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Response of Some Wheat (*Triticum aestivum* L.) Genotypes to Salinity at Germination and Early Seedling Growth Stages

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Abstract: Establishment of seedlings at early growth stages of crop plants as one of the most important determinants of high yield is severely affected by soil salinity. Therefore, high germination rate and vigorous early growth under salty soils is preferred. In this study 15 wheat (*Triticum aestivum* L.) cultivars were used to investigate the effects of three salinity concentrations including 4 and 8 and 16 ds m^{-1} NaCl solution and distilled water as control in a four replicated CRD on their germination percent, radicle and hypocotyle length, seedling fresh and dry weight, radicle and hypocotyle dry weight. Result showed that increasing concentrations of NaCl reduced germination percentage, radicle and hypocotyle length, seedling fresh and dry weight, radicle and hypocotyle dry weight. Germination percentage in all cultivars showed considerable decrease with increasing salinity up to 8 ds m^{-1} NaCl. The decrease in length of radicle was more pronounced as compared to root in all NaCl salt treatments; however this decrease was more prominent in Dez cultivar than others. Great inhibition in root length was also recorded in Yavarous. Inia, Kavir and Chamran cultivars indicated the highest salt tolerance while Dez, Vinak, Cross Adl and Star cultivars showed the lowest. In general, it can be concluded that to select cultivars for better salt stress tolerance at seedling stage coleoptile and root elongation may be used as breeding criterions. More vigorous cultivars like Inia and Kavir could be considered as plant materials which are useful to breeders for future development of salt tolerant wheat cultivars.

Key words: Germination rate · Seedling weight · Radicle · Hypocotyl

INTRODUCTION

Soil salinity is one of the most important factors that limit crop production in arid and semi-arid regions [1]. Salinity affects about 7% of the world's total lands area [2]. The percentage of cultivated land affected by salt is even greater, comprises 19% of 2.8 billion hectares of arable land on the earth [3-5]. Furthermore there is also a dangerous trend of a 10% per year increase in the saline area throughout the world. Soil salinity may be robbing the country of about 25% of its crop production. A major part of the salt-affected soils, about 3.5 million hectares is under rice, wheat, cotton, sugarcane and rapeseed cultivation [6]. Data collected at CIMMYT suggest that 8-10%of the area planted to wheat in India, Pakistan, Iran, Egypt, Libya and Mexico is affected by salinity [7]. Wheat is a mandatory salt tolerant crop and serves as a staple food in 43 countries [8, 6]; including Iran, where it is grown on a large area. On the other hand, Iran is one of the countries that suffer from sever salinity problems. For example 18 million ha or 10% of total land area in Iran is salinity or sodicity soil. Need to develop crops with higher salt tolerance have increased strongly due to increased salinity problems. Therefore seeds with more rapid germination under salt stress may be expected to achieve a high final germination percentage and rapid and vigorous seedling establishment [9].

Salt stress affects many physiological aspects of plant growth. Shoot growth was reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point [10]. Early flowering reduced dry matter, increased root: shoot ratio and leaf size caused by salinity may be considered as possible ways of decreasing yield in wheat under salt stress condition [11-13]. Toxic effects of salts may change enzymatic activity and hormonal balance of plants. It is reported

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that high transpiration rate at leaf surfaces cause high accumulation of salt in leaves which kill them before full maturity [14]. Net photosynthesis was decreased due to the reduction in photosynthesis and increasing in respiration per unit of leaf area. Studies on physiological salt tolerance mechanisms revealed that plants may reduce detrimental effects of salts by the control of salt uptake [15] reducing damage under excessive ion uptake [16, 17] and osmotic adjustment [18]. Growth inhibition by salt stress also occurs due to the diversion of energy from growth to maintenance [19]. To maintain under salt stress plants need to regulate ion concentration in various organs and within the cells by synthesis of organic solutes for osmoregulation or protection of macromolecules for maintenance of membrane integrity [14]. It has been reported that increasing salt concentration in growing medium of pea plants increased roots and stems respiration [19, 20]. However, results of many cases show that respiration decreases under water stress condition, though decrease in respiration is much less than photosynthesis. Salt stress was also showed to increase the activity of an alterative pathway along with the cytochrome pathway. A salt tolerant wheat cultivar was showed to produce more ATP than a salt sensitive one [21].

When the salt concentration of the soil solution increases, water potential decreases, the turgor potential of plant cells declines and cells ultimately cease to grow. Under these water stress conditions, in general, stomata close resulting in the reduction of photosynthesis. Protein breakdown is enhanced and plants show poor growth. The low osmotic potential of saline soils makes it necessary for plants growing on them to maintain a lower intracellular osmotic potential; otherwise, they would experience water stress due to the movement of water osmotically from the plant tissue into the soil. In order to achieve a lower osmotic potential, osmotic adjustment under saline conditions can occur in plants due to uptake of inorganic ions from the saline growth medium [22], or by internal synthesis of osmotically active organic solutes [23].

The establishment stage of the crop consists of three parts: germination, emergence and early seedling growth; that are particularly sensitive to substrate salinity [24-27, 6]. Germination is reactivation of growth triggered by environmental stimuli as simple as availability of water and oxygen, or as complex as temperature, light, endogenous inhibitor and promoter interactions. Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to seed germination and grow while soil moisture and osmotic potentials decrease [28, 29] Much information is available in literature about the effect of water quality, soil texture and soil salinity on germination and emergence [30-32, 27]. Retardation and reduction in seed germination have been reported under NaCl treatments in the literatures [33-35]. The decrease in germination rate particularly under drought and salt stress conditions may be due to the fact that seeds to prevent germination develop and osmotically enforced dormancy under water stress conditions. This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedling [34]. Several investigation of seed germination under salinity stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seeding phases of development [36, 37]. Crop plants are usually seeded within the top 10 cm layer of the soil where it usually contains highest amount of salt [38]. For winter crops such as wheat, soil may contain even more salts at sowing because of high rate of evaporation in the previous summer fallow during which salts migrate to the soil surface. To produce satisfactorily under saline conditions seeds must germinate and seedlings must vigorously pass through the salty layer of the soil and survive [39]. Under such condition vigorous seedling growth is very important for crop establishment. Rapid and uniform seed germination under saline condition not only increases early seedling establishment but also has the advantage of higher drought tolerance [40].

The present study was undertaken to study the response of fifteen wheat cultivars to different levels of salinity and to determine the genotypic variability in their tolerance to salinity both at germination and seedling stages and selecting cultivars for rapid and uniform germination under saline conditions can contribute towards early seeding establishment.

MATERIALS AND METHODS

Plant Material: Fifteen cultivars of wheat (*Triticum aestivum* L.), were used in this study. All seeds were obtained from the Seed and Plant Improvement Institute in Iran. Some plant features and origins of these cultivars have shown in Table 1. The experiment carried out in Islamic Azad University of Ramhormoz, Khuzestan, Iran in October 2010. Similar seed size and weight was selected to exclude effect of that on the seedling

Table 1: Characteristics of wheat cultivars used in the experiment

Cultivar	1000 grain weight (g)	Response to environmental stresses	Grain yield (ton ha^{-1})
Cross Adl	36.0	Relatively early maturing-Tolerant to cold, salt and drought stress	5.3
Pishtaz	42.0	Late maturing-Tolerant to cold and logging	4.9
Niknejad	37.0	Early maturing- Tolerant to drought stress	6.3
Shiraz	40.0	Late maturing-Tolerant to cold and logging	7.5
Marvdasht	37.0	Mid maturing- Tolerant to cold	6.7
Chamran	39.0	Relatively early maturing-Tolerant to heat and drought stress	6.2
Star	36.0	Relatively late maturing- Tolerant to logging	5.7
Darab2	37.5	Early maturing-Tolerant to heat stress and logging	5.9
Kavir	39.0	Early maturing-Tolerant to salt and drought stress	6.1
Yavarous	46.0	Early maturing-Tolerant to drought stress	5.6
Inia	38.0	Early maturing-Tolerant to salt stress	5.1
Dez	39.0	Early maturing-Tolerant to heat and drought stress	5.9
Vinak	40.0	Very early maturing- Tolerant to drought stress	3.7
Showa mald	48.0	Early maturing-Tolerant to drought stress	5.6
S78-18	39.0	Early maturing-Tolerant to drought stress	5.8

Table 2: Used amounts of sodium chloride to obtain different levels of salinity

Ψ os level (MPa)	NaCl (g L^{-1})
0	0.00
4	2.56
8	5.12
16	10.24

establishment. Seeds were surface sterilized in 1.5% (v/v) sodium hypochloride for 10 min and thoroughly washed with sterile tap water. Seeds were germinated in covered, sterilized, disposal pertri dishes containing Whatman No. 1 filter paper moistened with either distilled water (control), or different treatment solutions. Germination was assessed using three replicates of 50 seeds in a factorial laid out in Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, 4, 8 and 16 ds m^{-1} NaCl, Table 2).

Growth Conditions: Seeds were incubated in a growth chamber (Type 8194, VINDON) and were considered germinated with the emergence of the radical. Temperature was maintained during the 10-d duration of the germination tests at 25°C (±0.5). In order to maintain adequate moisture, 5 mL of the original salt solutions were added to each petri dish every three days. Germination was scored when a 2 mm radical emerged from the seed coat [41]. Every three days, the germinated seeds were removed from the petri dishes. The seeds to germinate in each replicate were retained for measurements of radical and hypocotyl lengths at the end of the experiment. After 240 h, final germination percentages were recorded and seedling fresh weights immediately determined. To determine the impact of the treatments on seed germination, all seedlings were separated from the remaining seeds. Seedlings were harvested after ten days and washed with deionized water after harvest. Five washed seedlings from each replication were separated into root and shoot for the determination of their fresh and dry weights. Dry weight was determined after oven drying the samples at 65°C. Stem diameter was measured above the first real leaf by using caliper ruler with 0.001 mm.

Growth Parameters: Germination rate, germination percentage, radicle and hypocotyl lengths, seedling fresh and dry weights, radicle and hypocotyls dry weights and total dry weight were measured.

Germination Rate: A germination index was calculated for each subpopulation as GR:

$$GR = X1/Y1+(X2-X1)/Y2+\ldots+(Xn-Xn-1)/Yn$$

Where Xn is the germination percentage on Nth day and Yn in the number of day from first day experiment [42].

Germination Percentage: Data were analyzed using the GLM procedure of SAS program [43]. Significant differences between treatments were determined using Duncans multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Germination Percentage: Salinity decreased germination percentage of all the cultivars (Table 3). Even at the lowest salinity treatment (4 ds m^{-1} NaCl). The average germination percentage of cultivars at 4, 8 and 16 ds m^{-1} NaCl salinity was reduced to about 13, 41 and 84% of controls (Table 4). There was a negative correlation (r = -0.995, P< 0.001) between salt concentration and germination percentage (Table 5). There were no significant differences between cultivars at different salt levels (Table 3). However, the highest and the lowest germination percentage were related to Inia + 16 ds m^{-1} NaCl and Dez + 16 ds m^{-1} NaCl, respectively (Data not shown).

Salinity decreases germination [44, 45, 33], dry matter accumulation, the rate of CO2 assimilation, relative growth, leaf cell expansion and ultimate leaf growth [46-48]. Salt also affects the cellular and nuclear volume and inhibits or stimulates nucleic acid and protein synthesis. Soil salinity either completely inhibits germination at higher levels or induces a state of dormancy at low levels [49].

It has been reported by several authors that salinity stress affects seed germination either by decreasing the rate of water uptake (osmotic effect) and or facilitating the intake of ions, which may change certain enzymatic or hormonal activities inside the seed (ion toxicity) [50]. Bewley and Black [51] suggested that the inhibition of the radicle under salt stress is due to a reduction in the turgor of the radicle cells.

Germination Rate: The data (Table 3) indicated that there is significant difference between cultivars and levels of salinity. On average over all the cultivars at 4, 8 and 16 ds m^{-1} NaCl salinity germination rate was about 22, 43 and 110 %, respectively of control (Table 4). Germination rate was inversely related to salt concentration (r = -0.972, P<0.001) (Table 5). With increasing salinity, germination rate decreased by 50-100% in all cultivars except in Inia, Kavir and Chamran at 4 ds m^{-1} NaCl in which it increased by 5, 7 and 6%, respectively, relative to the control (Fig. 1). Inia consistently had greater germination rate at all salinity levels. The salt tolerance of wheat cultivars regarding germination rate was not consistent between salt levels. Shiraz and Darab-2 cultivars performed better up to 8 ds m^{-1} NaCl but poorly at 16 ds m^{-1} NaCl.

Table 3: Analysis of variance for the traits investigated in 15 wheat cultivars in response to salinity stress

Sources of variation	df	Germination percentage	Germination rate	Radicle length	Hypocotyl length	Radicle dry weight	Hypocotyl dry weights	Seedling fresh weight	Seedling dry weight
Cultivar	14	*	**	*	*	**	**	**	**
Salt stress	3	*	*	**	*	**	*	*	**
Cult × S. stress	42	ns	*	*	ns	*	ns	*	*

ns Non- significant, * Significantly at p < 0.05, ** Significantly at p < 0.01

Table 4: Mean values of seed germination traits for fifteen wheat cultivars and different salinity stress

Treatment	Germination percentage	Germination rate	Radicle length (mm)	Hypocotyl length (mm)	Radicle dry weight (mg)	Hypocotyl dry weights (mg)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Salinity level ds m^{-1} NaCl								
0	90.05a	44.47a	79.36a	59.36a	10.56a	13.42a	180.18a	24.95a
4	79.32b	36.21b	55.44b	38.08b	8.25b	10.23a	156.40b	19.87a
8	64.16c	31.56c	24.22c	17.27c	5.46c	5.78b	112.87c	11.49b
16	49.24d	21.18d	10.98d	5.87d	1.98d	2.12c	71.36d	4.19c

Means with similar letters in each column are not significantly different at 5 % level of probability. (Duncan)

Table 5: Correlation coefficients between wheat seedling characteristics and respiration of wheat cultivars grown under control and salt stress conditions

Traits	Salinity	Germination percentage	Germination Rate	Radicle length	Hypocotyl Length	Radicle dry weight	Hypocotyl dry weight
Salinity	1						
Germination percentage	-0.995**	1					
Germination Rate	-0.972**	0.980**	1				
Radicle length	-0.981**	0.701*	0.723*	1			
Hypocotyl length	-0.995**	0.964**	0.930**	0.789**	1		
Radicle dry weight	-0.743*	0.946**	0.964**	0.695*	0.948**	1	
Hypocotyl dry weight	-0.036^{ns}	0.238^{ns}	0.177^{ns}	0.584*	0.487^{ns}	0.320^{ns}	1

*, ** Significant at 0.05 and 0.01 probability levels, respectively

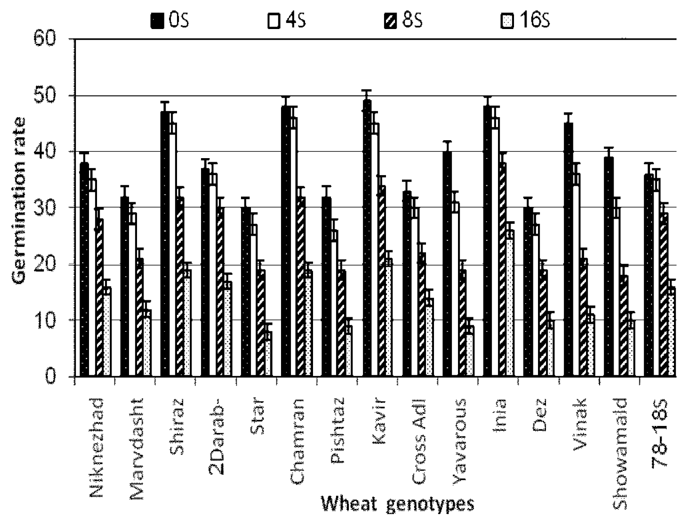

Fig. 1: Effect of salinity levels on germination rate of wheat genotypes

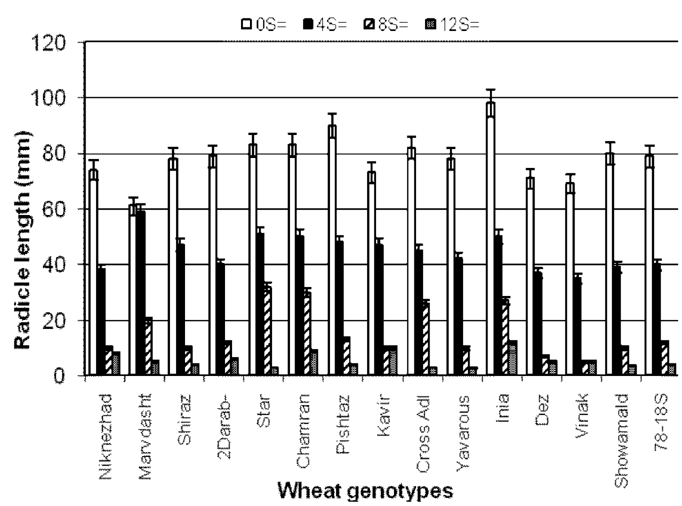

Fig. 2: Effect of salinity levels on radicle length of wheat genotypes

There are reports suggesting that salt may affect the germination rate to a greater extent than the germination percentage [52]. These results are also similar to Jamil and Rha [32]. They reported that germination of sugar beet and cabbage decreased as salinity concentration increased and salinity also delayed germination rate. Similar kinds of results were reported by Jeannette *et al.* [53]. They found that the mean time to germination of almost all Phaseolus species increased with the addition of NaCl and this increase in median germination time was greater in higher concentration as compared to low concentration.

Rapid seed germination and stand establishment are critical factors to crop production under salt stress conditions particularly critical in semi-arid areas where favorable conditions in the seed zone may be brief. In many crop species, seed germination and early seedling growth are the most sensitive stages to salinity stress [34].

It assumed that germination rate and the final seed germination decreased with the decrease of the water movement into the seed during imbibitions [54]. Similar declines in seed germination rate have been reported in the literature [33, 35].

Radicle and Hypocotyl Length: These two traits were highly sensitive to salt with about 45% reduction even at the lowest concentration of 4 ds m^{-1} NaCl (Table 4). With increasing salinity, radicle length decreased progressively and there was a negative correlation (r= -0.981, P <0.001) between salt concentration and radicle length (Table 5). There was also a negative correlation (r=-0.995, P< 0.001)

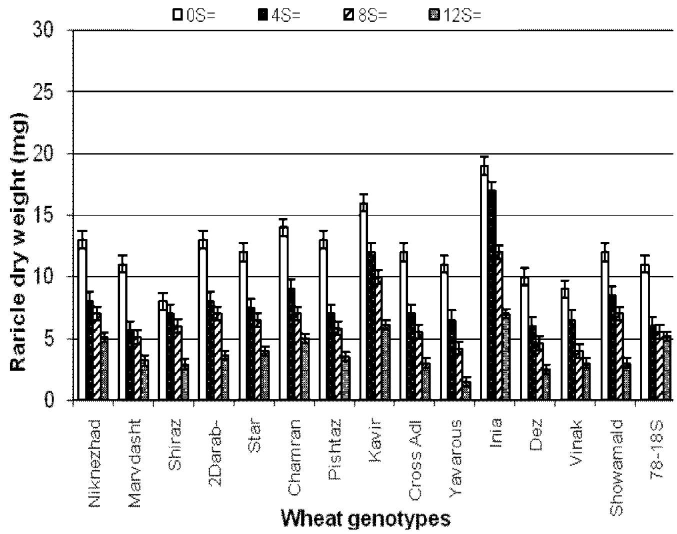

Fig. 3: Effect of salinity levels on radicle dry weight of wheat genotypes

between salt concentration and hypocotyle length (Table 5). On average over all the cultivars at 4, 8 and 16 ds m^{-1} NaCl salinity, radicle length were about 41, 300 and 700 % of the control. Hypocotyle length also decreased to 58, 350 and 600 % of the control at 4, 8 and 16 ds m^{-1} NaCl, respectively. Cultivars performances were not consistent with increasing salinity except Inia and Kavir cultivars. Inia and Yavarous had the greatest and lowest radicle length (12 and 2.7 mm) at the highest salinity level than the other cultivars (Fig. 2). Hypocotyle length was reduced greatly with further increase in salt (Table 4).

The reason for reduced shoot and root development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant [55]. Neumann [1] indicated that salinity can rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil. Salt stress inhibited the growth of shoot more than root in Brassica species [27]. Similar observations have been reported in barley (*Hordeum vulgare* L.) [50], bean (*Phaseolus acutifolius* L.) [56] and tomato (*Lycopersicon*) [57].

The shoot and root length are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress [32].

Radicle and Hypocotyls Dry Weight: Radicle and hypocotyl dry weight was also affected related to the salt concentration. There were also differences between cultivars in response to salinity (Table 3). On average over all cultivars at 4, 8 and 16 ds m^{-1} NaCl salinity radicle dry weight were about 30, 110 and 500 % of the control (Table 4). Hypocotyle dry weight also decreased to 30, 160 and 600 % of the control at 4, 8 and 16 ds m^{-1} NaCl, respectively. At the lowest salt concentration (4 ds m^{-1} NaCl) radicle dry weight of all the cultivars were reduced to about 30-70% to the control. The highest and the lowest radicle dry matter at 16 ds m^{-1} NaCl salinity produced in Inia and Dez, respectively (Fig. 3). Kavir and Chamran also showed the highest dry weight at 8 and 16 ds m^{-1} NaCl salt. The radicle and hypocotylel of Inia, Kavir, Chamran and Niknezhad did not appear to be as adversely affected by increasing salt as other cultivars. A negative correlation was found between radicle dry weight and salt concentration ($r = -0.743$, $P<0.01$) (Table 5).

Nieman [19] found that growth inhibited by salt partly due to the diversion of energy from growth to maintenance. Munns [58] reported that growth reduction of vascular plants at high salinity levels may be attributed to the reduction in photosynthetic rate of plant tissues, which could arise from adverse effects of Na^{+}, Cl^{-} and water stress on metabolism.

Growth reduction due to salinity is also attributed to ion toxicity and nutrient imbalance. Salt stress in addition to the known components of osmotic stress and ion toxicity, is also manifested as an oxidative stress [59].

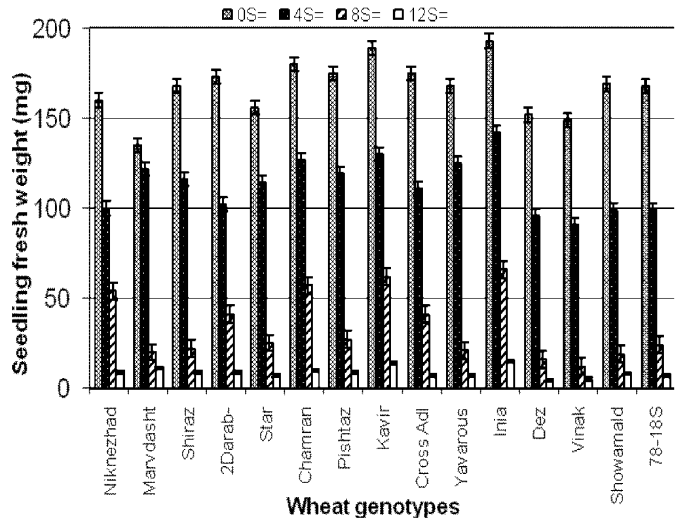

Fig. 4: Effect of salinity levels on seedling fresh weight of wheat genotypes

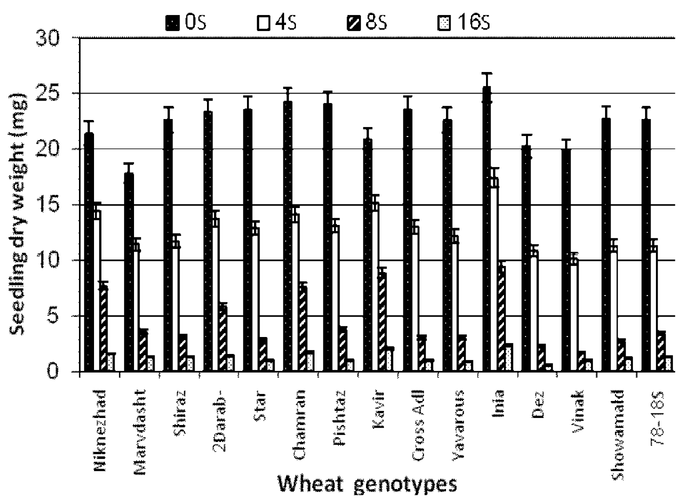

Fig. 5: Effect of salinity levels on seedling dry weight of wheat genotypes

However, ion content and salt tolerance are not often correlated and several studies indicate that acquisition of salt tolerance may also be a consequence of improving resistance to oxidative stress [60].

Seedling Fresh and Dry Weight: Significant difference in seedling fresh and dry weight between salinity levels and cultivars was observed in this study (Table 3). On average over all cultivars at 4, 8 and 16 ds m^{-1} NaCl salinity seedling fresh weight were about 16, 60 and 150 %, respectively of the control (Table 4). A deleterious effect of salt on seedling dry weight was observed at all salt concentrations on all the cultivars except Inia at all salinity levels. However, on average over all cultivars at 4, 8 and 16 ds m^{-1} NaCl salinity seedling dry weight was reduced to about 25, 110 and 600 %, respectively of the control. Cultivars also differed in the response of seedling fresh and dry weight to salinity levels (Table 3). Shoot fresh weight of Inia and Kavir cultivars was not adversely affected at all salinity levels, whilst in all other cultivars seedling fresh weight was reduced and ranged from 52 to 85% relative to the control (Fig. 4). Inia and Kavir consistently maintained higher seedling fresh and dry weight at all salt levels, respectively (Fig. 4, 5). Lower seedling fresh and dry weight was observed in Dez, Vinak, Crros Adl and Star (Fig. 4, 5). The seedling dry weight of Inia, Kvir and Chamran cultivars did not appear to be as adversely affected as other cultivars at 8 and 16 ds m^{-1} NaCl. This indicates the existence of genetic diversity of these traits among the wheat cultivars and it is in agreement with the results obtained by Hemati and Pakniyat [61], who reported variation of these traits in bread and durum wheat cultivars in response to salt stress. Reduced seedling growth has also been reported by Huang and Reddman [50] on barley, Foolad and Jones [62] on tomato and Jeannette *et al.* [53] on phaseolus under salt stress condition.

The response of plants to salt stress is a complex phenomenon that involves biochemical and physiological processes as well as morphological and developmental changes [63, 64]. Difference among species and cultivars for salinity tolerance may depend on their differences in salinity tolerance mechanism. Exploitation of these useful genetic variations in salinity tolerance particularly of crop plants is an economical approach for proper utilization of salt-affected agricultural lands [8, 3].

CONCLUSION

Among Iranian genotypes, Inia, Kavir and Chamran cultivars indicated the highest salt tolerance while Dez, Vinak, Cross Adl and Star cultivars showed the lowest. Furthermore, Dez and Vinak were more sensitive at moderate and high salinity levels and to become more tolerant at low salinity levels, it is suggested that maintaining the salinity at low levels is an important strategy for improving the growth of these two varieties. The data on the average length of shoot and shoot shows that all wheat genotypes revealed a strong inhibition with the increasing level of salt solution. There was considerable reduction in the size of shoot and root at highest level of salinity (16 ds m NaCl). These results showed sigh of grate inhibition of shoot and root growth with NaCl treatments. The decrease in length of radicle was more pronounced as compared to root in all NaCl salt treatments; however this decrease was more prominent in Dez cultivar than others. Great inhibition in root length was also recorded in Yavarous.

Finally, it can be concluded that to select cultivars for better salt stress tolerance at seedling stage coleoptile and root elongation may be used as breeding criterions. More vigorous cultivars like Inia and Kavir could be considered as plant materials which are useful to breeders for future development of salt tolerant wheat cultivars. Longer coleoptiles may increase their potential to emerge under salt stress condition. Results therefore could be used for prediction of sowing rates depending upon expected conditions. This study showed that salt stress inhibits coleoptiles growth more than root growth. Similar results were found by Foolad [57], Huang and Reddman [50] and Jeannette *et al.* [53]. The results in this study also indicates that the ranking among genotypes for salt tolerance based on the germination rate, dry weight of total plant, root and shoot length show close correlated with their tolerance on salinity levels.

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