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Salinity (NaCl) Tolerance of Wheat Genotypes at Germination and Early Seedling Growth

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Abstract: Salinity tolerance during germination and early seedling growth was evaluated for nine wheat cultivar (*Triticum aestivum* L.) in six treatments of salinity including 0 (control), 75, 150, 225, 300 and 375 mM NaCl in a 3 replicated RCBD. The results showed that different treatments of salinity had considerable effect on the germination percentage, germination rate, elongation of the first leaf and root, total dry weight and root/shoot dry weight. Germination percentage in all cultivar showed considerable decrease with increasing salinity up to 375 mM NaCl. This reduction was more in Pishtaz and Ghods as compared to Tajan and Karchia. The seedling growth of nine genotypes was significantly inhibited by all salinity levels. During early seedling growth, applied NaCl salinity significantly affected the RGR of all the genotypes, particularly at 150-375 mM NaCl but there was no significantly difference at the low salinity treatments ($p<0.05$). The effect of salinity on RGR values varied according to the salt tolerance of the genotypes. First leaf and root length of all genotypes were considerably reduced. Elongation of the first leaf was more affected as compared to root growth at all salinity levels. Also, the rate of reducing of total dry matter was slower than reducing of leaf elongation. Cluster analysis with multiple parameters simultaneously to evaluate the salt tolerance revealed three groups. Karchia was used as a standard for the salt tolerance test. Among 9 genotypes, Tajan was the most tolerant and Pishtaz and Ghods were the most sensitive genotypes.

Key words: *Triticum aestivum* L. (wheat), germination, seedling growth, salt, NaCl tolerance

INTRODUCTION

Soil salinity is one of the most important factors that limit crop production in arid and semi arid regions (Neumann, 1995). Salinity affects about 7% of the world's total lands area (Flowers *et al*., 1997). The percentage of cultivated land affected by salt is even greater, comprises 19% of the 2.8 billion hectares of arable land on earth (Ponnamierumo, 1984; Pessarakli and Szabolcs, 1999; El-Hendawy *et al*., 2004). 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 (Raza, 2005). Wheat is a moderately salt tolerant crop and serves as a staple food in 43 countries (Pervaiz *et al*., 2002; Raza, 2005); including Iran, where it is grown on a large area. On the other hand, Iran is one of the countries that suffer from severe salinity problems. For example 18 M ha or 10% of total land area in Iran is salinity or sodicity soil.

Most crop plants are glycophytes, which have evolved under low salt condition. The mechanisms they have evolved for uptake, transport, recirculation and utilization of minerals may not function optimally under saline conditions. Salinity decreases germination (Murillo-Amador *et al*., 2000a; Gehlot *et al*., 2005; Sharma *et al*., 2004), dry matter accumulation, the rate of net CO_2 assimilation, relative growth, leaf cell expansion and ultimate leaf growth (Murillo-Amador *et al*., 2000b; Cramer *et al*., 2001; Saqib *et al*., 2004; Mansour *et al*., 2005). The plant growth is ultimately reduced by salinity stress but plant species have different responses to salinity. Salt tolerance of crops may vary with their growth stage (Munns and Termaat, 1986; Mass *et al*., 1994; Rogers *et al*, 1995; Flowers *et al*., 1997).

The establishment stage of the crop consists of three parts: germination, emergence and early seedling growth; that are particularly sensitive to substrate salinity (Mariko *et al*., 1992; Baldwin *et al*., 1996; Grieve and Suarez, 1997; Jamil *et al*., 2005; Raza, 2005). Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the

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seed species to germinate and grow while soil moisture and osmotic potentials decrease (Welbaum *et al*., 1990; Roundy, 1985). Much information is available in literature about the effects of water quality, soil texture and soil salinity on germination and emergence (Grillot, 1957; Maas, 1986; Jamil and Rha, 2004; Jamil *et al*., 2005). Retardation and reduction in seed germination have been reported under NaCl treatments in the literatures (Sharma *et al*., 2004; Gill *et al*., 2003; Garrciarrubio *et al*., 2003). The decrease in germination rate particularly under drought and salt stress conditions may be due to the fact that seeds seemingly develop an 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 seedlings (Gill *et al*., 2003). During early seedling growth, salinity and soil texture affected the development of the seedlings that showed symptoms of water stress. The consequence of water stress could already be observed some days after emergence, the higher water stress, expressed by lower leaf water potential, stomatal conductance and evapotranspiration and the lower the leaf area and dry matter production (Katerji *et al*., 1994; El-Hendawy *et al*., 2005).

Salinity stress is an important characteristic when selecting a variety for salinity tolerance (Konak *et al*., 1999). Numerous traits related to salt tolerance that have been used to screen germplasm include germination percentage, seedling root and shoot attributes, rates of Na^+ or Cl^- accumulation in leaves (Munns *et al*., 1995, 2006), ion concentration in root cells (Flowers and Hajibagheri, 2001; Munns *et al*., 2006). Munns *et al*. (2006) discussed physiological mechanisms and selectable indicators of gene action, with the aim of promoting new screening methods to identify genetic variation for increasing the salt tolerance of cereal crops, particularly with respect to wheat. They reported that precise phenotyping is the key to finding and introducing new genes for salt tolerance into crop plants.

Furthermore, Growth rate is a key parameter, but the Relative Growth Rate of plants (RGR) under saline condition has been considered to allow more appropriate comparisons of growth among species or genotypes than absolute growth rate (Cramer *et al*., 1994; Jaradat *et al*., 2004). The RGR is a function of the Net Assimilation Rate (NAR), which is an index of the photosynthetic-assimilatory capacity of the plant per unit leaf area. At the level of the whole plant, therefore, this parameter may make it possible to clarify whether genotypic variation in salt tolerance can be attributed to morphological changes or photosynthetic response (Ishikawa *et al*., 1991; El-Hendawy *et al*., 2005).

Studies on a range of grass species showed that leaf elongation rate reduced by salinity. The relative elemental growth rate of leaf and the length of elongation zone respond in various ways to environmental constraints on the plant (Delane *et al*., 1982; Bernstein *et al*. 1993a, b; Hu *et al*., 2000; Neves-Piestun and Bernstein, 2005). Leaf growth of grasses is of central importance to their development, not only is the expansion of leaves crucial to early seedling establishment by providing a continuous supply of energy and carbon through photosynthesis, it also facilitates development of other organs such as tillers, ears and grains (Hu *et al*., 2005).

Genetic variation for desirable plant traits is fundamental to any plant improvement program and dictates potential progress. Researchers are trying to get the salt resistant crop on which human's food depend. Thus, screening for salt-tolerant wheat germplasm is important to determine whether there is a genetic basis for selection and breeding purposes and to whether there are useful genotypes or new genes for tolerance to salt stress. Although there are extensive studies of salinity effects on wheat, research about the effects of salinity on wheat and critical thresholds of responses is still limited at early seedling growth. The present study was undertaken to study the responses of nine wheat cultivars to different levels of salinity (75-375 mM NaCl) and to determine the genotypic variability in their tolerance to salinity both at the germination and seedling stage. For screen the different wheat genotypes, we used multiple physiological parameters.

MATERIALS AND METHODS

Plant material and growth conditions: Nine cultivars of wheat (Triticum aestivum L.), were used in this study. All seeds (Chamran, Ghods, Karchia, Pishtaz, Shahryar, Shahpasand, Shiraz, Tabasi and Tajan variety) were obtained from the Seed and Plant Improvement Institute in Iran. The experiment was carried out in Azzahra University, Tehran, Iran in September 2004. Karchia is the most tolerant wheat genotype and is used as a standard for the salt resistance test of wheat worldwide (Sharma *et al*., 1994; Ashraf, 2002).

Similar seed size and weight was selected to exclude effect of that on the seedling establishment. Seeds were surface sterilized in 1.5% (v/v) sodium hypochloride for 10 min and thoroughly washed with sterile tap water. Although field screening for salt tolerance has the advantage of testing germplasm under natural conditions, it is less efficient at germination and early growth stages and also it is more expensive than screening under controlled conditions (Shannon and Noble, 1990). For this

reasons, fifteen seeds were placed in 10 cm sterile petri dishes on filter paper supplemented with salt solutions. Salt stress was applied by subjecting the seeds to 5 mL of control (no added NaCl), 75, 150, 225, 300 and 375 mM NaCl solutions. Plates were sealed with parafilm and placed under controlled conditions (25±2°C during the day; 16/8 h Light/ dark; irradiance 4500 Lux) Seeds were considered to have germinated when the radicle measured in excess of 2 mm.

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

$$\text{Germination Rate} = X1/Y1 + (X2-X1)/Y2 + \ldots + (Xn-Xn-1)/Yn$$

Where Xn is the germination percentage on the nth day and Yn is the number of day from first day experiment (Maguire, 1962). The mean germination percentage was calculated from number of seedlings, at 7th day of growth, having axes at least 5 mm long derived from each Petri dish.

Growth parameters: The fresh and dry weight of the shoots and roots, the length of shoots and roots, length of first leaf and coleoptile were measured immediately after the end experiment of stress treatment. The dry weights were measured by drying the shoot and root at 75°C for 48 h, to give a constant weight.

Relative Growth Rate (RGR): Plant growth was evaluated in terms of RGR. Root and shoot growth of the nine genotypes was assessed by measuring dry weights at the 3 day of start and end of the treatments and calculating Relative Growth Rates (RGR). These were estimated as mean values over the time interval T1 to T2 according to Hunt (1990) using the following equations:

$$RGR = \frac{\ln(W_2) - \ln(W_1)}{T_2 - T_1}$$

where, T and W represent time (days) and total plant dry weight (g) respectively. Subscripts 1 and 2 refer to the values of the variable measured at two successive harvests (1, initial and, 2, final). Relative growth rate is expressed in g g^{-1}/day.

Standard errors (SE) were calculated for each variable.

Statistical analysis: All the experiments were conducted by using a Randomized Complete Block Design (RCBD) with three replications. For isolation of intrinsic growth potential of genotypes from their tolerance, all data from every experiment divided to its control value of every genotype. As data were corrected, data related to controls are equal and have no effect in total variance thus they deleted in statistical analysis. Then remaining data transformed into arcsin form and were subjected to analysis of variance (ANOVA) to examine the effects of cultivar, salinity treatment and their interactions after testing for normal distribution and homogenous variance. Significant differences between treatments were determined using Duncans multiple range test at the 0.05 level. Analysis of variance was performed by using the SPSS software version 9 and Microsoft Excel 2002.

For grouping the lines that show similar growth characteristic, different clustering methods as well as ordination based on principal components analysis was performed (Chatfield and Collins, 1995).

RESULTS

At early growth stage of seedling, the relative salt tolerance indicates for all the measured parameters varied among genotypes. The 9 genotypes used in this study were classified into 3 groups: salt-tolerant (Karchia, Tajan, Shiraz), moderately tolerant (Chamran, Shahryar, Tabasi) and salt-sensitive genotypes (Shahpasand, Pishtaz, Ghods) based on the ranking of 7 characters of these genotypes (Fig. 1). This study revealed a remarkable reduction in the germination rate, germination percentage, total dry weight and length of root, coleoptile and first leaf at early stage of seedling growth (Table 1). The low salinity treatment (75 mM) reduced these parameters to a lesser degree than high salinity treatment and even related to some parameters, such as root/shoot dry weight ratio, they increased (Table 2).

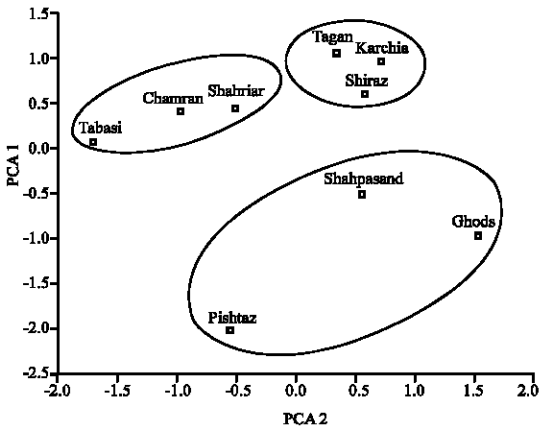

Fig. 1: Cluster analysis and ordination of lines that treatment by different concentration of NaCl, PCA represented Principal Component Analysis

Table 1: Means of some studied characters of 9 wheat genotypes at early stage of growth seedling

Character	Salinity (mM NaCl)	Mean±SD	Character	Salinity (mM NaCl)	Mean±SD
Leaf length	0	62.99±14.835	Root/Shoot dry weight	0	0.599±0.172
	75	53.667±11.453		75	0.657±0.111
	150	37.727±11.241		150	0.668±0.167
	225	18.775±9.169		225	0.768±0.196
	300	7.626±5.925		300	0.823±0.159
				375	1.094±0.201
Root length	0	53.475±12.854	Germination rate	0	42.460±6.721
	75	61.592±19.567		75	36.772±9.975
	150	42.471±22.214		150	34.193±11.297
	225	27.364±10.927		225	31.164±12.351
	300	21.713±6.131		300	28.446±13.411
	375	13.398±4.491		375	21.951±11.775
Coleoptile length	0	21.457±3.936	Germination percentage	0	90.0±12.247
	75	22.086±4.623		75	80.37±18.962
	150	19.648±4.696		150	73.518±21.056
	225	17.756±4.146		225	67.222±22.837
	300	16.361±5		300	62.037±26.455
	375	8.96±3.435		375	48.148±25.103
Total dry weight	0	0.041±0.007	RGR	0	0.131±0.048
	75	0.039±0.006		75	0.167±0.052
	150	0.03±0.006		150	0.157±0.049
	225	0.022±0.007		225	0.108±0.052
	300	0.018±0.005			
	375	0.011±0.004			

Table 2: Salt tolerance indices of physiological parameters in wheat genotypes under different salinity levels at germination and early growth stage

	Karchia	Tagan	Shiraz	Tabasi	Chamran	Shahriar	Shahpasand	Pishtaz	Ghods
Germination Rate (mM NaCl)									
0	46.667a	48.214a	43.929ab	39.048bc	46.25a	48.571a	36.786c	28.036d	44.643ab
75	44.464a	43.214ab	40.476ab	37.679a	45.179a	46.131a	29.286b	17.5c	27.024c
150	43.155ab	42.738ab	37.143b	38.988a	44.345ab	42.560ab	21.905c	15.595cd	21.31d
225	42.857a	43.214a	34.226a	34.821a	41.25a	38.155a	17.44b	12.679b	15.833b
300	40.833a	42.321a	36.369a	25.952b	39.821a	35.357ab	9.881d	10.655c	14.821cd
375	38.571a	34.94ab	25.952a-c	21.369b-d	19.226c-e	31.726ab	9.405ef	10.238de	6.131f
Germination percentage (mM NaCl)									
0	96.667a	98.333a	100.0a	81.667b	93.333a	96.667a	78.333b	65.0c	100.0a
75	91.667ab	91.667ab	91.667ab	83.333a	90.0ab	96.667ab	66.667bc	36.667d	75.0c
150	88.333a	88.333a	83.333a	78.333a	90.0a	90.0a	50.0b	31.667b	61.667b
225	85a	91.667a	80.0a	68.333a	85.0a	78.333a	41.667b	26.667b	48.333b
300	81.667a	88.333a	86.667a	48.333bc	83.333a	75.0ab	25.0d	23.333d	46.667cd
375	76.667a	83.333a	63.333ab	38.333bc	43.333bc	66.667ab	20.0cd	23.333cd	18.333d
Dry weight (mM NaCl)									
0	0.049a	0.033c	0.052a	0.036bc	0.038bc	0.038b	0.049a	0.034c	0.041b
75	0.044a	0.046b	0.047c	0.035c	0.033c	0.034c	0.046c	0.034c	0.038c
150	0.039a-c	0.029ab	0.038a-d	0.021e	0.026c-e	0.025de	0.034c-e	0.03a	0.029b-e
225	0.033a	0.026a	0.026b	0.014c	0.021b	0.022b	0.028b	0.011c	0.02b
300	0.023bc	0.023a	0.021cd	0.013d	0.017bc	0.021b	0.022bc	0.008e	0.015d
375	0.016ab	0.014a	0.012cd	0.011bc	0.007de	0.013ab	0.016ab	0.004e	0.008de
(Root/shoot) dry weight (mM NaCl)									
0	0.500bc	0.384c	0.628ab	0.353c	0.484bc	0.759a	0.751a	0.720a	0.808a
75	0.622ab	0.545a	0.714a-c	0.479a	0.62a	0.699bc	0.624c	0.769a-c	0.839a-c
150	0.669a	0.543ab	0.635a-c	0.437ab	0.609ab	0.777a-c	0.575c	1.018a	0.749bc
225	0.701a	0.599a	0.807a-c	0.518bc	0.81a	0.878a-c	0.65c	1.195a	0.753bc
300	0.673a-c	0.612ab	1.097a	0.656a	0.869a	0.842bc	0.947a-c	0.773c	0.936bc
375	1.184ab	1.313a	1.419ab	0.902ab	1.178ab	1.155bc	0.921c	0.889c	0.888c
RGR (mM NaCl)									
0	0.097cd	0.105cd	0.151a-c	0.140b-d	0.071d	0.086cd	0.215a	0.129b-d	0.189ab
75	0.109bc	0.254a	0.217a-c	0.131c	0.152ab	0.112bc	0.192c	0.128c	0.206bc
150	0.088b	0.149b	0.124b	0.122b	0.182a	0.147b	0.158b	0.177b	0.262b
225	0.063a-c	0.144ab	0.070c	0.029a-c	0.077a-c	0.141a	0.165bc	0.101a-c	0.183a-c
300	-0.021c	0.171ab	0.128bc	0.069bc	0.162a	0.145ab	0.188bc	0.113bc	0.287ab
375	0.139a	0.148a	0.079a	0.099a	0.06a	0.103a	0.113a	0.0b	0.0b
First leaf length (mM NaCl)									
0	68.545b	54.167c	54.583c	39.0d	51.917c	62.583bc	69.111b	82.25a	84.778a
75	53.417cd	67.75a	51.417bc	41.333ab	41.583cd	44.500cd	54.75cd	53.083d	75.167bc
150	36.167bc	48.75a	38.333b	19.222cd	32.083bc	33.167bc	46.091bc	29.222d	56.417b
225	28.583b	30.417a	20.667bc	13.333bc	20.0bc	22.909bc	12.889d	0e	20.182cd
300	2c	17.25a	9.583b	2.667c	13.222c	12.417b	7.833b	0d	3.667c
375	0b	0.0b	0.0b	0.0b	0.0b	3.571a	2.667a	0b	0.0b

Table 2: Continued

	Karchia	Tagan	Shiraz	Tabasi	Chamran	Shahriar	Shahpasand	Pishtaz	Ghods
Root length (mM NaCl)									
0	55.5b	44.667bc	51.571bc	39.7bc	37.4c	50.333bc	55.875b	73.9a	72.333a
75	67.167a-c	68.917a	60.333a-c	44.111bc	48.667a-c	37.5d	49.5cd	77.5bc	100.636ab
150	39.889bc	45.750b	42.333b-d	15.917e	24.833cd	30.333d	43.083b-d	45.1d	95.0a
225	50.25a	35.667a	29.667b	17.333bc	21.25bc	21.333cd	19.111de	18.417e	33.250b-d
300	30.583a	25.167a	27.583a	16.083bc	22.0a	17.667c	26.333ab	12.167e	17.833d
375	17.833bc	17.667ab	18.333ab	18.0a	9.0cd	10.083de	11.833de	7.333f	10.5ef
Coleoptile length (mM NaCl)									
0	28.667a	19.0de	19.0de	20.917cd	17.833de	17.0e	21.111cd	26.25ab	23.333bc
75	24.833a	21.25b	20.583b	28.444a	16.833a	15.667a	19.583a	28.75b	22.833bc
150	22.083c	18.25ab	20.667a	15.917c	16.25b	17.917a	13.25d	28.583a	23.917ab
225	24.667ab	19.083ab	19.75a	13.25c	15.167ab	15.75ab	17.222b	12.333d	22.583ab
300	24.75bc	19.0ab	20.583a	13.417e	14.583b-d	16.333ab	14.0de	7.083f	17.5c-e
375	12.167a-c	11.667a	8.0a-c	12.0a	4.667c	10.667a	10.833ab	2.556d	8.083bc

Value followed by different letter(s) differ significantly at p>0.05

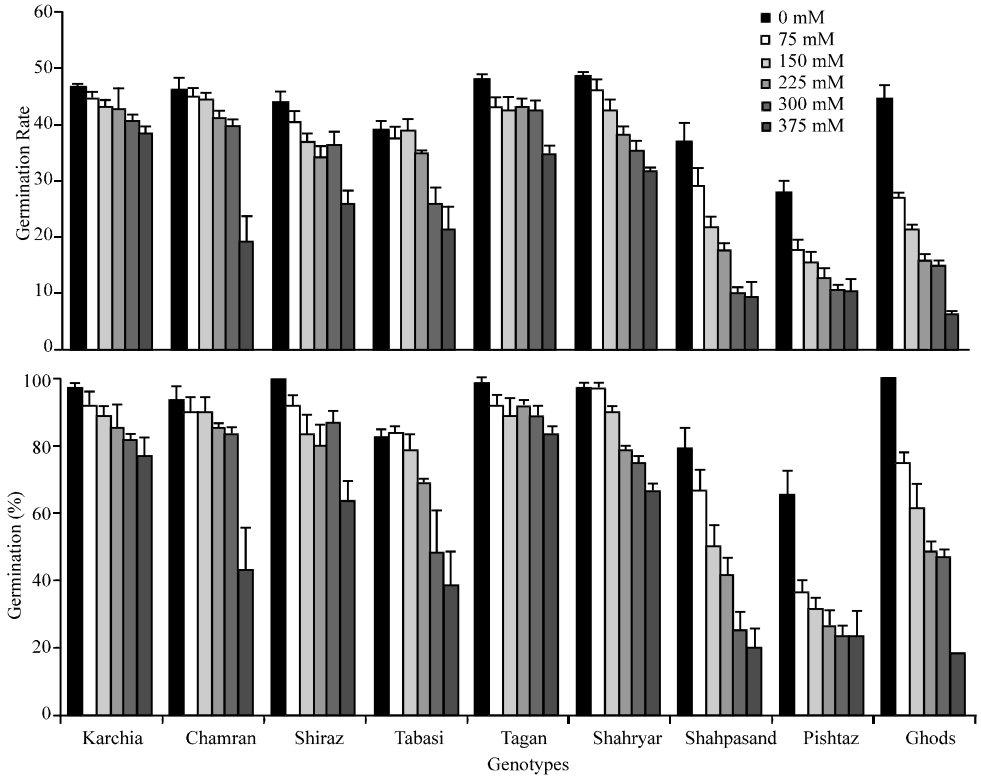

Fig. 2: Effect of different salinity levels on the germination percentage (A) and germination rate (B) for different wheat genotypes. Error bars represent standard deviations

Karchia, Tajan, Shiraz and chamran genotypes displayed the greatest germination percentage, dry weight and leaf area and leaf length in high salinity treatment. This indicates that salinity affected on the germination, emergence and early seedling growth and there was different response to salt stress among genotypes.

GENOTYPIC VARIATION IN GERMINATION

Although genotypes indicated a reduction in the final seed germination under increased salinity, analysis of variance revealed that significant differences were evident among the wheat genotypes for germination percentage and germination rate under different salinity treatments ($p<0.01$) germination percentage was high level among tolerant and then moderate genotypes (Fig. 2) and salinity treatments, with the exception of 375 mM NaCl, show no significant effect on it. Karchia and Tajan locates in grouping a, because they showed good germination response at all salinity levels among all lines. Also rate and percentage of germination of Karchia, Tajan, Chamran and Shiraz varied very low by increased salinity treatment until 300 mM NaCl.

Percentage of germination in the Pishtaz, Shahpasand, Ghods and Tabasi genotypes was strongly affected by all salinity treatments. Germination response to NaCl was significantly different in case of Pishtaz; its final germination was lesser than 70% at the control (0 mM NaCl) and by increased concentration of salt reduced to half at 150 mM. The reduction being strongest particularly at the highest level of salt concentration compared to control. Germination percentage of Tabasi, Pishtaz, Shahpasand and Ghods reached to 39, 36, 25 and 18%, respectively, as compared with the controls at 375 mM NaCl treatment. While final germination in Karchia, Tajan and Shiraz at 375 mM NaCl was 79, 84 and 63% of the control, respectively.

In view of germination rate, there was considerable reduction in this character in Pishtaz, Shahpasand and Ghods genotypes at all salinity levels compared to others, this parameter among 3 lines mentioned above restricted to 6-10% at 375 mM NaCl (Table 2). Although Ghods cultivar shows suitable germination at control level (percentage and rate germination was 100 and 44.6% respectively) but this line considered as sensitive cultivar because by increasing of salt concentration induced a significant decrease in these parameters. For example, percentage and rate of germination of Ghods genotype reduced to 18.3 and 13.7% at 375 mM NaCl than control.

GENOTYPIC VARIATION IN GROWTH

Dry matter production is an important criterion to evaluate salt tolerance in plants since it permits direct estimations of economic returns under specified saline conditions (Maas, 1986). Much less information is available about effect of salt stress on growth of leaf area and dry matter during early seedling growth, because young seedlings are rather delicate material for such measurements.

Effects of different salinity levels on dry weight of total plant and root/shoot dry weight ratios of different genotypes show in Fig. 3. Analysis of dry weight of the varieties showed that all the genotypes had decreased levels of dry matter production and concomitantly increased root/shoot ratio with increased substrate salinity (Fig. 3). The difference among the genotypes and salinity levels and their interaction was significant ($p<0.01$). Regarding to applied different salinity treatment in each genotype, there was no significantly different between 0-75 mM NaCl treatments however by increased salinity from 150 to 375 mM NaCl revealed that significant differences among the salinity levels ($p<0.05$). There were obvious differences among the 3 genotype groups in total dry matter, root/shoot biomass and RGR values. The salt tolerant group had higher dry matter at moderate and high salinity levels, reduced dry matter per plant for the salt tolerant genotypes were about 21% at 150 mM NaCl, 54% at 300 mM NaCl and 63% at 375 mM NaCl lesser than the controls, respectively, whereas in sensitive genotypes this value reduced about 58% at 150 mM NaCl, 68% at 300 mM NaCl and 81% at 375 mM NaCl compared with the controls, respectively.

Shiraz, Karchia and Shahpasand genotypes produced more dry matter than other genotypes under control condition but at high concentration of NaCl, ratio of root/shoot dry matter in Shahpasand was lower while Karchia and Tajan genotypes had significantly higher dry matter compared with other genotypes (Table 2). The effects of different salinity levels on the chlorophyll content revealed that total chlorophyll in the leaf of wheat seedlings was not significantly different from 0 to 150 mM NaCl in the tolerant and moderate groups but it was plunged with increased salinity levels from 150 to 300 mM NaCl. Among sensitive genotypes, chlorophyll content of Shahpasand was higher near to tolerant genotypes (data don't show) therefore, it seems that higher dry matter and higher RGR in this variety may be caused for this reason. Response of biomass production in Pishtaz genotype to salinity was completely depended on salinity level and this genotype located in latest class among other lines at all salinity treatment.

Applied NaCl salinity significantly affected the RGR of all the genotypes, but there was no significantly difference among the salinity treatments at $p<0.05$ levels, interaction of the different genotype and salinity was significant at $p<0.1$ levels. The effect of salinity on RGR values varied according to the salt tolerance of the genotypes. RGR values was increased at low salt concentration (75 mM NaCl) in the tolerant and moderate groups, whereas the opposite was found in the salt sensitive group. In this group, RGR values not changed or reduced. For example, compared with the control, RGR value at 75 mM NaCl was increased by about 2.4-fold in Tajan, 2.1 fold in Chamran, 1.4 fold in Shiraz, 1.1 in Karchia and that decreased about 0.9 fold in Tabasi and Shahpasand lines. At higher salinity levels, RGR values were decreased in three genotypic groups, reduce of RGR was obvious especially in Shiraz and Tabasi at 225 mM NaCl level.

At early seedling growth, RGR value in sensitive genotypes of Ghods and Shahpasand was determined higher under non-saline condition, as compared with Karchia. In spite of this, Ghods genotype was reported as a salt sensitive line in Iran in previous studies (Kafi *et al*., 2003). It seems that higher relative growth rate in both

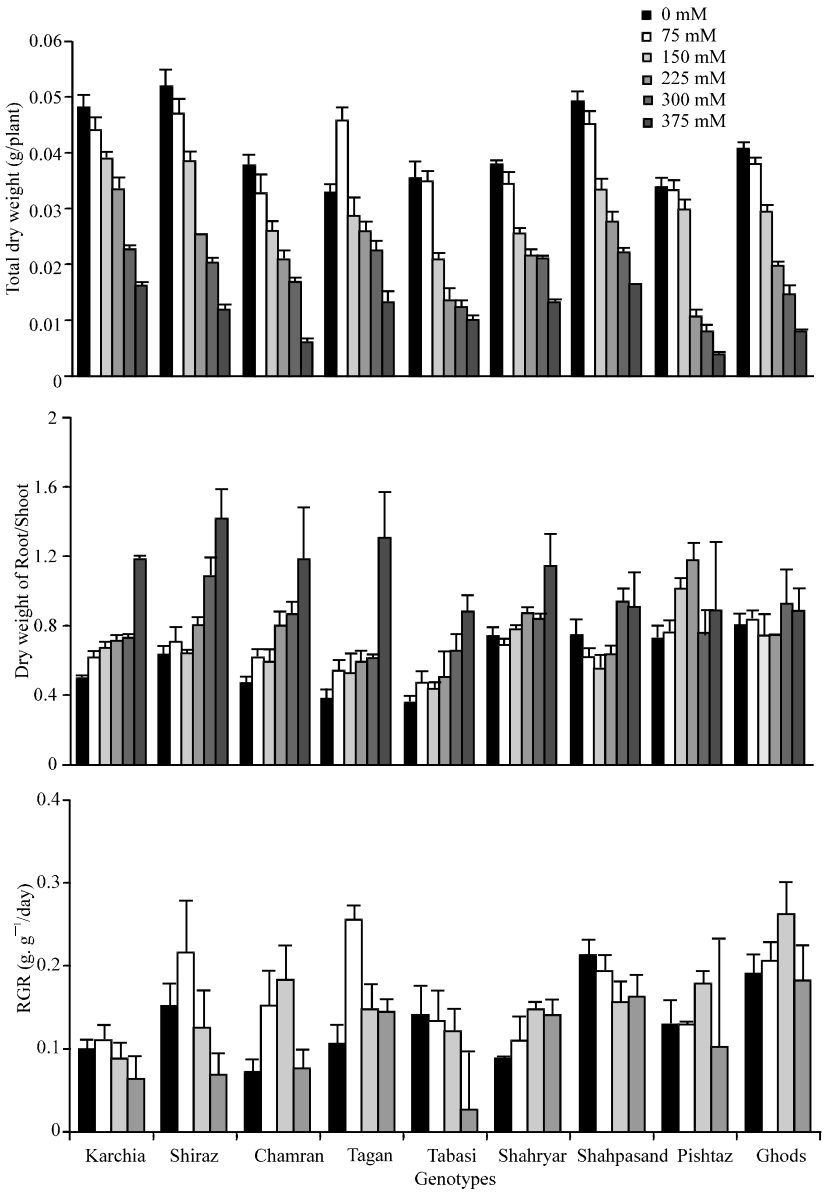

Fig. 3: Effect of different salinity levels on the total dry weight (A), root/shoot dry weight (B) and RGR (C) for different wheat genotypes. Error bars represent standard deviation

lines which mentioned above was depended on genotypic traits and caused harmful ions accumulated at toxic levels during future growth stage and so that decreased growth of these genotypes.

Concerning R/S ratio, there was a highly significant increase at Tajan, Karchia, Shiraz, Chamran and there was a non-significant change at sensitive genotypes such as Shahpasand, Shahryar and Ghods. The increase in this ratio results from relatively greater decrease in shoot than in root growth under salt stress (Fig. 4). The results in Table 2 and Fig. 4 show that there was remarkably increased in R/S ratio of tolerant and moderates groups at 375 mM NaCl treatment. The R/S ratio were increased by an average of 2.8 fold in tolerant genotypes, as compared with the control. Genotype Tajan indicated the highest (3.4 fold) while Ghods showed the lowest (1.1 fold) R/S ratio at the highest salinity treatment. These results are in agreement with the reports by Malik *et al.* (1979). The increase in root/shoot dry matter ratio often implies the development of a larger ratio of root length density to leaf area, which translate into a better capacity for sustaining plant water status under a given evapotranspirational demand (Malik *et al.*, 1979). The classical explanation of water stress in plants growing in a saline environment is

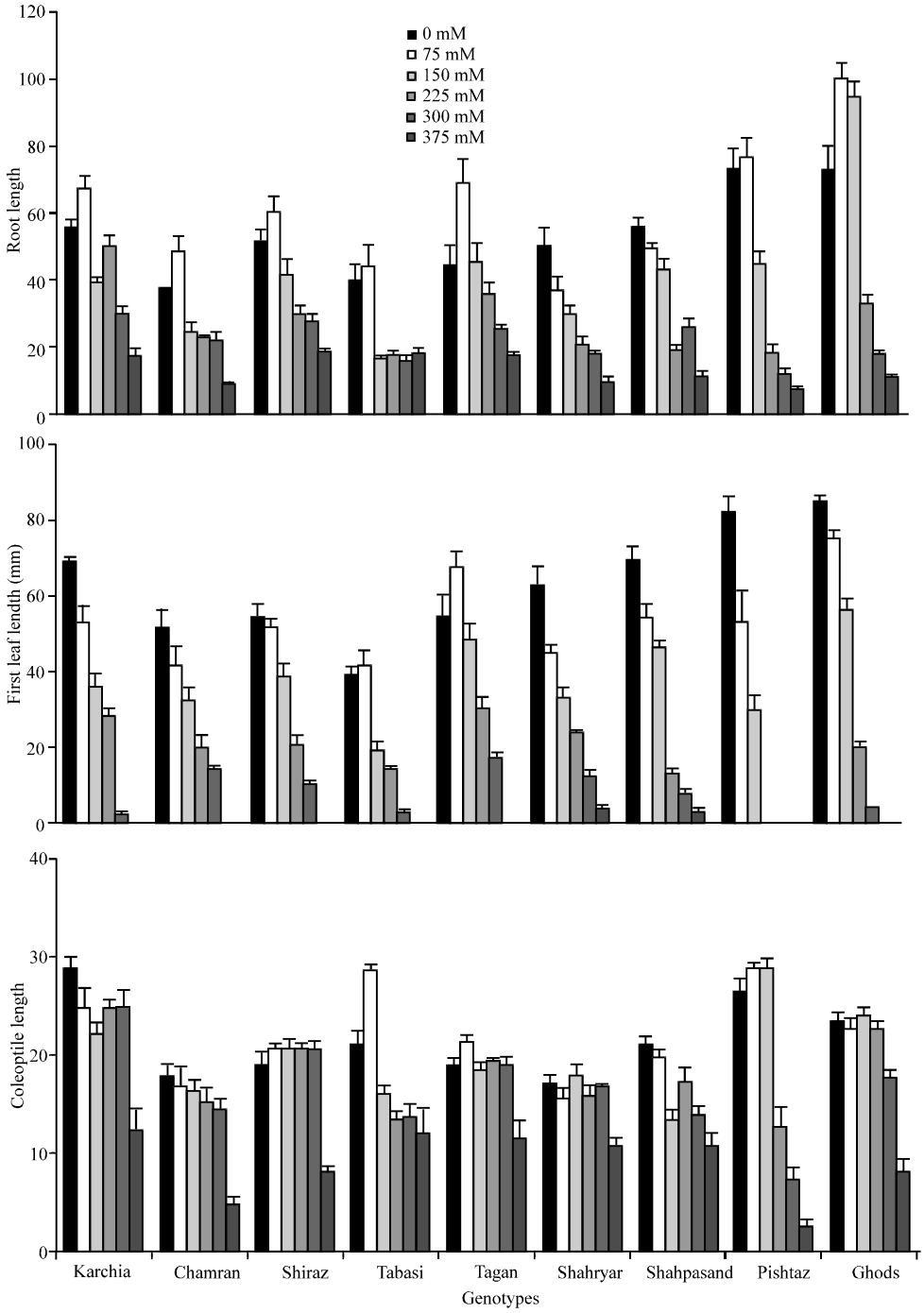

Fig. 4: Effect of different salinity levels of the root length (A), first leaf length (B) and coleptiles length (C) for different wheat genotypes. Error bars represent standard deviations

the reduced availability of soil water due to its osmotic potential. Osmotic adjustment and turgor maintenance in growing region was also important in sustaining root growth at low water potential (Morgan, 1995).

GENOTYPIC VARIATION IN PLANT HEIGHT

Plant height was measured in wheat cultivars and compared across salinity treatments. Height was found to

decrease significantly ($p<0.05$) as the external salinity level increased. Root length of most genotypes at low salinity (75 mM NaCl) was increased, but at high salinity it was decreased by average 21% at 150 mM NaCl, 49% at 225 mM NaCl, 60% at 300 mM NaCl and 75% at 375 mM NaCl, as compared with the control (Table 1). The growth rate of root system in two sensitive genotypes (Ghods and Pishtaz) was approximately 1.46 fold of tolerant-line group under non-saline condition but it decreased remarkably at once plant pass through threshold concentration (150 mM NaCl). Therefore both genotypes located in the end of Duncan's grouping at high concentration levels (Table 2). However as compared with sensitive salinity group, the tolerant-salinity group keep growth rate of root at higher level although they which had lower rate growth under non-saline condition. Root length of tolerant-group was 55 and 33% of the control at 300 and 375 mM NaCl, respectively, while it was 20.5 and 12% of the control at 300 and 375 mM NaCl within sensitive group genotypes.

Same to root length, elongation of the first leaf decreased significantly when salinity elevated ($p<0.05$). Development of leaf was strongly inhibited at 375 mM NaCl and leaves of most genotypes cannot grow. Compared with the control, first leaf length at 150, 225 and 300 mM NaCl was reduced by average 70, 45 and 17% in tolerant genotypes and by 66, 24 and 4% in sensitive genotypes, respectively. Statistically analysis showed that lowest leaf length was noted in genotype Pishtaz and the highest was in genotype Tajan at 300 mM NaCl, (Table 2). Salinity delays leaf emergence and reduced the leaf size of plants both longitudinally and laterally. Although, elongation of the first leaf decreased by enhanced salinity levels from 0 until 300 mM NaCl but the rate of reducing of total plant dry matter was slower (Fig. 3 and 4). This suggests that the relative effect of salinity stress on leaf elongation is significantly larger than that on photosynthesis. Leaf expansion is very sensitive to salt and water stress and it is completely inhibited under mild stress level that hardly affected photosynthetic rate (El-Hendawy *et al*., 2005; Taiz and Ziger, 2002). Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot. Simply state, a shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth; conversely, root will grow until their demand for photosynthate from the shoot equals the supply. This functional balance is shifted if the water supply decreases (Taiz and Ziger, 2002).

Response of coleoptiles elongation to salinity treatment was not significantly different within varieties than control. There was non-considerable reduction in growth of coleoptiles among all genotypes at all salinity levels particularly at 0-300 mM NaCl. At the highest level of salt concentration, the reduction was lower in tolerant and moderate genotype groups but it being strongest in sensitive-genotype group compared to control (Fig. 4). Among these genotypes, the reduction of coleoptile's length was more in Pishtaz. It decreased by about 47, 27 and 10% at 225, 300 and 375 mM NaCl, respectively, While it was 77, 86 and 42% in Karchia at 225, 300 and 375 mM NaCl, respectively, as compared with the control.

CONCLUSION

Little is know about seed germination responses of Iranian wheat cultivars to salinity stress. The studies were carried out to observe the influence of salinity on germination and seedling growth of germinating seeds of 9 wheat cultivars. The wheat genotypes in this study revealed significant difference for germinability and seedling survival under saline condition. Final germination of all genotypes decreased as the salinity level increased and salinity also delayed germination rate. It is assumed that germination rate and the final seed germination decrease with the decrease of the water movement into the seeds during imbibitions (Hadas, 1977). Increasing salinity concentrations in germination often cause osmotic and/or specific toxicity which may reduce or retard germination percentage (Waisel, 1972; Basalah, 1991). Similar declines in seed germination have been reported in the literature (Sharma *et al*., 2004; Gill *et al*., 2003; Garrciarrubio *et al*., 2003). Highly significant differences were observed among the sensitive and tolerant accessions for seed germination at high salinity treatments. These results are also similar to Jamil and Rha (2004). They reported that germination of sugar beet and cabbage decreased as the salinity concentration increased and salinity also delayed germination rate. Similar kind of results was reported by Jeannette *et al*. (2002). 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.

The result indicated that an increased salinity concentration caused delayed emergence of shoot compared to control. A continuous increase in length of shoot and root was detected in frequent days of germination in the control as well as salt treatments. The data on the average length of shoot and root 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 (375 mM NaCl). These results showed sign of great inhibition of shoot and root growth with NaCl treatments. The decrease in length of shoot and first leaf length was more pronounced as compared to root in all NaCl salt treatments; however this decrease was more prominent in Pishtaz than others. Great inhibition in root length was also recorded in Pishtaz. 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 (Jamil and Rha, 2004). It was observed that the degree of the seedling growth reduction increased with the increasing concentration of salt. 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 (Werner and Finkelstein, 1995). Neumann (1995) 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 (Jamil *et al*., 2005). Similar observations have been reported in barley (*Hordeum vulgare* L.) (Huang and Redmann, 1995), pigeon pea (Cajanus cajan) (Subbarao *et al*., 1991), tepary bean (Phaseolus acutifolius) (Goertz and Coons, 1991) and tomato (Lycopersicon) (Foolad, 1996).

The results in this study indicate that the ranking among genotypes for salt tolerance based on the germination rate, dry weight of total plant, root/shoot dry weight ratio and first leaf length at the early growth stage show close correlated with their tolerance on salinity levels, whereas reduction of RGR, specially at low and moderate concentrations of NaCl, cannot revealed differences during early growth stage. This is probably due to short time period between two sampling times. Also, variation in storage content of the seed genotypes may be another reason for this. RGR can again be used as critical parameter by comparisons of their biomass production over a long growth period. Munns and James (2003) suggested that Salinity tolerance was defined as genotypic differences in biomass production in saline versus non-saline conditions over prolonged periods, of 3-4 weeks. In short-term experiments (1 week) measuring either biomass or leaf elongation rates revealed large decreases in growth rate due to the osmotic effect of the salt, but little genotypic differences, although there were genotypic differences in long-term experiments.

Among Iranian genotypes, Tajan indicated the highest salt tolerance while Pishtaz showed the lowest. Furthermore, Ghods and Shahpasand 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. Also, among moderate genotypes Tabasi is more sensitive under saline condition at early growth stage.

Salt tolerance of plants varies during their successive growth stages (Bernstein and Hayward, 1958). The first stage, during which the crop is established, is regarded as particularly difficult, even for tolerant crops (Bernstein and Fireman, 1957; Maas and Hoffman, 1977). Although, studies of salt tolerance at early growth of seedling is important but it is not sufficient, the reproductive stage is the most important in terms of economic yield (Taiz and Zeiger, 2002; Wahid and Rasul, 2004). Therefore, assessment of the actual salt tolerance of the genotypes required to complete by studying of numerous physiological parameters at the reproductive stage for determining the salt tolerance of 9 genotypes that grown in soil under saline conditions within the same experiment.

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