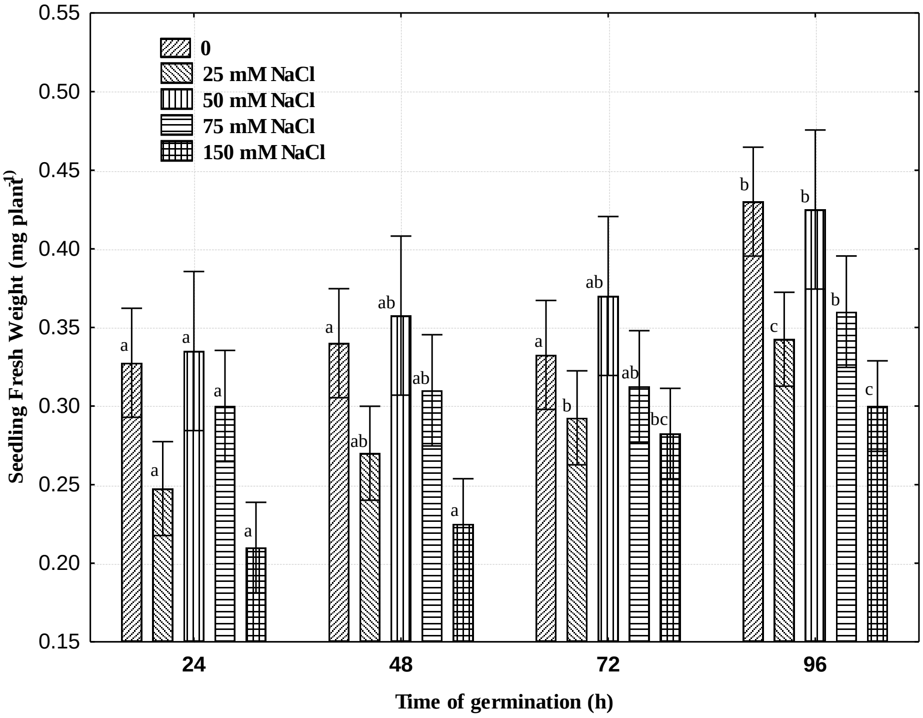


Fig 1. Germination percentages of field pea at different osmotic potentials of PEG and different concentrations of NaCl. Values are mean ± SE (n = 5). One-way ANOVA was used to test differences among treatments. Different letters indicate significant differences among concentrations of NaCl and PEG 6000 at $P<0.05$ (Tukey's test). Control treatment was distilled water

3.2. Effect of PEG and NaCl in seedling fresh weight, dry weight, water content and length of hypocotyl and radicle

Under salinity and osmotic stress, fresh weight of pea seedlings was evidently increased in NaCl and PEG solutions. In the control sample, fresh weight of an early seedling after 72 h of germination was about 0.34 g, whereas in the samples subjected to salt stress it reached 0.27 g (Figure 2). When early seedlings were exposed from water stress to optimal conditions, they were accompanied by an increase in fresh weight of about 8 mg (from 0.20 to 0.28 g). This increase, however, was smaller than in the control sample after 96 h of germination (10 mg) (Figure 2). Decreasing water potential by NaCl and PEG caused an increase in seedling fresh weight (Figure 2). Differences determined among the treatments were significant. Although the field pea showed similar responses to each NaCl and PEG concentration, the highest values were observed from pea in PEG -0.03 MPa and 75 mM in NaCl after 96h,. Seedling fresh weight of *P. sativum* showed a trend similar in NaCl and PEG, and, depending on the treatment, fresh weight increased with increasing NaCl and PEG concentration.



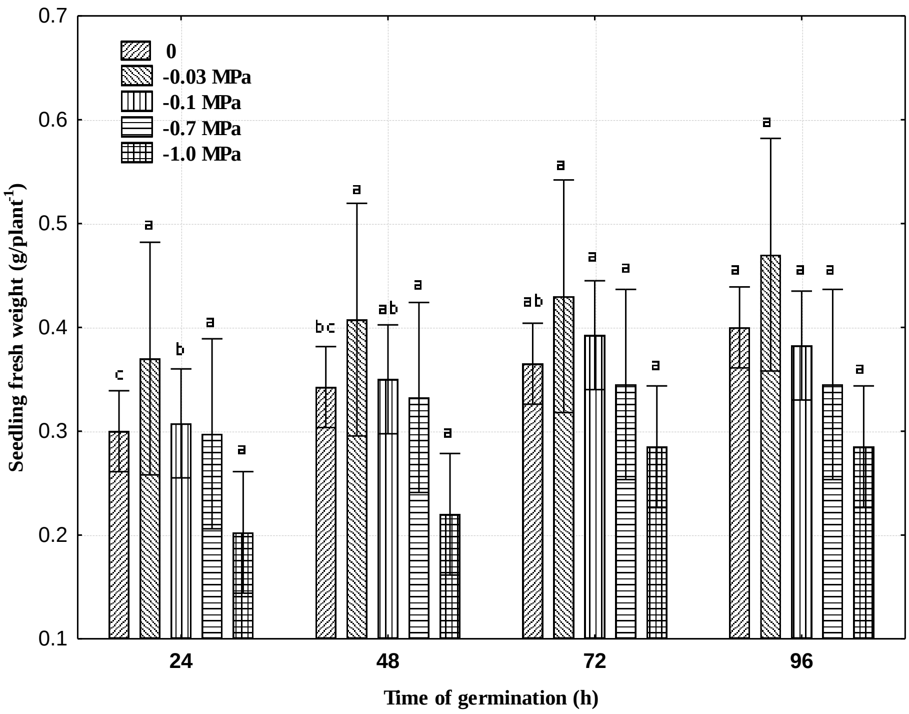


Fig 2. Seedling fresh weight of field pea at different osmotic potentials of PEG and different concentrations of NaCl. Values are mean ± SE (n = 5). One-way ANOVA was used to test differences among treatments. Different letters indicate significant differences among concentrations of NaCl and PEG 6000 at P<0.05 (Tukey's test). Control treatment was distilled water.

A significant reduction in water content of field pea was observed at higher concentrations of NaCl (150 mM), while in the experiments with osmotic potentials of PEG, no significant decrease of water content is observed at different concentrations of PEG (Figure 3).

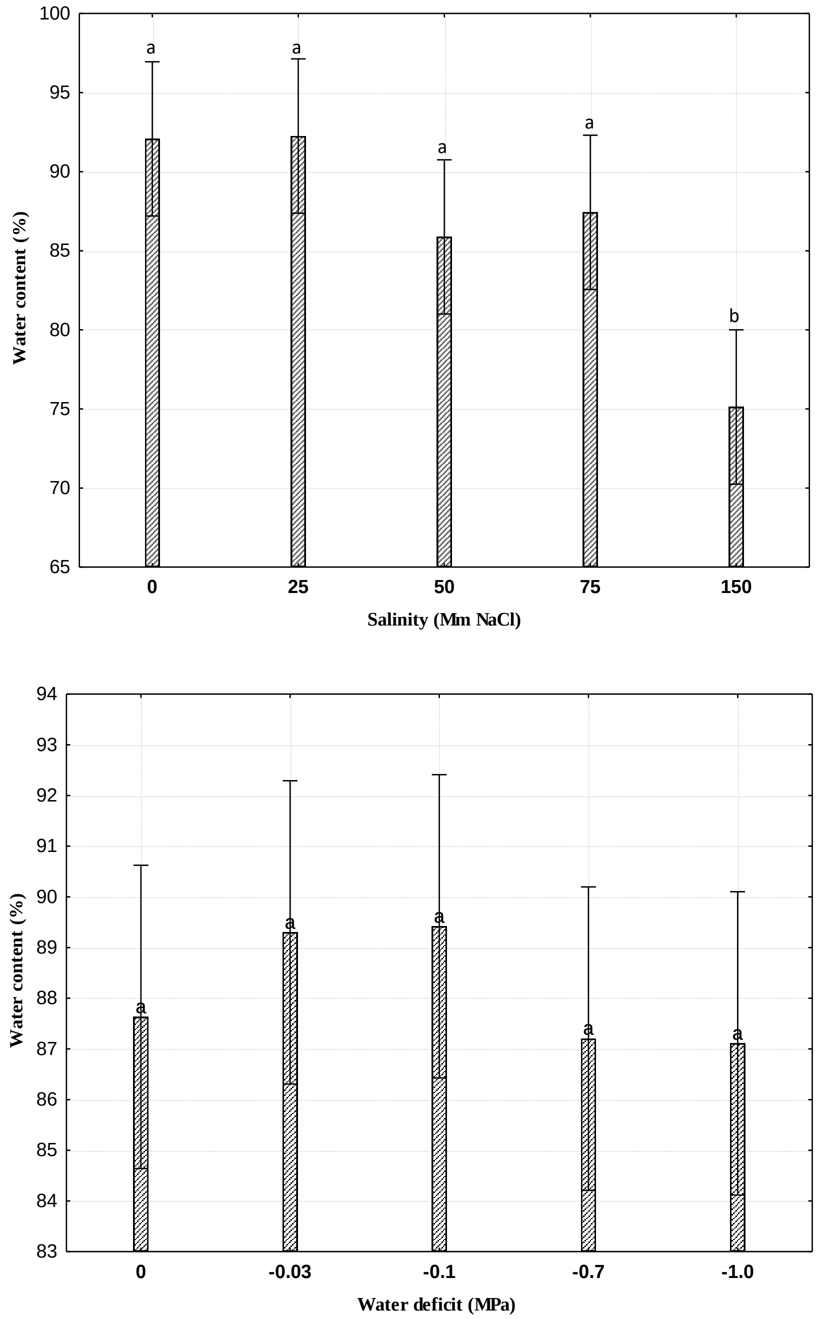
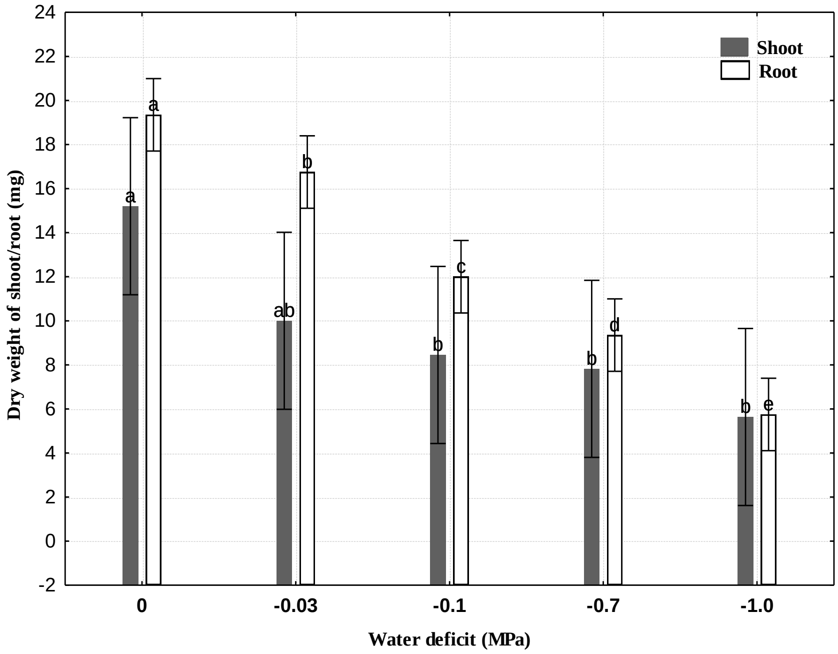


Fig 3. Water content of field pea at different osmotic potentials of PEG and different concentrations of NaCl. Values are mean ± SE (n = 5). One-way ANOVA was used to test differences among treatments. Different letters indicate significant differences among concentrations of NaCl and PEG 6000 at P<0.05 (Tukey's test). Control treatment was distilled water.

Fresh and dry weights of seedling of *P. sativum* grown in different concentrations of NaCl and PEG are presented in Figure 4 and 5. Fresh weight of seedling significantly decreased due to the increase in the salinity and drought levels compared to control (Figure 4).



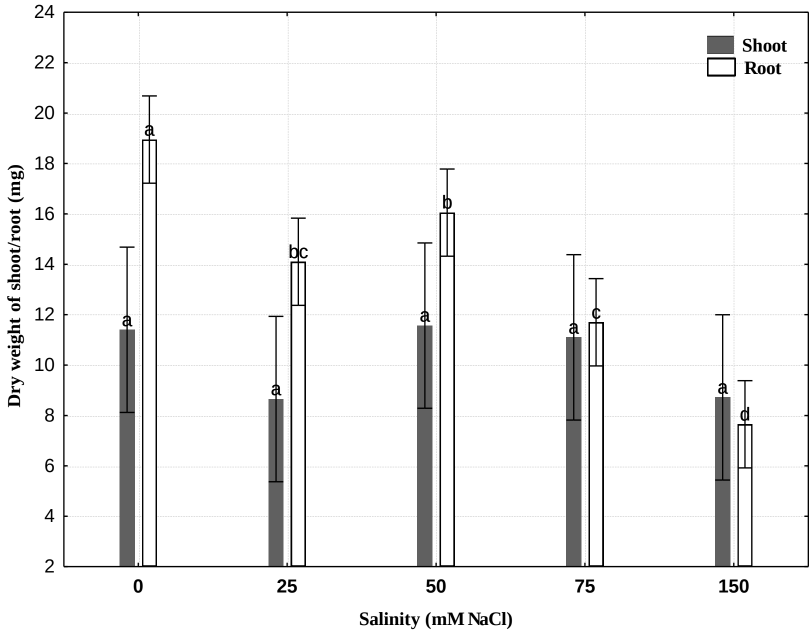
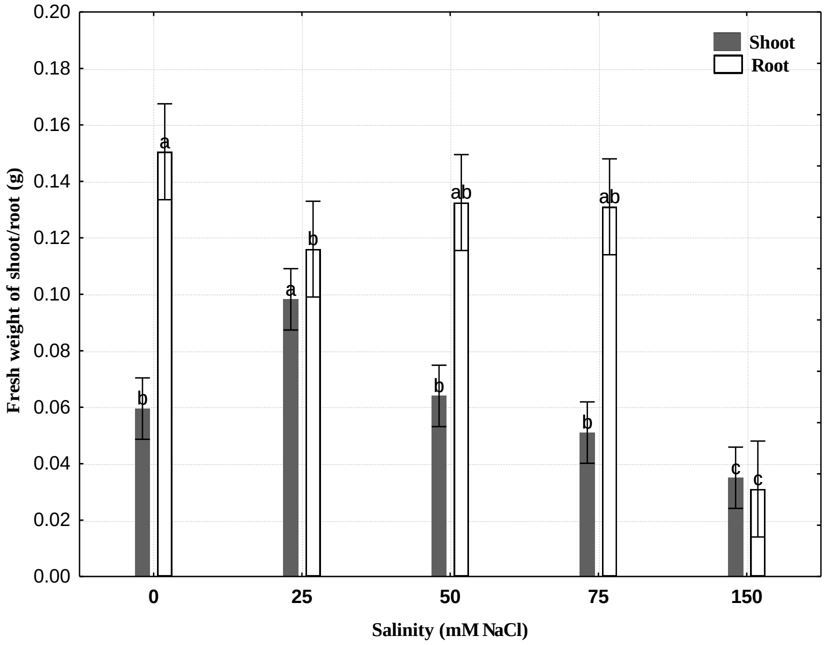


Fig 4. Dry weight of shoot and root of field pea at different osmotic potentials of PEG and different concentrations of NaCl. Values are mean ± SE (n = 5). One-way ANOVA was used to test differences among treatments. Different letters indicate significant differences among concentrations of NaCl and PEG 6000 at P<0.05 (Tukey's test). Control treatment was distilled water.

Though, at the different PEG and NaCl concentration the dry weight deceased significantly with increasing salinity and drought levels (P <0.05) compared to the control (Figure 5).



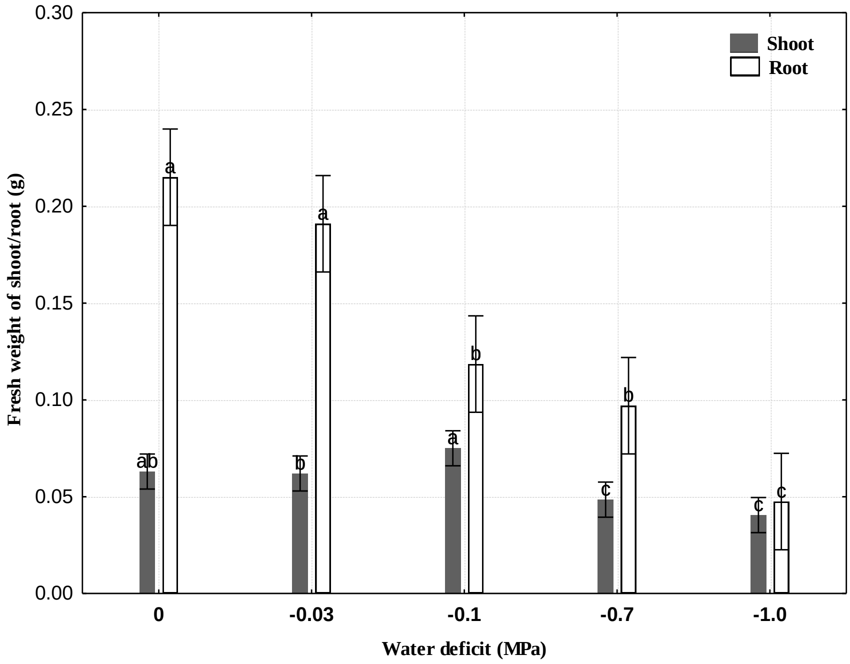
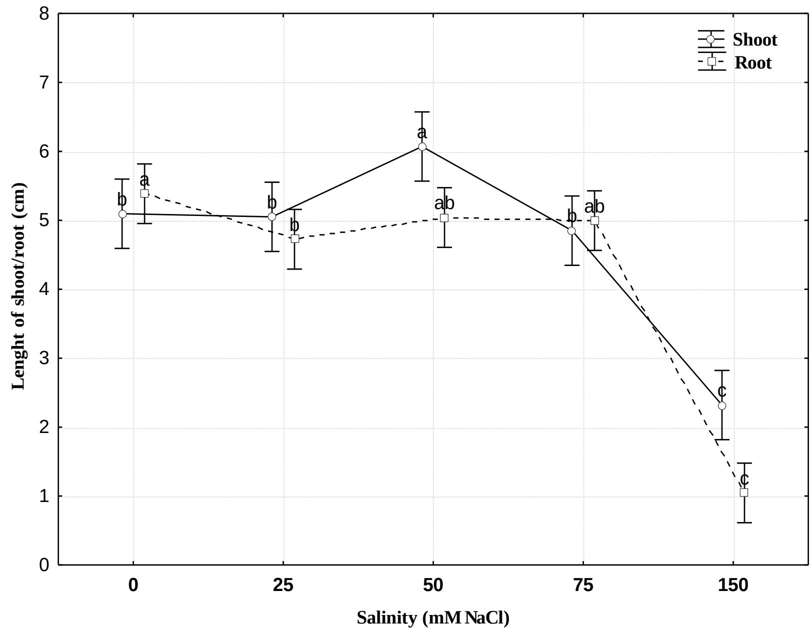


Fig 5. Fresh weight of shoot and root of field pea at different osmotic potentials of PEG and different concentrations of NaCl. Values are mean ± SE (n = 5). One-way ANOVA was used to test differences among treatments. Different letters indicate significant differences among concentrations of NaCl and PEG 6000 at P<0.05 (Tukey's test). Control treatment was distilled water.

For the length of hypocotyl and radicle of field pea at different osmotic potentials, the salt solutions had a stronger negative effect than the PEG solutions generating the same osmotic potentials (Figure 6).



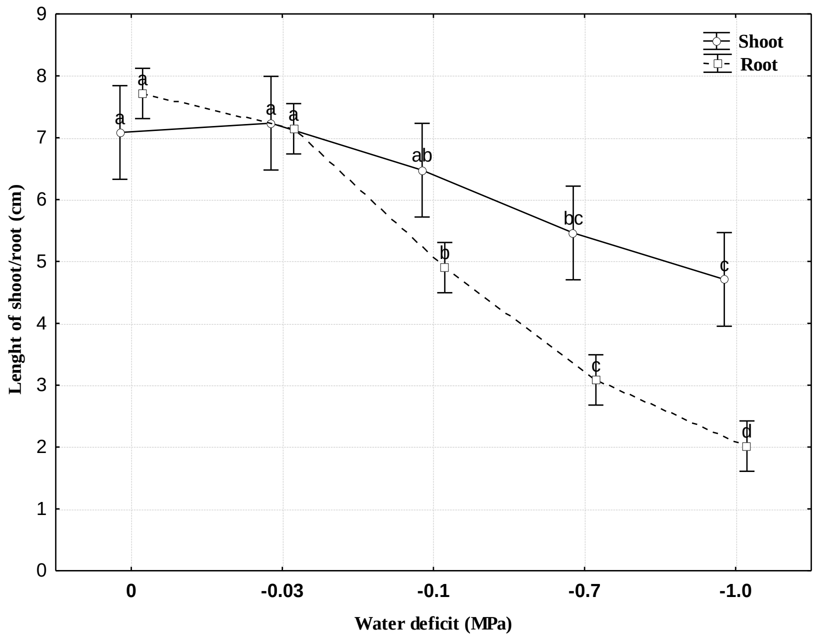


Fig 6. Lenght of shoot and root of field pea at different osmotic potentials of PEG and different concentrations of NaCl. Values are mean ± SE (n = 5). One-way ANOVA was used to test differences among treatments. Different letters indicate significant differences among concentrations of NaCl and PEG 6000 at P<0.05 (Tukey's test). Control treatment was distilled water.

3.3. Effect of PEG and NaCl in vigor index

The data presented in Figure 7 revealed that the average seedling vigor index of different concentrations of PEG was ranging between 1500 to 700 and average seedling index of different concentrations of NaCl varied between 1100 to 300. Significant differences were observed between NaCl and PEG treatments for seed vigor index of field pea (Figure 7). Seedling vigor index is considerably decreased with increasing NaCl and PEG concentrations. The highest Vigor Index was recorded in control (1500), whereas the lowest one (300) was noted at 150 mM NaCl (Figure 7). The highest Vigor Index was achieved with control associated with their more shoot lengths as compared to other treatments. There was an indirect relationship between NaCl and PEG levels and germination characteristics, i.e. the germination was influenced with increasing NaCl levels especially under salinity of 150 mM NaCl. In general, all determined germination and seedling parameters (seedling fresh weight, radical length, hypocotyl length and vigor index) decreased in response to water and salt stress.

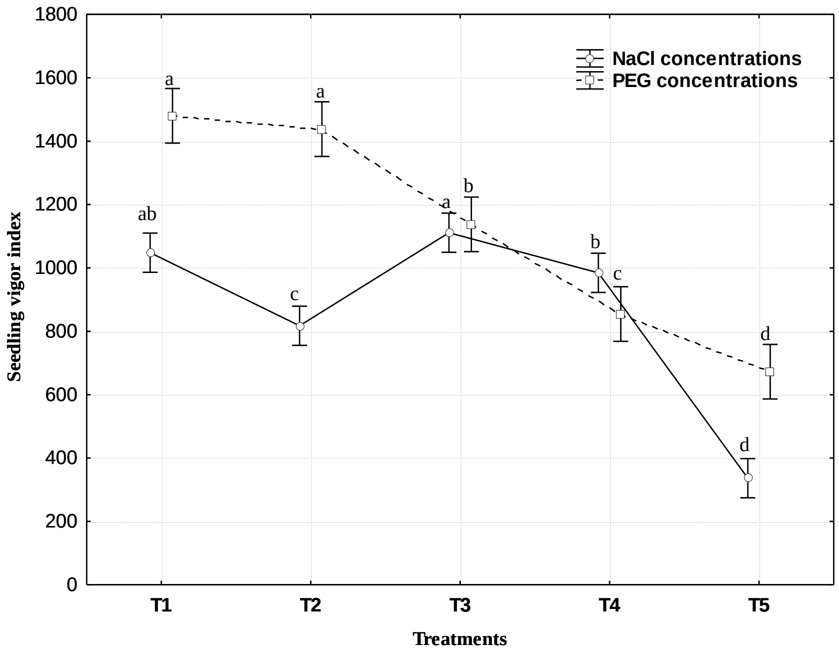


Fig 7. Seedling vigor index of field pea at different osmotic potentials of PEG and different concentrations of NaCl. Values are mean ± SE (n = 5). One-way ANOVA was used to test differences among treatments. Different letters indicate significant differences among concentrations of NaCl and PEG 6000 at $P<0.05$ (Tukey's test). Control treatment was distilled water.

4. Discussion

Seed germination is the critical stage for species survival. Drought and salinity affect germination and seedling growth and yield of several crop species such as field pea.

Salinity and drought are two of the most important soil abiotic limiting factors limiting germination and seedling growth (Atak *et al*., 2006). These associated abiotic factors can have a profound effect on germination rates and, consequently, on seedling establishment. Therefore, the study of the role of these factors on seed germination is necessary to get information about environmental factors affecting seedling recruitment in the field, which can be very valuable for conservation and restoration purposes (Hu *et al*., 2013). Seed germination and seedling growth are the stages most sensitive to these stresses. Water deficit and salt stress causes adverse physiological and biochemical changes in germinating seeds. In this study, the maximum of germination was not significantly decreased by increasing salt stress and PEG solutions, which approached a final value 100% at all treatments in three day.

Germination percentage of field pea was not significantly decreased by NaCl and PEG solutions, many authors demonstrated the opposite effect of salt and water stress in different species.The depressive effects of an increased salt concentration or soil water deficit upon germination percentage and rate and following seedling emergence have been extensively shown in many crops (Allen *et al*., 1986, Murillo-Amador *et al*., 2002, Okc¸u *et al*., 2005, Kaya *et al*., 2006).

Furthermore, drought and salinity stress severely affect seed germination by preventing water uptake and through the toxic effect of sodium and chloride ions. These factors result in inhibited or delayed seed germination and seedling growth (Ashraf and Foolad, 2005). Incubating seeds of *P. sativum* with a saline solution of NaCl and PEG solution had no adverse effect on their germinability, the percentage germination of seeds is about 100%.

Fresh weight of seedling significantly decreased due to the increase in the salinity and drought stress compared to control. The same effect was reported by Entesari et al. (2012) and Baghizadeh (2011) indicated that drought and salinity stress caused reduction of fresh and dry weight of radical and shoot in comparison with seedling control.

Previous studies have analyzed the germination of certain pea species, 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; Gordana *et al*., 2016). Okçu *et al*. (2005) has found that -0.6 MPa osmotic potential decreased *Pisum sativum* seed vigor and the reduction was more significant on seed with low vigor.

For the length of hypocotyl and radicle of field pea, the salt solutions had a stronger negative effect than the PEG. This can be easily explained by the "ion toxicity" component of salt stress that, in addition to the osmotic component, has a number of deleterious effects in plants, inhibiting many enzymatic activities and cellular processes, directly inactivating proteins or interfering with mineral nutrition (Munns and Tester, 2008; Zhu, 2001). In contrast, Kumar *et al*. (2011) found that PEG induced osmotic stress caused more growth inhibition as compared to NaCl induced osmotic stress. 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.

Taking into account physiological processes that differ between plants we can expect that studies on legumes will unravel specific mechanisms involved in abiotic stress resistance/tolerance (Reddy *et al*., 2012). This is a common behaviour of plants and, in fact, these early phases of the life cycle are generally the most sensitive to stress (Vicente *et al*., 2004; Okcu *et al*., 2005; Franco *et al*., 2011). In our experiment, seedling vigor index was reduced by osmotic stress; however, the decreased values of seedling length and germination percentage with PEG and salinity indicate that the osmotic stress affects seedling elongation more than germination percentage as an adaptive response to osmotic stress.

Drought and salinity stress are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri *et al*., 2001; Farooq *et al*., 2009). These stresses share similar physiological mechanistic responses during plant growth.

5. Conclusion

Existing knowledge on seed germination and seedling growth of leguminous species such as *P. sativum* under various environmental conditions remains inadequate. Field pea had a considerable tolerance to osmotic stress at germination and early seedling growth stage. In conclusion, the increasing NaCl and PEG doses caused detrimental effects on all parameters except germination percentage. Germination indices of this plant were favorable up to -1.0 MPa stress severity. In addition, field pea appropriately germinated up to salinity level of 150 mM. Moreover, pea germination and seedling growth was satisfactory until drought stress of -1.0 MPa and salinity stress of 150 mM. Field pea continues to be an important crop worldwide both for food and feed and as a rotational crop with other cultures. Totally, *P. sativum* showed a better germination especially under osmotic stress; therefore it seems to be a suitable candidate for breeding programs. In addition, future research should focus on molecular, physiological and metabolic changes induced by priming agents under salt and water stress of pea seeds.

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