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Annals of Biological Research, 2013, 4 (7):120-126
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Assessment of salinity-related traits in the recombinant inbred lines population derived from the cross wheat varieties of Roshan and Sabalan

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ABSTRACT

The effect of salinity stress on some agronomical and physiological traits was evaluated for 272 F₇ recombinant inbred lines (RILs) derived from a cross between Roshan and Sabalan wheat varieties. Experiment was conducted using a randomized completely block design with three replications. Genotypes were grown in tap water (EC=0.5dsm⁻¹) and saline water (EC=18dsm⁻¹) as control and salt stress treatment with hydroponics in greenhouse. Saline-related traits including sodium and potassium concentrations in shoot and roots, chlorophyll content, plant height, dry and fresh weight of shoot were measured at seedling stage. Significant differences were observed between salinity treatments for all measured traits, except for the chlorophyll content. Differences among RILs were significant for all traits. Salinity stress decreased K⁺ concentration and K⁺/Na⁺; however Na⁺ concentration was increased in Roshan and Sabalan genotypes and all RILs under saline conditions. Also, there was a lower transfer of Na⁺ from root to the shoot and higher ability of leaves for exclusion Na⁺ in the salt tolerant genotypes. Roshan had high amounts of K⁺ and K⁺/Na⁺ in shoot under stress treatment. Moghan3 (as sensitive control variety) showed the most reduction in shoot dry matter under saline conditions, so it can be considered as the most sensitive genotype. One sensitive and tolerant line founded among 272 RILs compared with parental and control varieties. Tolerant line (line 90) had less Na⁺ and more K⁺, resulting in higher K⁺/Na⁺ in shoot, and produced more dry matter compared with tolerant Roshan and Arg (as tolerant control variety) varieties. In contrast, the sensitive line (line 33) had higher Na⁺ and less K⁺/Na⁺, dry matter compared with sensitive Sabalan and Moghan3 varieties.

Keywords: RILs, K⁺/Na⁺ ratio, Salinity, Wheat

INTRODUCTION

Wheat is one of the most important food crops in the world, and it is a part of daily diet of over 70% of the world's population [35]. Salinity is one of the most important limiting factors for crop production in irrigated and rain-fed environments around the world. Salinity stress adversely affects seedling establishment at early growth stages and causes yield reduction [6]. Salinity increase toxic levels in the older transpiring leaves, causing premature senescence and reduce leaf photosynthesis to level that stop growth [28]. Main mechanisms of tolerance to salinity stress contain Na⁺ exclusion from leaves, sequestration Na⁺ and Cl⁻ in the roots and shoots vacuoles and processes

that resulting in growth maintenance despite the osmotic stress [27]. Salinity reduces plant capacity to take up water that resulting in reduction photosynthetic, growth rate and metabolic changes [28]. High selectivity for K^+ over Na^+ during root uptake is an important trait contributing to salt tolerance and, therefore, K^+/Na^+ ratio in plant tissue is a widely used in distinguishing genotypes for their tolerance to NaCl toxicity in wheat and other cereal species [22]. High pH can cause reduction K^+ uptake, even though, it might not affect Na^+ uptake [27]. Different screening methods have been reported by Munns and James (2003), however, it's necessary to test these methods in the field [15]. Field conditions vary from site to site, not only in soil salinity, but also in soil physical and chemical properties such as solidity, high pH, boron and interactions between these stresses can occur. Screening methods based on hydroponics or supported hydroponics has become preferred method for most researchers, because it gives a high degree of control and reproducibility [20]. Hydroponic method were carried out at early growth stage because limitations of space and time [16, 17, 25, 37]. El-Hendawy *et al.* (2007, 2009) reported that physiological traits such as Na^+ and K^+ concentration in shoot and root, chlorophyll content and agronomic traits such as dry matter production, leaf area were well screening criteria for salt tolerance under field and controlled conditions. Screening genotypes for salinity tolerance included K^+ to Na^+ ratio, Na^+ and Cl^- exclusion [2, 25]. Tolerance to high saline concentration in bread wheat related to reduce accumulation of Na^+ , to maintain adequate levels of K^+ and to enhance capacity of osmotic adjustment [7]. K^+/Na^+ ratio [21, 36, 39], sodium exclusion [29] and chlorophyll content of wheat genotypes could be considered as indexes for salt tolerance under saline conditions. The development of salt-tolerant crops is an important on salt-affected soils, so should use strategies which reduce salt accumulation, such as improved agronomic practices and landscape management [38]. In this study, 272 recombinant inbred lines (RILs) with their parents and two varieties as control (Arg and Moghan3) were evaluated and screened for salinity related to physiological and agronomical traits in hydroponic system.

MATERIALS AND METHODS

Plant materials

A Population of 272 F_7 recombinant inbred lines (RILs), derived from a cross between Roshan \times Sabalan by single seed descent were used in current study. Roshan is a native variety of IRAN that is relatively tolerant to salinity stress [31]. Sabalan was originally introduced from (908*FnA12)*1-32-4382 that is generally considered susceptible to salinity.

Phenotyping

RILs and their parents with a tolerant control variety-Arg and a sensitive control variety-Moghan3 were grown in tap water ($EC=0.5\text{dsm}^{-1}$) and saline water ($EC=18\text{dsm}^{-1}$) as control and salt stress treatment with hydroponic in greenhouse. Trial was arranged in randomized completely block design with three replications under each of control and salinity stress. The experiment was conducted with 16/8 day/night photoperiod, 27 day/20 °C night temperature and relative humidity of about 60%. Seeds of parents and RILs and two control varieties were sterilized in 1% hypochlorite for 15 min and germinated in petri dishes according to Munns and James (2003). After two days, germinated seeds were transferred to holes made in sheets of 2cm styrofoam, which were floated on distilled water on 12-liter plastic tray. Two day after transplanting, half-strength Hoagland solution was applied for three days. Then full-strength Hoagland solution was used. No salt was applied at the germination stage to ensure that all the lines germinated evenly. Ten days after transplanting, salt treatment started. NaCl was added to the solution 50mM daily over 3 days to final concentration of 150mM, with supplemental calcium as $CaCl_2 \cdot 2H_2O$. Supplemental calcium was added to the salt treatment giving a $Na^+:Ca^{2+}$ ratio of 15. This ratio was identified by Gen *et al.* (2007, 2010) that it is optimum for growth under saline conditions. The nutrient solution was changed once a week. The pH was monitored daily with pH meter. The pH of solution was maintained at 5.6-5.8 and adjusted using either HCL or NaOH every day. The EC of the nutrient solution was monitored using an EC meter. After three weeks of treatment with 150 mM NaCl, the chlorophyll content of base, middle and tip [25] of leaves measured using a SPAD-502 chlorophyll meter. Shoots was separately harvested, and rinsed with distilled water. Shoot height and fresh weight were recorded, and then the materials were oven-dried (48 h, 72°C) for dry weight measurement. The control experiment was conducted in the same way without adding salt. For measuring Na^+ and K^+ concentrations of shoots, RILs and parents and two control genotypes were harvested and rinsed with distilled water and dried. Then 0.1 gram each of them weighed and extracted in 0.1 M acetic acid at 90 °C for 4 h. Na^+ and K^+ concentrations were measured using standard flame photometry procedure [30].

Statistical analysis

Data were analyzed using SAS (version 9.0) statistical package. Mean comparisons were performed using least significant difference (LSD) test (P<0.05). Pearson’s correlation coefficients were calculated for each trait under saline and control conditions using SPSS 16. Analyses of the frequency distribution for traits among the 272 recombinant inbred lines in the salinity treatment were performed using SPSS 16.

RESULTS

Frequency distribution of population for evaluated traits in this experiment showed approximately normal distribution for all traits (Fig. 1). Observing different range of data for evaluated traits in RILs showed that there is transgressive segregation on both ends of distribution. In first combined analysis of variance two environment (control and stress) was performed (Table 1). There were significant differences at levels P<0.05, P<0.01 and P<0.001 between genotypes for all traits, except for the chlorophyll content (Table 1). Interaction effect of location and genotype were also significant for all traits, except for chlorophyll content and plant height (Table 1). Non-significant effects of location-genotype on plant height and chlorophyll content indicate that differences among genotypes in terms of salt tolerance may not be related directly to the response of the vegetative phase of growth [31]. Significant location×genotype interaction for sodium and potassium concentration and K⁺/Na⁺ ratio in shoot and root showed that the RILs acted differently in sodium and potassium absorption under salt stress (Table 1). The grand mean of parents and 272 RILs and two control varieties are shown in Table 2. As shown in this table, the parental and control varieties differed for all estimated traits. The salt tolerant parent, Roshan, had a more K⁺, K⁺/Na⁺ concentrations, dry weight in shoot and less shoot Na⁺ concentration than the salt sensitive parent (Sabalan) and two control varieties (Arg and Moghan3) (Table 2). In addition, the mean value of RILs decreased under saline conditions except for chlorophyll content (Table 2). Moghan3 had higher shoot sodium concentration than Arg and two parents. Height, shoot fresh weight, K⁺ concentration and K⁺/Na⁺ ratio in shoot and root decreased in response to increasing concentration of NaCl to 150mM, however shoot sodium concentration increased for all RILs (Table 2). Roshan, as a resistance parent, showed low sodium concentration in shoot and high sodium concentration in root under salinity treatment. So, this cultivar restricted Na⁺ uptake and stored most of it in it's roots. Also, it absorb more K⁺, resulting in higher K⁺/Na⁺ in shoot (1.85) which is a good salinity tolerance index. Line 33 showed lower K⁺ concentration and K⁺/Na⁺ in shoot, but higher shoot Na⁺ concentration compared with Moghan3 (Figure 2). In contrast, line 90 had significantly higher dry matter, shoot K⁺ and K⁺/Na⁺ concentration, and lower shoot Na⁺ concentration compared with Roshan under stress treatment (Figure 2). The correlations between physiological and agronomic traits under control and saline conditions are presented in table 3. These results indicate that these traits were good critical for screening salt tolerance.

Table 1. Analysis of variance for evaluated traits in parental, control varieties and the 272 RILs

Source of variation	Df	Mean square									
		PLH ¹	FWS ²	DWS ³	Chl ⁴	NaS ⁵	NaR ⁶	KS ⁷	KR ⁸	K/NaS ⁹	K/NaR ¹⁰
Location	1	65067.3**	78.243**	2.86**	10712.52	496.46**	0.196	570.06**	74.479**	13461.52**	594.926
Error	4	1891.2	1.228	0.109	96084.08	0.157	0.677	5.836	0.383	119.51	0.357
Genotypes	275	61.31***	0.046***	0.009***	24.51**	0.735***	5.695***	0.571***	0.354***	4.667***	1.809***
Location × Genotypes	275	34.3	0.016*	0.007*	14.649	0.688***	6.12***	0.596***	0.363***	4.435***	1.78***
Error	1100	33.004	0.017	0.006	17.823	0.007	0.001	0.039	0.003	0.596	0.027
CV%		13.17	10.2	8.39	18.08	9.2	2	10.39	10.18	19.57	20.9

*Significant at P<0.05 level; **significant at P<0.01 level; ***significant at P<0.001. ¹ plant height; ² shoot fresh weight; ³ shoot dry weight; ⁴ chlorophyll content; ⁵ shoot Na⁺ concentration; ⁶ root Na⁺ concentration; ⁷ shoot K⁺ concentration; ⁸ root K⁺ concentration; ⁹ shoot K⁺/Na⁺ ratio; ¹⁰ root K⁺/Na⁺ ratio.

Table 2. Means (± standard error) of traits for parental, control varieties and 272 F₇ RILs under control and saline conditions

Traits	HS	FWS	DWS	Chl	NaS	NaR	KS	KR	K/NaS	K/NaR
Control										
Roshan	51.01±5.2	3.98±1.69	1.01±0.26	28.02±5.1	0.527±0.04	0.469±0.03	2.1±0.11	0.84±0.12	4.2±0.35	1.76±0.214
Sabalan	48.39±6.5	3.1±8.8	0.95±1.9	30.55±5.2	0.61±0.092	0.41±0.017	1.879±0.22	0.78±0.135	3.34±0.529	1.86±0.292
Arg	46.4±0.49	2.45±0.16	0.77±0.016	27.5±0.39	0.87±0.24	0.37±0.018	2.02±0.122	0.53±0.12	3.24±0.41	1.3±0.25
Moghan3	44.6±0.72	1.86±0.11	0.74±0.015	28.23±0.45	0.53±0.043	0.42±0.025	2±0.121	0.78±0.124	3.99±0.36	1.75±0.23
RILs	50.43±5.47	3.43±2.37	1.15±2.57	30.04±3.6	0.39±0.087	1.49±1.97	2.29±0.58	0.68±0.43	5.94±1.45	1.31±1.09
Stress										
Roshan	37.09±7.2	1.5±0.84	0.94±0.47	42.67±7	1.11±0.267	1.74±0.11	1.38±0.87	0.33±0.069	1.853±0.4	0.187±0.03
Sabalan	35.55±4.4	1.32±2.9	0.86±0.5	40.03±6.7	1.17±0.193	1.57±0.178	1.34±0.075	0.4±0.051	1.46±0.24	0.27±0.02
Arg	31.69±0.38	0.72±0.04	0.67±0.02	36.05±0.56	2.08±0.341	1.25±0.12	1.22±0.128	0.21±0.038	0.76±0.188	0.16±0.023
Moghan3	30.16±0.49	0.64±0.03	0.54±0.03	28.2±0.78	2.4±0.24	1.37±0.16	1.31±0.08	0.27±0.057	0.61±0.082	0.186±0.03
RILs	37.73±4.8	1.55±3.59	1.12±3.7	38.04±5.2	1.45±0.68	1.37±0.43	1.35±0.23	0.28±0.23	1.13±0.55	0.21±0.197

Table 3. Simple correlation coefficients between mean of traits measured on RILs population derived from a cross between the Roshan × Sabalan in A) Stress and B) Control conditions

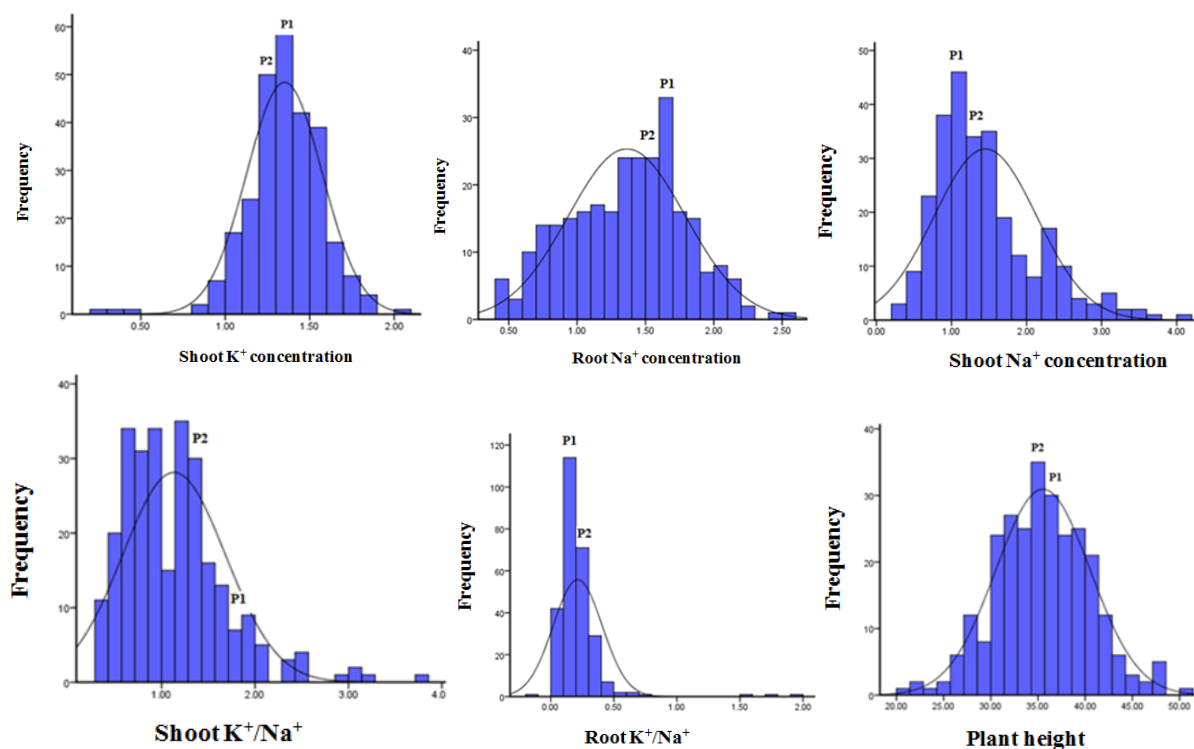
A)										
Traits	HS	FWS	DWS	Chl	NaS	NaR	KS	KR	K/NaS	K/NaR
HS		-0.026	0.098	0.048	0.049	-0.598**	0.007	0.043	0.032	0.19**
FWS			0.17**	0.13*	-0.36**	0.156**	-0.06	0.015	0.006	0.08
DWS				0.178**	0.07	0.63**	-0.137*	0.041	0.09	0.188**
Chl					0.83**	0.067	-0.046	0.291**	0.29**	0.06
NaS						-0.025	-0.015	0.26**	0.281**	-0.009
NaR							-0.145*	-0.039	0.021	0.02
KS								0.131*	0.063	0.095
KR									0.881**	0.039
K/NaS										0.056
K/NaR										

B)										
Traits	HS	FWS	DWS	Chl	NaS	NaR	KS	KR	K/Na S	K/Na R
HS		-0.23**	0.3**	0.02	0.196**	-0.063	0.456**	-0.03	-0.039	-0.001
FWS			-0.226**	0.078	-0.785**	-1.63**	-0.515**	0.018	0.05	-0.435**
DWS				-0.078	0.11	0.9**	0.26**	0.001	-0.01	0.14*
Chl					0.239**	-0.108	0.027	0.03	0.028	-0.018
NaS						0.039	0.461**	-0.034	-0.061	0.32**
NaR							0.138*	-0.004	-0.013	0.159**
KS								-0.023	-0.051	0.197**
KR									0.99**	0.084
K/NaS										0.067
K/NaR										

*Significant at $P < 0.05$ level; **significant at $P < 0.01$ level

Table 4. Salinity tolerance of parental and control varieties, tolerant and sensitive RILs under control and stress conditions

Genotypes	Shoot dry weight (g)		Salt tolerance (%)
	Control	Stress	
Roshan	1.01	0.94	93.1
Sabalan	0.95	0.86	90.5
Arg	0.77	0.67	87
Moghan3	0.74	0.54	73
90	0.69	0.78	113
33	0.8	0.56	70



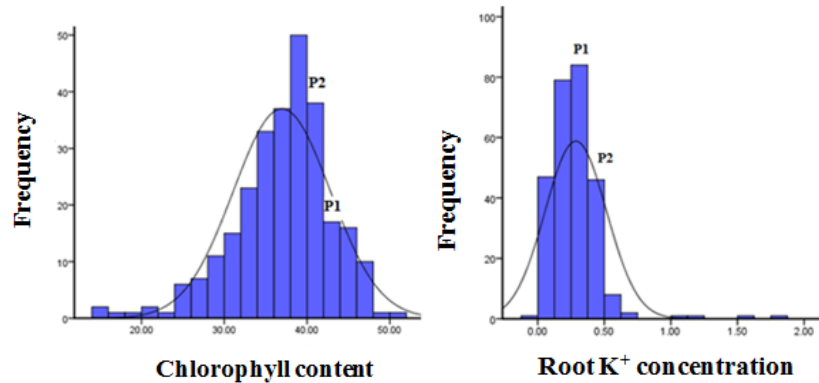


Figure 1. Frequency distribution of traits and parents positions among the 272 F₇ recombinant inbred lines under saline conditions

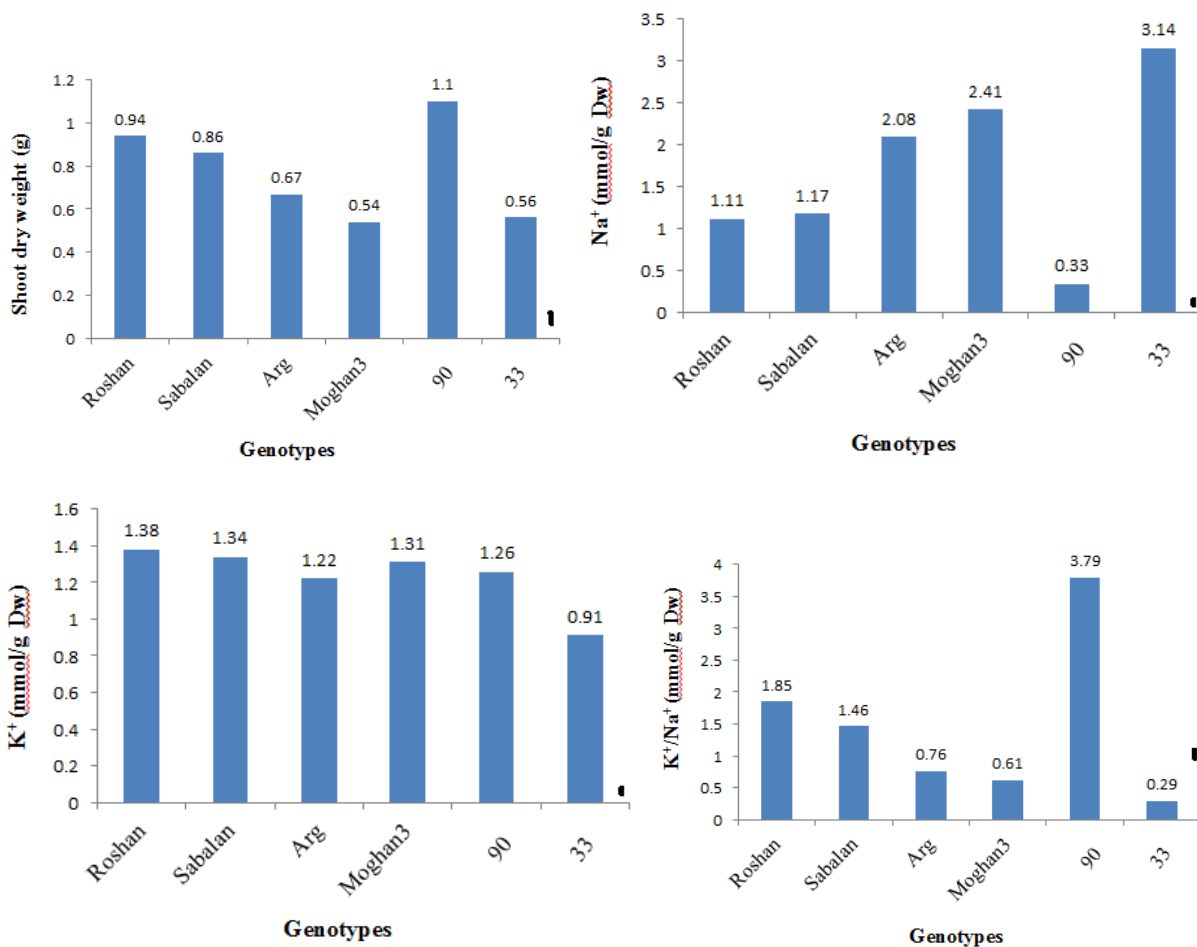


Figure 2. Na⁺ and K⁺ concentrations, K⁺/Na⁺ and shoot dry weight were observed for parental, control varieties and sensitive and tolerant lines under saline conditions. █ : Bar indicates LSD value

DISCUSSION

Among parental and control varieties, Roshan cultivar had the lowest leaf Na⁺ concentration, relatively high K⁺ concentration and high K⁺/Na⁺, thus it showed higher tolerance under salinity conditions. The high salinity tolerance of Roshan reported in previous studies [5, 10, 31]. The important position Na⁺ toxicity for most plants is the leaves blade, thus excluding Na⁺ from the leaves blades is important for salt tolerance [26]. Salinity was caused dry matter

reduction and Na^+ concentration enhancement in plant tissues for parental, control varieties and lines, whereas K^+ concentration decreased, resulting in significant decrease in K^+ to Na^+ ratios in root and shoot. RILs showed 371.7% increase in sodium concentration. Researchers reported that salt tolerance is associated with sodium accumulation [22, 24]. In general, the sensitive lines had higher Na^+ and transported it to the shoot, whereas, the tolerant lines had less Na^+ and maintained it in their roots, thus produced higher dry matter. Among 272 RILs, lowest Na^+ concentration were detected for line 90. Genotypes with the lowest Na^+ concentrations had the greatest dry matter and fewest injured leaves [25, 27]. The salinity tolerance of the parental, control varieties and two lines 90, 33 were shown under control and saline conditions in table 4. Salinity tolerance as a percentage of control shoot dry weight was reported by Munns and James (2003). A salt tolerance as control shoot dry weight for the line 90 was obtained 113%, compared with Roshan (as tolerant variety, 93.1%) (Table 4). The line 33 showed lowest shoot dry weight (70%), compared with Moghan3 (as sensitive variety, 73%). Potassium concentration of parents (Roshan and Sabalan) and RILs decreased due to the increasing salinity. The concentration of K^+ in the cytoplasm was related to Na^+ concentration [26]. High levels of Na^+ inhibit the K^+ uptake and resulting in K^+ deficiency, it causes a reduction in the K^+/Na^+ [23]. The line 90 showed low Na^+ and high K^+ concentration in salinity treatment. In this way, this line had high K^+/Na^+ after three weeks of 150mM NaCl treatment, compared with the others. A high K^+/Na^+ is more important than low Na^+ concentration [13]. Salt tolerance of the line 90 under saline conditions showed that this line could be introduced and used for future experiments. This line could be evaluated under field conditions for yield. K^+ concentration and K^+/Na^+ have been shown a strong positive correlation with the seed yield at the three leaf stage [36]. As an index, K^+/Na^+ , rather than Na^+ alone has been used for cultivars salt tolerance of wheat and rice [25, 39]. Correlation coefficients between K^+/Na^+ in shoot and root, height and chlorophyll content were significantly positive ($r=0.32^{**}$, $r=0.159^{**}$ and $r=0.197^{**}$, respectively). This result is in agreement to results of other studies [33]. It is indicating that enhancement K^+/Na^+ caused enhancement chlorophyll and duration of vegetative growth [10]. The significant negative correlation between K^+/Na^+ and Na^+ concentration in root and shoot indicates that increase in Na^+ will decrease K^+/Na^+ ratio (Table 3). This result obtained by literatures [11, 16, 31]. Na^+ and K^+ concentration in root and shoot had adverse effect, as negative correlations were observed between these two traits in root and shoot (Table 3). Negative relationship between Na^+ and K^+ in root and shoot were documented [3, 9]. Plant height decreased for parental, control varieties and RILs under saline conditions than control. Boubaker (1996) found that increasing salinity reduced plant height. There was a significant negative correlation between Na^+ concentration, K^+/Na^+ , height, chlorophyll content and root potassium concentration. Chlorophyll content increased under saline conditions for parental, control varieties and RILs. Enhancement in chlorophyll content under salt stress has already been reported [1]. Root sodium concentration was more than shoot sodium concentration for Roshan parent under saline stress. Also, there was a positively and strongly correlation between shoot dry weight and root Na^+ concentration under saline ($r=+0.63$) and control ($r=+0.9$) conditions. In the salt tolerance genotype was revealed a lower rate of Na^+ transfer from root to the shoot and higher capacity of the leaf sheath for exclusion Na^+ [7]. Correlation analysis showed that plant height significantly and positively correlated with shoot potassium concentration and negatively with shoot sodium accumulation. Reduction of wheat growth associated to reduction of potassium concentration and the increase sodium accumulation [34]. Based on results, Roshan had root K^+ content lower than shoot K^+ content under stress conditions. Salt tolerance is associated with low rates of transport of Na^+ to shoots with high K^+ to Na^+ ratio is suggested as an important factor for metabolism and growth [7]. The K^+/Na^+ to be controlled by a single locus (Knal) on chromosome 4D in bread wheat [12, 15], thus this is as an important trait for confer salinity tolerance in the field. Also, higher increase of Na^+ concentration in Moghan3 indicated sensitivity of this cultivar in salt stress conditions. Cultivars consist of low capacity of sodium exclusion introduced as sensitive cultivars [31].

CONCLUSION

Seedling growth of all RILs was reduced by adding salinity to 18dsm^{-1} . Significant positive correlations between shoot dry matter and K^+/Na^+ ($r=0.29$) were detected. According to data obtained on some agronomical and physiological, Roshan was more tolerant to salinity stress among parental and control varieties. Higher potassium concentration and dry matter in tolerant lines, resulting in lower sodium accumulation, contribute to their salinity stress tolerance. Salt tolerance was associated with a low rate of transport of Na^+ from root to shoot with high K^+/Na^+ and high capacity of leaves for exclusion Na^+ .

Acknowledgements

This research was supported by the Agricultural Biotechnology Research Institute of Iran (ABRII).

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