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Salinity Tolerance of Persian Clover (*Trifolium resupinatum* Var. *Majus* Boiss.) Lines at Germination and Seedling Stage

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Abstract: This investigation was carried out in the Field Crops Department, Agriculture Faculty, Namik Kemal University, Tekirdag, Turkey. The aim of the research was to characterize the effect of salinity on seed germination of three Persian clover lines namely Y, S and AY were evaluated for their salt tolerance/sensitivity on the basis of magnitude of seed germination, seedling vigor (length of root and shoot), plant growth (dry and fresh weight), sodium (Na^+) and potassium (K^+) content during seed germination and seedling growth stage. The seeds were not germinated by the 200 mM salinity level. However in the presence of salinity the seed germination decreased in all the lines at 150 mM salinity level. The germination of line S seeds was found to decrease significantly at 150 mM NaCl salinity. 100 and 150 mM salinity treatments resulted in decreasing the shoot fresh and dry weights of the lines as compared to that of shoot in the lines at other salinity treatments. However, the shoot and root water contents were not affected in all salinity treatments. The roots of salt-treated plants accumulated more Na^+ and K^+ than shoots of plants. The line S exhibited higher values than the other lines for tolerance indices of shoot and root dry weights under 100 (shoot, 0.72; root, 0.61) and 150 mM (shoot, 0.51; root, 0.34) salinity levels. Results suggest possible different behaviors of lines differing in salt tolerance with respect to germination, seedling vigor, plant growth, water content and Na^+:K^+ contents.

Key words: Persian clover · salt stress · seedling vigor · tolerance index · *Trifolium resupinatum* L.

INTRODUCTION

The total area of saline soils is 397 million ha. Of the current 230 million ha of irrigated land, 45 million ha are salt-affected soils (19.5%) and of the almost 1 500 million ha of dry land agriculture, 32 million are salt-affected soils (2.1%) to varying degrees the result of human-induced processes. Salt-affected lands are reflected as saline seeps in dry land agriculture and secondarily salinized irrigated lands. Globally more than 77 million ha of land is salt-affected by human-induced salinization [1].

Soil salinity designates a condition in which the soluble salt content of the soil reaches a level harmful to crops through the reduced osmotic potential of the soil solution and the toxicity of specific ions. These soluble salts may be from those present in the original soil profile or transported to the profile by irrigation water containing an unusual high concentration. Salinity largely affects the uptake of water through increased water potentials; however it also can affect the hydrologic processes of infiltration and redistribution through chemical induced changes of structure and aggregation. Salinity affects soil water by adding osmotic potential to the soil matrix potential as seen by plant roots as they abstract soil water by osmosis. Matrix potential is a property of the soil texture related to capillary tension and assumed constant as salinity changes. The net result is that while soil water content and matrix potentials are not affected for hydrologic budget computations, plant uptake and crop water stress are affected as a result of the increased total water potentials and reduced plant available water [2].

Plants differ in their tolerance to salt. Generally, fruits, vegetables and ornamentals are more salt sensitive than forage or field crops [3]. However, saline soils and water shortage severely limit the productivity of forage crops and pastures in semiarid and arid environments. The response of plants to excess sodium chloride (NaCl) is complex and involves changes in their morphology, physiology and metabolism [4]. Germination is one of the

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most critical periods for a crop subjected to salinity. Germination failures on saline soils are often the results of high salt concentrations in the seed planting zone because of upward movement of soil solution and subsequent evaporation at the soil surface. These salts interfere with seed germination and crop establishment [5, 6]. Lower levels of salinity delayed germination whereas higher levels in addition, reduced the final percentage of seed germination [7, 8].

Persian clover is an important source of nutrients for ruminant livestock [9]. It is adapted to a wide range of soil types, but it is best suited for low-lying areas. However, the development of cultivars with the ability to germinate under high salt stress would be useful in reclamation of saline soils. Besides, information on germination and seedling stage of *Trifolium resupinatum* var. *majus* and other varieties to salinity is not available. The aim of the research was to characterize the effect of salinity on seed germination of three Persian clover lines namely Y, S and AY were evaluated for their salt tolerance/sensitivity on the basis of magnitude of seed germination, seedling vigor (length of root and shoot), plant growth (dry and fresh weight), sodium (Na^+) and potassium (K^+) content during seed germination and seedling growth stage.

MATERIALS AND METHODS

Persian clover lines were provided by the Department of Field Crops, Agriculture Faculty, Namik Kemal University, Tekirdag, Turkey. Seeds (Y, AY and S lines) were well screened lab. These are reached lines proposed for adaptation in humid and semiarid regions [9]. Seeds were surface sterilized with 2% sodium hypochlorite for 15 min. After extensive rinses with running tap water and dematerialized water, the seeds were germinated in rolls of neutral pH "germ test" paper partially immersed in 1/5 strength Clark's nutrient solution, pH 5.5 [10]. Seven concentrations of NaCl namely, 0, 1, 10, 50, 100, 150 and 200 mM were used for lines [11]. The experiments were performed with four replicates of each. Starting with 4 h socked seeds (0 h of seed germination) the germinated seeds were taken out at 24 h intervals up to 7 days, root and shoot (along with cotyledons) were separated from the seeds [3]. The seeds were not germinated by the 200 mM salinity treatment. Shoot and root lengths, fresh weight (FW, root and shoot) and dry weight (DW, root and shoot) were evaluated using twenty seedlings. Length of root, shoot and FW of these seed parts was measured. For the determination of dry weight these seed parts were dried at 55°C for 48 h in an oven. Water Content (WC) as percentage of FW was calculated using following formula:

$$\text{RWC (\%)} = \left(\frac{\text{FW-DW}}{\text{FW}}\right) \times 100$$

Furthermore, a Tolerance Index (TI) was calculated for each line; i.e., the shoot DW was calculated by adopting the following formula [12]:

$$\text{Tolerance Index (TI)} = \frac{\text{DW of shoot in salinity stress}}{\text{DW of shoot in control}}$$

The oven dried samples were ground to fine powder and 15 mg of this transferred to a digestion flask (25 ml) containing an acid mixture of HNO_3 and $HClO_4$, in the ratio 2:1 (v/v). The flask was heated gently over a sand bath. The cooled digest was then diluted by adding distilled water and the volume was made up as required. The K^+ and Na^+ contents were determined using flame photometer following the methods described by Ates and Tekeli [13]. Each treatment was analyzed with at least four replicates and Standard Deviation (SD) was calculated [14].

RESULTS AND DISCUSSION

It was observed that in 0, 1, 10, 50 and 100 mM level of salinity almost 100% germination was observed from day 1 onwards in the lines S, AY and Y. The seeds were not germinated by the 200 mM salinity level. However in the presence of salinity the seed germination decreased in all the lines at 150 mM salinity level (Fig. 1). The decrease was more prominent at the beginning, which progressively became less prominent during subsequent days of germination at 150 mM salinity level in the lines. The germination of line S seeds was found to decrease significantly at 150 mM NaCl salinity ($p<0.05$, Fig. 1). Genetic variability within a species offers a valuable tool for studying mechanism of salt tolerance. One of these mechanisms depends on the capacity for osmotic adjustment that allows growth to continue under saline conditions. With increasing salinity there was a decrease in germination of seeds, seedling vigour, plant growth and water uptake in the lines. Inhibition of germination due to salinity has been reported earlier [15, 16]. It is suggested that decrease in seed germination and depression in seedling vigour under saline stress is attributed to decrease water uptake followed by limited hydrolysis of food reserves from storage tissues as well

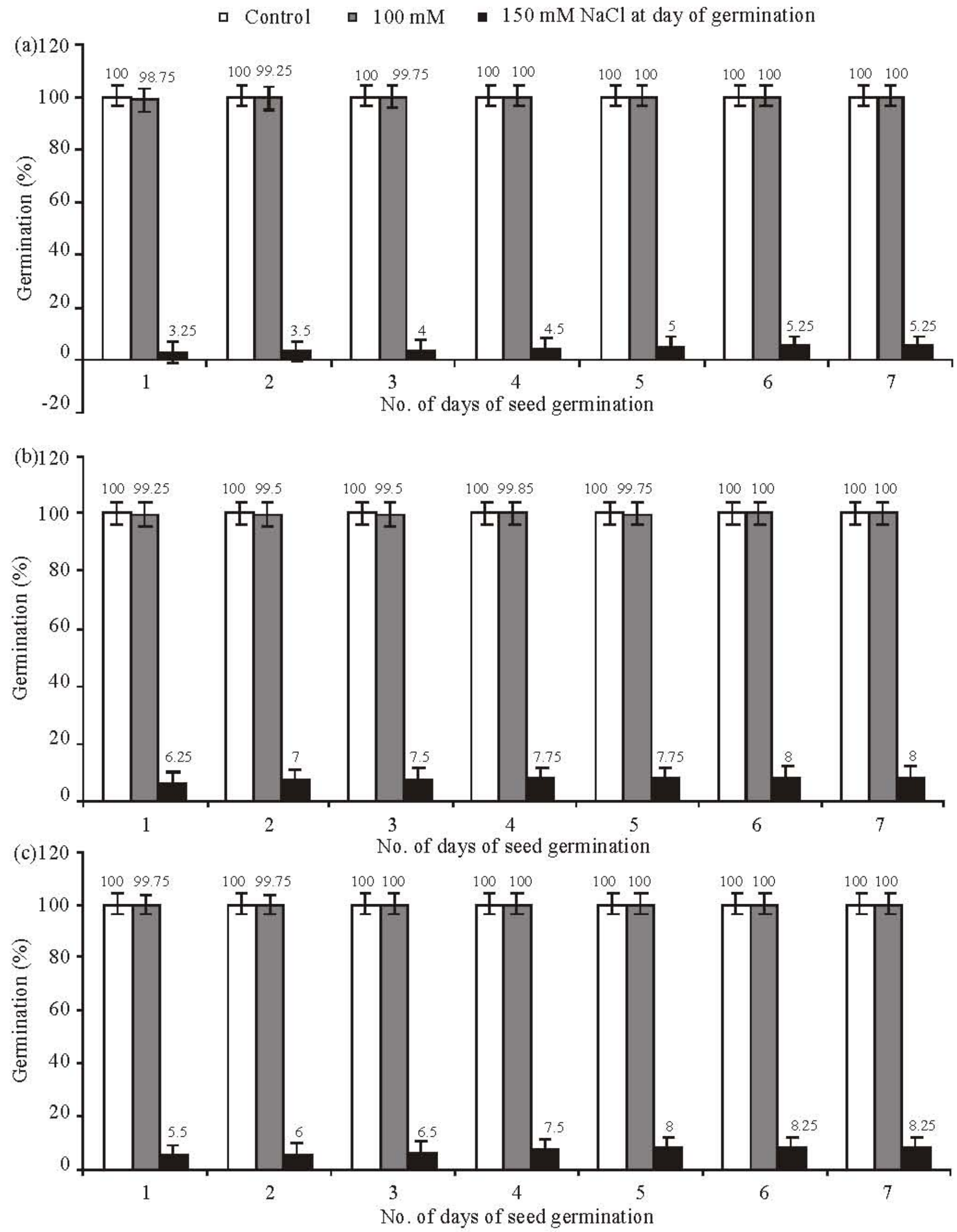

Fig. 1: Effect of salinity on germination percentage of Persian clover line S (a), line AY (b), line Y (c), Each value represents mean of four independent observations and SD determined

as due to impaired translocation of food reserves from storage tissue to developing embryo axis [17].

The shoot and root lengths increased gradually in all the lines with 1-7 days of seed germination under the conditions of absence (control) and presence of various levels of salinity. However, salinity treatment resulted in decreasing the root and shoot in the lines as compared to their respective control values (maximum concentration of salinity, 150 mM NaCl for lines; Figs. 2 & 3). Furthermore, root was determined to be more responsive towards salinity than that shoot as decrease in root length was more pronounced as compared to that of shoot in the lines at 150 mM NaCl of salinity. 100 and 150 mM salinity treatments resulted in decreasing the shoot fresh and dry

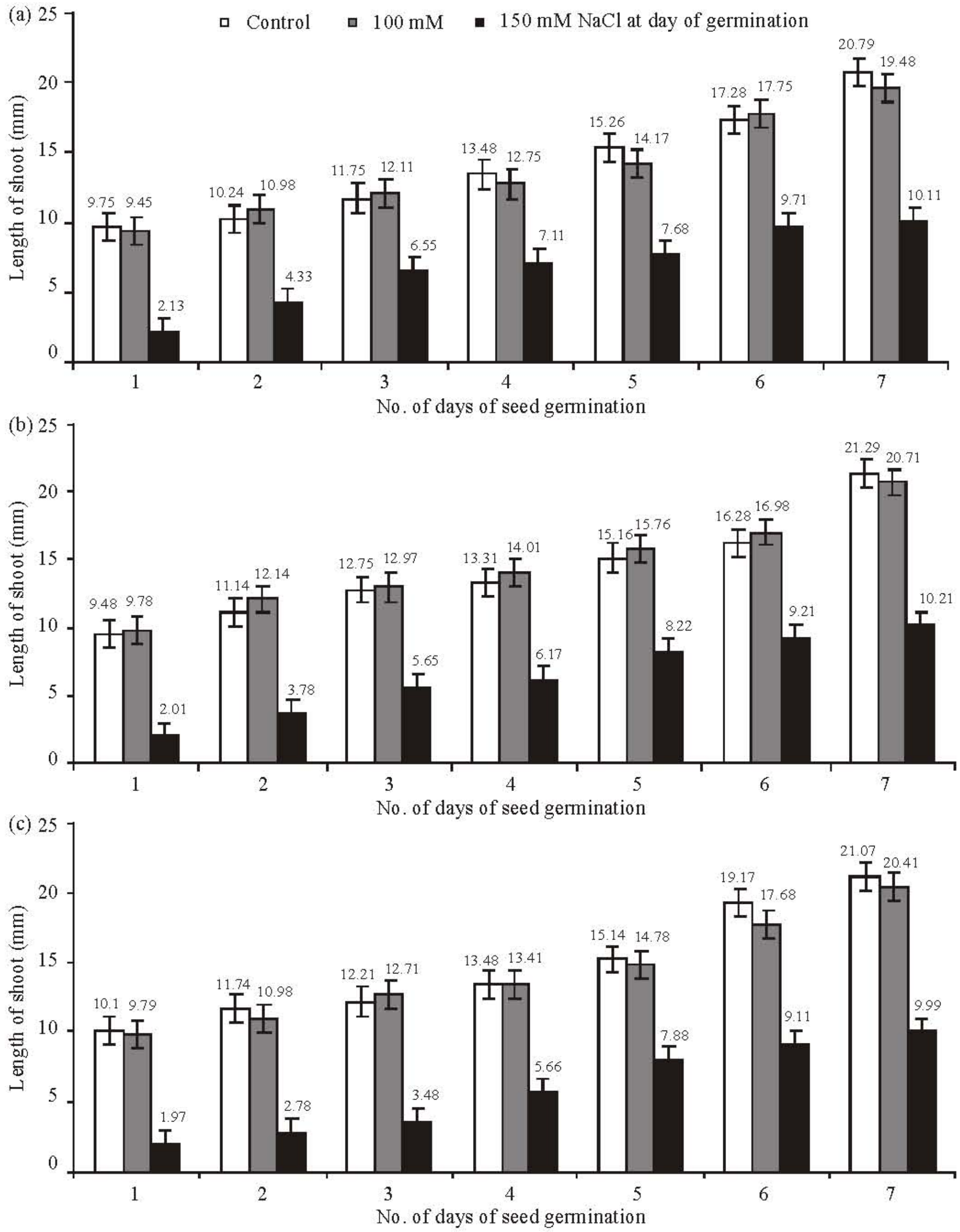

Fig. 2: Effect of salinity on shoot lengths of Persian clover lines S (a), AY (b), Y (c) control; 100 mM; 150 mM NaCl at 1-7 days of seed germination. Each value represents mean of four independent observations based on average lengths of 20 shoots and SD determined

weights of the lines as compared to that of shoot in the lines at other salinity treatments (Table 1). Some of the morphological changes are reduction of shoot and root length and restricted rooting [18-20]. Reduced growth rates as a result of salt stress were initially related to loss of turgor and its accompanying relaxation of cell wall tension. However, the relationship appears to be more complex. Reductions of growth rate occur even without loss of turgor and it appears likely that growth is actively controlled, independently of the sensing of turgor pressure, via changes in cell wall extensibility [21]. Such changes appear to be related to changes in protein

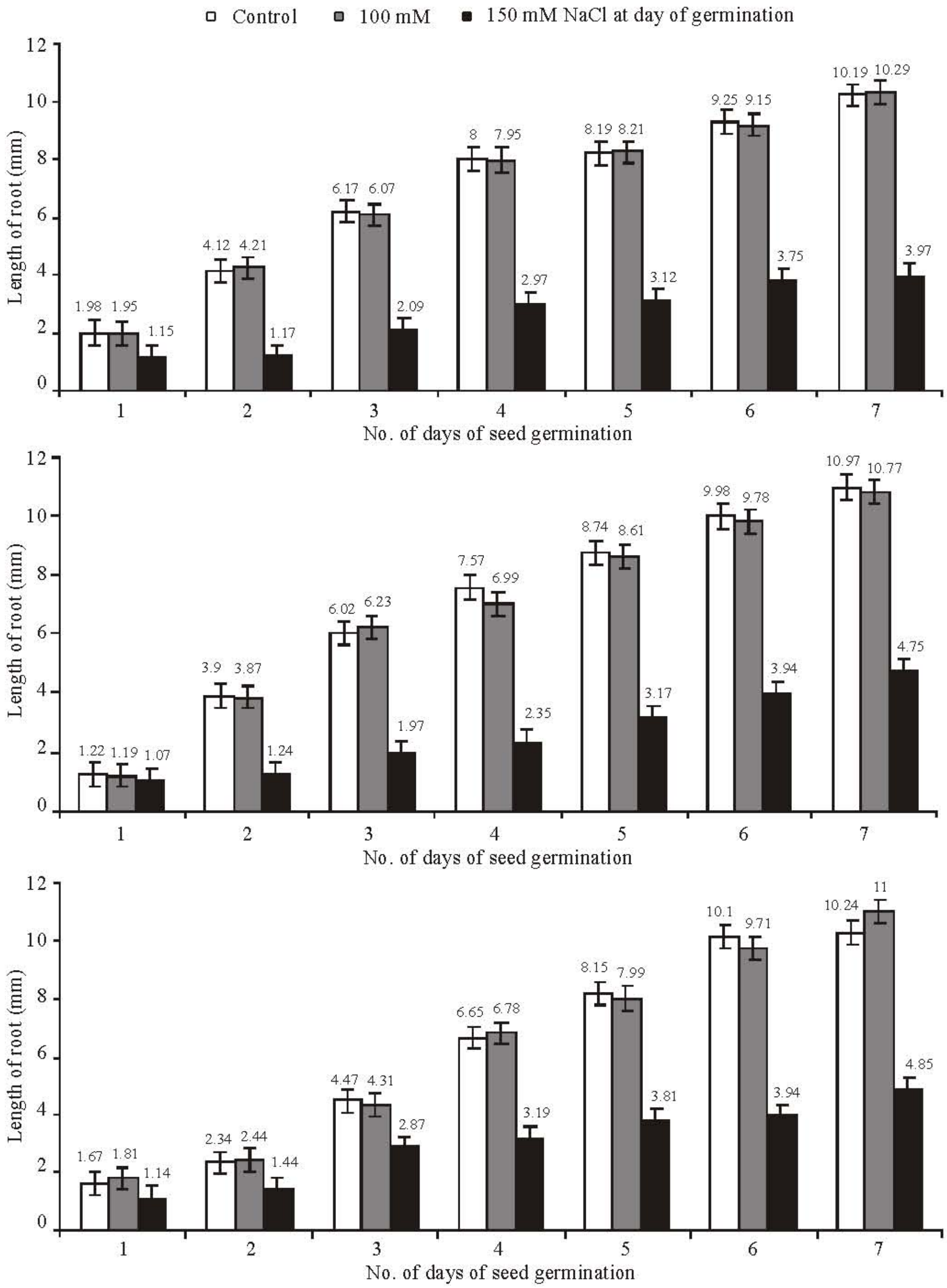

Fig. 3: Effect of salinity on root lengths of Persian clover lines S (a), AY (b), Y (c) control; 100 mM; 150 mM NaCl at 1-7 days of seed germination. Each value represents mean of four independent observations based on average lengths of 20 roots and SD determined

composition of cell walls [22]. Thus, adverse effects of salinity on growth and metabolism may be due to osmotic inhibition of water availability, toxic effects of high salt ions and disturbance of the uptake and translocation of nutritional ion [23]. Marcum *et al.* [24] investigated the tolerance of some grasses to salinity. The more salt tolerant grasses had a greater rooting depth and a greater total root dry weight under salinity stress. They defined salt tolerant grasses; and the osmotic potential was controlled, more than in susceptible plants. Increased

Table 1: Effect of salinity on FW, DW, RWC and Na^+: K^+ ratios of shoot and root of Persian clover lines at day 7 of seed germination

Lines	NaCl salinity (mM)	Shoot ZFW (mg)	Shoot DW (mg)	Shoot RWC (%)	Shoot Na:K	Root FW (mg)	Root DW (mg)	Root RWC (%)	Root Na:K
S	0 (contol)	11.90±2.3	3.98±0.3	67±1.6	0.38±0.03	4.21±0.4	1.30±0.04	69±1.2	0.55±0.02
	1	11.90±2.3	3.87±0.3	67±1.6	0.41±0.04	4.42±0.3	1.40±0.04	68±1.2	0.60±0.04
	10	17.40±3.4	4.21±0.4	76±1.8	0.41±0.04	4.44±0.4	1.38±0.03	69±1.4	0.66±0.06
	50	15.90±3.1	3.97±0.3	75±1.7	0.44±0.05	2.70±0.2	0.80±0.01	70±1.6	0.67±0.06
	100	9.50±1.4	2.86±0.2	70±1.4	0.46±0.05	2.07±0.2	0.79±0.01	62±1.1	0.65±0.05
	150	6.70±1.2	2.03±0.1	70±1.4	0.54±0.07	1.24±0.1	0.44±0.009	64±1.2	0.68±0.07
	200	-	-	-	-	-	-	-	-
AY	0	13.00±2.1	4.33±0.4	67±1.6	0.36±0.02	5.12±0.6	1.67±0.06	67±1.1	0.60±0.04
	1	12.60±2.0	4.20±0.4	67±1.6	0.39±0.03	3.73±0.3	1.26±0.04	66±1.1	0.62±0.04
	10	14.90±3.2	4.27±0.5	71±1.6	0.39±0.03	3.41±0.3	1.16±0.04	66±1.1	0.68±0.07
	50	17.00±3.0	4.10±0.3	76±1.8	0.44±0.05	2.30±0.2	0.78±0.02	66±1.1	0.67±0.06
	100	8.00±1.7	2.42±0.2	70±1.5	0.45±0.05	1.91±0.1	0.75±0.01	61±1.0	0.66±0.06
	150	4.10±1.1	1.12±0.09	73±1.7	0.57±0.09	1.08±0.08	0.38±0.008	65±1.2	0.69±0.08
	200	-	-	-	-	-	-	-	-
Y	0	11.70±2.2	3.94±0.3	66±1.1	0.38±0.03	4.00±0.3	1.43±0.04	64±1.2	0.59±0.04
	1	12.10±2.4	3.99±0.3	67±1.6	0.40±0.04	4.20±0.4	1.51±0.05	64±1.2	0.58±0.03
	10	16.50±3.1	4.09±0.4	75±1.7	0.39±0.03	3.60±0.3	1.23±0.03	66±1.1	0.63±0.05
	50	13.30±2.7	3.87±0.3	71±1.6	0.47±0.05	2.70±0.2	0.90±0.01	67±1.1	0.66±0.06
	100	8.00±1.3	2.39±0.2	70±1.4	0.46±0.05	1.60±0.1	0.62±0.009	61±1.0	0.64±0.05
	150	4.98±1.2	1.07±0.08	73±1.7	0.55±0.07	1.00±0.08	0.40±0.008	60±1.0	0.69±0.08
	200	-	-	-	-	-	-	-	-

Each value represents mean of four replicates. Values are rounded up to nearest whole figure and SD determined

NaCl levels results in a significant decrease in root, shoot and leaf growth biomass and increase root/shoot ratio in cotton [25]. Orak and Ateş [3] and Arin and Kiyak [26] stated that the fresh weight and seedling diameter was increased by salinity stress conditions, but these results were in accordance with Alyari *et al.* [15] and Kaya *et al.* [27]. They reported that the fresh weight and dry weight in seedlings were deceased by salinity. Parida *et al.* [28] speculated that plant height, fresh weight and dry matter of plant may not be affected by different salinity levels.

The shoot and root water contents were not affected in all salinity treatments (Table 1). These results were distinct with Khan [29] and Romeroaranda *et al.* [30]. They reported that the water content and osmotic potential of plants become more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity. With increasing salinity, leaf water potential and evaporation rate decrease significantly in the *Suaeda salsa* L. while there are no changes in leaf relative water content [31]. A greater decline in osmotic potential compared with the total water content led to turgor maintenance in plants under progressive or prolonged salinity stress [32]. Parida *et al.* [28] stated that the water content was decreased by salinity stress conditions, but Gebauer *et al.* [33] reported that the leaves of salt-treated plants accumulated more water than leaves of control plants.

The Na^+:K^+ ratio was also significant different ($p<0.05$). The Na^+:K^+ ratio was very small in the control plants, increased substantially in the three lines after the plants were exposed to high level of NaCl, particularly in the shoots (Table 1). But, the roots of salt-treated plants accumulated more Na^+ and K^+ than shoots of plants. The roots had the highest Na^+:K^+ ratio while the shoots had the lowest Na^+:K^+ ratio. The salt tolerant cultivar of reed plants always maintained low Na^+ contents in the shoot [34]. It was reported that the Na^+:K^+ ratio might serve as an indicator of crop tolerance to stress as the increase of Na^+ in salt tolerant species is generally associated with a decrease in K^+ [35]. However, salt tolerance is actually often associated with particular capacities to maintain K^+ content high [8]. Salinity enhances the content of Na^+ in broad bean (*Vicia faba* L.) and the ratio of Na^+:K^+ decreases [36].

Tolerance indices of shoot and root lengths, shoot and root dry weights for saline treatments are shown in

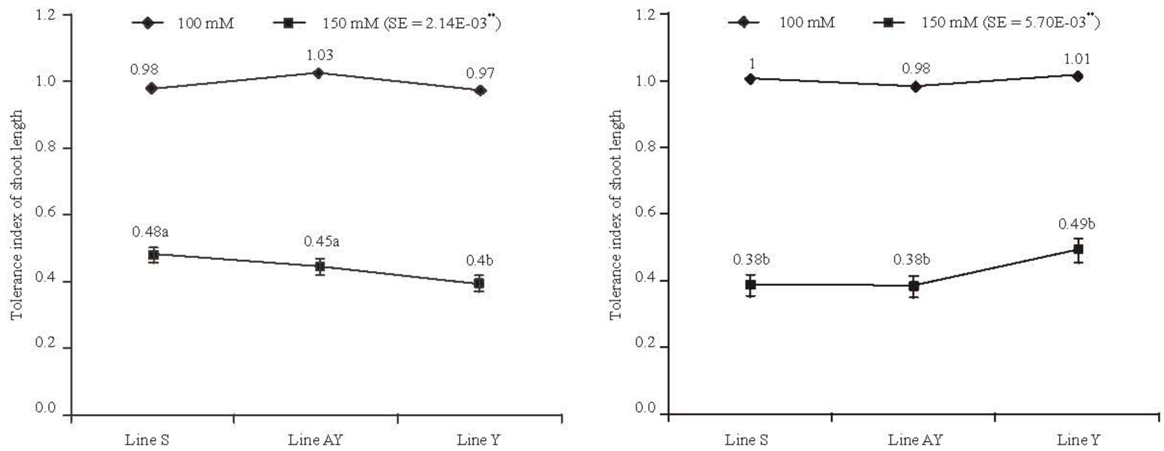

Fig. 4: Tolerance indices of shoot and root lengths under 100 and 150 mM salinity levels, **p<0.01

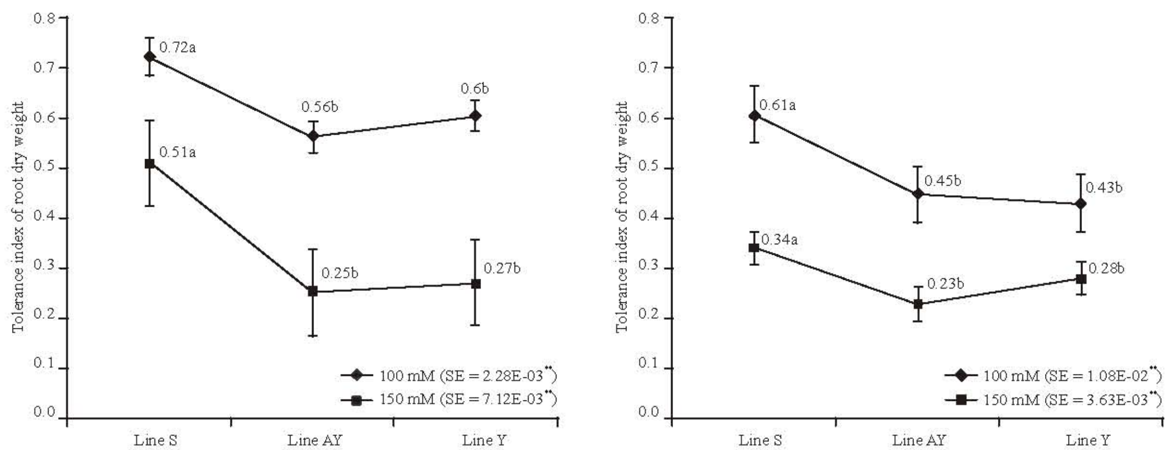

Fig. 5: Tolerance indices of shoot and root dry weights under 100 and 150 mM salinity levels, **p<0.01

Figs. 4 & 5, respectively. The Persian clover lines showed diversity in tolerance indices under saline stress treatments. The lines showing a high tolerance index for shoot length under 150 mM salinity were line S (0.48) and line AY (0.45) ($p<0.01$). For root length in the 150 mM salinity treatment, high tolerances were shown by line Y (0.49). The line S exhibited higher values than the other lines for tolerance indices of shoot and root dry weights under 100 (shoot, 0.72; root, 0.61) and 150 mM (shoot, 0.51; root, 0.34) salinity levels ($p<0.01$). These results were distinct with De La Rosa-Ibarra and Maiti [37] and Maiti *et al.* [12]. They reported that the tolerance index of root dry weight was increased by salinity stress conditions.

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