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Evaluation of triticale genotypes for salt tolerance using physiological traits

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Abstract

Salinity is one of the major constraints to plant production worldwide. The effect of terminal salinity on the physiological traits of 18 triticale (*X. triticosecale*) lines comprising nine doubled haploid (DH) and nine corresponding advanced lines (F_8) and two bread wheat cultivars (control) was investigated under field conditions. Salt-stressed experiment was irrigated using saline water with EC = 16 ds m-1 from mid-jointing growth stage (Zadoks 43) onward. Na^+ concentration, K^+/Na^+ ratio, Ca_2^+/Na^+ ratio, chlorophyll content, carotenoids content, proline content and grain yield were assessed. A high correlation coefficient (r=0.84**) was obtained between K^+/Na^+ ratio and grain yield under saline field conditions. The results also revealed an inverse and significant relationship between grain yield loss due to salinity with K^+/Na^+ ratio, proline and carotenoids content under saline stress provided evidence supporting the role of K^+/Na^+ ratio in salinity tolerance. F_8 line number 6 possessed the highest values for carotenoids content, Ca_2^+/Na^+ ratio and proline content as well as the least Na^+ concentration under salt-stressed conditions, accompanied by having the least grain yield loss due to salinity, was ranked this line as superior salt-tolerant genotype.

Key words: Physiological traits, Salt tolerance, *X. triticosecale*

Introduction

Salinity in soil or water is one of the major abiotic stresses, especially in arid and semi-arid regions, can severely limit plant growth and productivity. The increasing occurrence of dry periods in many regions of the world and the salinity problems associated with irrigated areas frequently lead to the consecutive incidence of drought and salinity on cultivated land (Islam et al., 2011).

Ion toxicity, osmotic stress and nutrient imbalance are the factors associated with the deleterious effect of salinity on plant growth and productivity. Therefore, understanding the salt tolerance mechanisms such as Na^+ exclusion, K^+/Na^+ discrimination and osmotic adjustment is essential to improve salt tolerance in crop plants. Low Na^+ accumulation and high K^+/Na^+ discrimination have been found to be strongly contribute to enhanced salinity tolerance in bread wheat (Santa-Maria and Epstein, 2001) and in durum wheat (Houshmand et al., 2005).

Osmotic adjustment is a fundamental adaptive response of plant cells to salinity, helping the maintenance of turgor under saline conditions (Serrano et al., 1999). The significance of proline accumulation in the osmotic adjustment has been extensively studied but, its role is still a matter of debate and varies according to the species. However, it has been postulated that proline acts as a compatible solute, an osmoprotectant, and a protective agent for cytosolic enzymes and cellular organelles (Bohnert et al., 1995). Proline has also been considered a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules as well as a free radical scavenger (Jain et al., 2001).

Photosynthesis and photosynthesis-related parameters have been affected by salinity stress (Arzani, 2008). Carotenoids are lipid-soluble and non-enzymatic antioxidants produced by most photosynthetic organisms and belong to two classes of the carotens and xanthophylls. In plants, carotenoids act as light collectors shielding against photosensitization in the chloroplasts.

For a complex trait such as salinity tolerance screening highly homozygous genotypes like

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doubled haploid (DH) lines will be a significant advantage. This is the case for field grown plants for which genotype by environment interactions can be very large due to the heterogeneity in soil salinity (Arzani, 2008).

Triticale (X. *Triticosecale* Wittmack) is one of the most successful man-made cereal as it combines the unique grain quality of wheat (*Triticum* ssp.) with rye (*Secale* spp.) tolerance to harsh environment (Lelley, 2006). Triticale seems to be an interesting alternative to other cereals, particularly bread wheat, in environments where growing conditions are unfavorable or for in the low-input systems (Erekul and Kohn, 2006).

The objectives of this study were: (1) to evaluate salt tolerance of triticale DH and their corresponding F_8 lines as well as two bread wheat cultivars (Roshan and Kavir), using physiological traits, and (2) to determinate the associations of physiological traits with grain yield under salt stress conditions in triticale.

Materials and Methods

Plant materials and growth conditions

Two separate experiments were carried out under salt stressed and non-stressed conditions at a research farm of the Isfahan University of Technology, located at Lavark, Iran (40 km south west of Isfahan, 32˚32´N, 51˚23´ E, 1630 m asl), during the 2008-2009 growing season. The soil is silty clay loam, typic Haplargids of the arid tropic, with pH=7.3-7.8, EC=1-1.2 dS m^{-1} and contained 1.3% of organic matter. Mean annual precipitations and mean annual temperature were 140 mm and 15°C, respectively. Plant materials used in this study included 9 doubled haploid (DH) lines and 9 corresponding F_8 lines of triticale derived from Polony Q / TW179 cross (Arzani and Darvey, 2002) and two local bread wheat cultivars ('Roshan' and 'Kavir'). 'Roshan', as a drought tolerant, and 'Kavir' as a salt tolerant cultivars (Daei et al., 2009), were included as control. DH line number 3 was registered in Australia as the 'Eleanor' cultivar (Anon, 2001).

A randomized complete block design with three replications was used in each of the two experiments. Each plot consisted of four rows with 4 m long and 25cm apart spaced. At the salt stressed and non-stressed experiments, irrigated water with an EC of 1 dS m^{-1} was used until mid-jointing stage (43 growth stage of Zadoks scale), and afterward salt-stressed experiment was irrigated using saline water by dissolving salt in water (1% NaCl). The electrical conductivity (EC) of the irrigation water was nearly 16 dS m^{-1}.

EC and the chemical properties at 30 cm depth of soil of saline and non-saline experiments were determined. Na^+ concentration, K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio, the contents of photosynthetic pigments [chlorophyll $a+b$ (chl $a+b$), carotenoids (xanthophylls and carotenes ($x+c$)], and the weight ratio of chl a and b to total carotenoids (chl ($a+b$)/($x+c$))), proline and grain yield were evaluated.

To measure the Na^+, K^+ and Ca^{2+} concentrations, plant samples were dried (75 ºC to constant mass) and weighted. leaf samples were ashed, at 550 ºC, for 3 h. Inorganic ions were then extracted with 10 ml 1 M H_2SO_4, and the volume of each sample was standardized to 100 ml. Na^+ and K^+ concentrations of the solutions were measured using a 410-Corning flame photometer. Ca^{2+} concentration was estimated using a Perkin-Elmer, 2380 atomic absorption spectrophotometer.

To measure the photosynthetic pigments**,** 100 mg of tissue from 10 leaves obtained randomly from each plot was used.

Concentrations of leaf chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids (C x+c) were spectrophotometrically determined, using the redetermined extinction coefficients and equations of Lichtenthaler and Buschman (2001) in 80 % acetone extract.

Free proline content was extracted from 0.5 g of leaf samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using ninhydrin reagent according to the method of Bates et al. (1973).

Statistical analysis

The data from two experimental conditions (control and saline) were subjected to combined analysis of variance (ANOVA) using PROC GLM of SAS (SAS Institute, 1999). All the effects were considered random. Mean comparisons were conducted using the Fisher's least significant difference ($LSD_{0.05}$) test. Linear regression was conducted to determine the relationship between physiological traits and grain yield with two experimental groups of saline and non-saline (control) field conditions.

Results and Discussion

A significant influence of salinity on the physiological traits with the exception of Ca^{2+}/Na^+ ratio, was found (Table 1). The tested genotypes varied significantly ($P \leq 0.01$) for the physiological traits. Overall, the orthogonal comparison showed a comparable performance of both triticale lines and wheat cultivars in physiological response to salinity stress (data not shown). This occurred in spite of

using one salt tolerance and one-drought tolerance wheat cultivars to compare with 18 triticale lines selected under normal growth conditions.

Na^+ concentration increased in response to salinity stress (Table 2). Increase of Na^+ concentration in plant tissues is one of the primary plant responses to salinity stress (Meneguzzo et al., 2000). Under normal conditions, means of Na^+ concentration ranged from 0.49 for F_8 line number 1 to 1 for DH line number 6. DH line number 2 (2.69) and F_8 line number 4 (1.14) exhibited the highest and the lowest Na^+ concentration under salt-stressed experiment, respectively (Table 2). DH lines had higher Na^+ concentration than F_8 lines under both conditions. Na^+ concentration had negative and significant correlation with chl $a+b$ (r = -0.48*) and grain yield (r = -0.45*) and positive correlation with grain yield loss (r =0.48*) under salinity stress conditions. It is evident that salt tolerance is associated with low uptake of Na^+ (Schachtman and Munns, 1992; Santa-Maria and Epstein, 2001), partial exclution (Colmer et al., 1995), its removal from the cytoplasm and compartmentalization into the vacuoles (Ashraf, 1994). Schachtman and Munns (1992) described the association of low shoot Na^+ concentration with salt tolerance in wheat.

High K^+/Na^+ discrimination was described as a physiological index for salinity tolerance in wheat (Dvorak et al., 1994). Our results followed a similar trend which K^+/Na^+ ratio ranged from 1 for DH line number 2 to 1.78 for F_8 line number 4 under non-stressed and from 0.22 for DH line number 3 to 0.8 for F_8 line number 1 under salt stressed experiment (Table 2). The leaf K^+/Na^+ ratio of genotypes was significantly ($P \leq 0.01$) reduced as plants were subjected to salinity stress (Table 2). Decrease of K^+/Na^+ ratio in response to salinity stress has been previously observed in bread (Dvorak et al., 1994) and durum wheat (Meneguzzo et al., 2000; Houshmand et al., 2005). Comparatively, in our study it was found that F_8 lines had higher K^+/Na^+ ratio than DH lines under both growth conditions (Table 2). Due to the impact of K^+ concentration on reducing toxicity of Na^+ in plants under salinity stress, the K^+/Na^+ ratio has been proposed, as a saline tolerance indicator in wheat (Dvorak et al., 1994). Our data supported the positive role of potassium in salinity tolerance, observing a strong relationship between the leaf K^+/Na^+ ratio and the grain yield in triticale under saline field conditions (Figure 1). A negative correlation coefficient was found between K^+/Na^+ ratio and grain yield loss due to salinity (r=-0.55*) providing, further evidence that supports the role of K^+/Na^+ ratio in salt tolerance in triticale.

Ca^{2+}/Na^+ ratio was not significantly affected by the salinity in the tested genotypes (Table 1). According to Cachorro et al. (1994), a high Ca^{2+} concentration could decrease the Na^+ toxicity. Houshmand et al. (2005) reported that shoot Ca^{2+}/Na^+ ratio increased with increase in NaCl concentration of the growth medium. Our data revealed significant differences between triticale lines and wheat cultivars for Ca^{2+}/Na^+ ratio with triticale lines being significantly ($P \leq 0.01$) superior under both conditions. F_8 line number 4 and DH line number 8 had the highest Ca^{2+}/Na^+ ratio and grain yield under both growth conditions (Table 2, 3). Higher Ca^{2+}/Na^+ ratio in these genotypes may be accounted for the high grain yield of the F_8 line number 6 (Table 2, 3) that accompanied with the least grain yield loss. Accordingly, evidence was provided supporting the role of the Ca^{2+}/Na^+ ratio in the salt tolerance mechanisms in triticale.

Table 1. Result of combined analysis of variance of the tested traits in triticale and wheat genotypes grown under salinity and normal field conditions.

		Mean Square							
Source of variation	df	Na^+ concentration	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	chl a+ b	x + c	a+b/x+c	Proline	Grain yield
Environment(E)	1	22.77**	24.56**	0.0004	2.30**	0.056**	11.27**	919.40**	28161560**
Replicate	4	0.02**	0.04**	0.00009	0.0001	0.002*	0.30*	0.37	306947
Genotype (G)	19	0.24**	0.18**	0.006**	0.04**	0.007**	0.61**	37.61**	7003525**
G×E	19	0.16**	0.02**	0.0008**	0.02**	0.002**	0.30**	17.98**	1611394**
Residual	76	0.003	0.004	0.0002	0.0001	0.0006	0.106	0.19	610371
CV%		4.74	6.45	10.95	1.2	10.4	10.3	5.99	14.63

* $P \leq 0.05$, ** $P \leq 0.01$

Table 2. Means of the Na^+ concentration, K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio and chlorophyll a+b (chl a+ b) of triticale and wheat genotypes grown under salinity stress and normal field conditions.

Traits	Na^+		K^+/Na^+ ratio		Ca^{2+}/Na^+ ratio		chl a+ b (mg/g fw)	
Genotype	Non stress	Stress	Non stress	Stress	Non stress	Stress	Non stress	Stress
F_8 lines								
1	0.49i	1.46hi	1.74ab	0.8a	0.09fgh	0.11d-g	0.89g	0.56hi
2	0.58gh	1.36jk	1.68a-d	0.66cd	0.15bc	0.13cd	0.84ij	0.56h
3	0.73cd	1.64ef	1.16h	0.43gh	0.06i	0.07h	0.77n	0.54i
4	0.57ghi	1.14l	1.78a	0.64cde	0.16abc	0.16ab	0.82l	0.52j
5	0.76bcd	1.29k	1.20h	0.40gh	0.15bc	0.14bc	0.96e	0.63ef
6	0.70cde	1.17l	1.48fg	0.63de	0.17ab	0.18a	0.81l	0.64d
7	0.60gh	1.79c	1.64bcd	0.64cde	0.09fgh	0.07h	0.84j	0.66c
8	0.76bcd	1.39ij	1.48fg	0.50fg	0.10d-g	0.11d-g	0.78mn	0.55hi
9	0.52hi	1.32jk	1.71abc	0.77ab	0.11de	0.11efg	1.03c	0.59g
DH lines								
1	0.73cd	1.37ijk	1.50efg	0.60def	0.12d	0.12c-g	0.79m	0.60g
2	0.78bc	2.69a	1i	0.33h	0.16bc	0.14c	0.68o	0.38l
3	0.84b	1.90b	1.13h	0.22i	0.07hi	0.10g	1.08b	0.46k
4	0.69de	1.50gh	1.42g	0.60def	0.11def	0.07h	0.99d	0.74a
5	0.62efg	1.60f	1.42g	0.54ef	0.14c	0.1fg	0.94f	0.66c
6	1a	1.65ef	1.58def	0.63de	0.16abc	0.10fg	0.85hi	0.70b
7	0.68def	1.77cd	1.40g	0.60def	0.12d	0.11d-g	0.95ef	0.64de
8	0.59gh	1.16l	1.63bcd	0.67bcd	0.18a	0.18a	0.82kl	0.67c
9	0.59gh	1.58fg	1.60cde	0.74abc	0.09e-h	0.12c-f	1.09a	0.67c
Roshan	0.70cde	1.70de	1.40g	0.50fg	0.08ghi	0.08h	0.86h	0.62f
Kavir	0.60gf	1.48h	1.60de	0.54ef	0.10d-g	0.13cde	0.83jk	0.67c
LSD	0.08	0.09	0.11	0.11	0.02	0.02	0.014	0.016

Means followed by the same letter within a column are not significantly different ($LSD_{0.05}$).

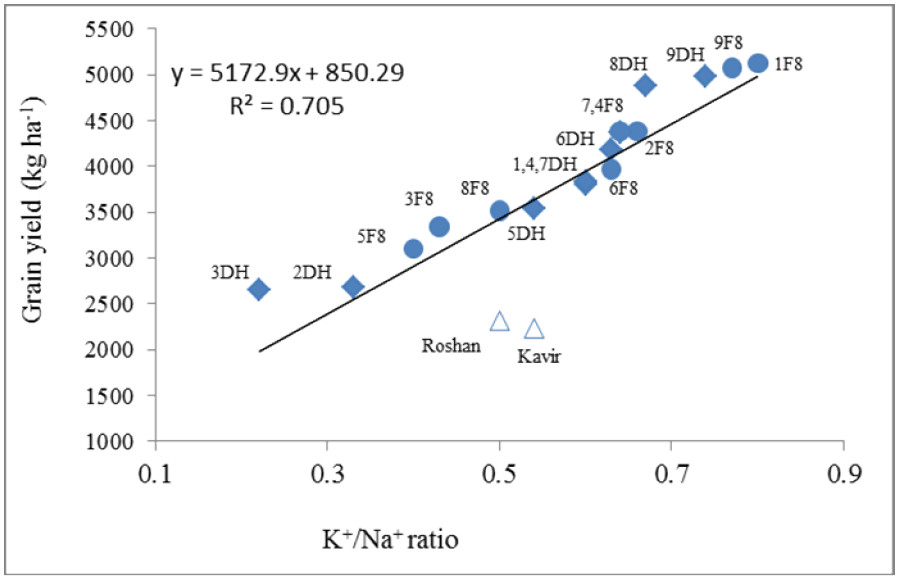

Figure 1. Relationship between K^+/Na^+ ratio and grain yield observed under saline field conditions.

Our data further revealed the significant influences of both salinity and genotype on photosynthesis related parameters (Table 1). Indeed, chl *a*+*b* and carotenoids content decreased due to salinity stress (Table 2, 3). Chl *a*+*b* ranged from 0.68 mg/g_{fw} for DH line number 2, to 1.09 mg/ g_{fw} for DH line number 9 under non-stressed experiment, and from 0.38 mg/g_{fw} for DH line number 2 to 0.74 mg/g_{fw} for DH line number 4 under salt-stressed experiment (Table 2). Carotenoids content ranged from 0.20 mg/g_{fw} (DH line number 2) to 0.39 mg/g_{fw} (DH line number 9), and 0.14 mg/g_{fw} (DH line number 2) to 0.27 mg/g_{fw} (F_8 line number 7) for non-stressed and salt-stressed environments, respectively (Table 3). Carotenoids displayed a positive and significant correlation with chl *a*+*b* (r = 0.65**) under both growth conditions. A negative and significant correlation coefficient was observed between grain yield loss and carotenoids content (r = -0.54*) under salt stressed conditions. Akbarian et al. (2011) observed a negative and significant correlation coefficient between grain yield loss and carotenoids content under field drought stress. Chl (*a*+*b*)/(*x*+*c*) ratio ranged from 2.84 to 4.27 in non-stressed, and from 2.20 to 3.50 in salt stressed experiment (Table 3). The ratio of chl (*a*+*b*)/(*x*+*c*) of the genotypes were 3.45 and 2.84 in non-stressed and salt stressed environments, respectively. The weight ratio of chl (*a*+*b*)/(*x*+*c*) is an indicator of greenness (Lichtenthaler and Buschman, 2001). Indeed, lower values for the ratio of chl (*a*+*b*)/(*x*+*c*) are an indicator of senescence, stress, and damage to the plant and the photosynthetic apparatus, which is expressed by a faster breakdown of chlorophyll than carotenoids.

Table 3. Means of the chl a+b content, carotenoids content (x+c), proline content and grain yield of triticale and wheat genotypes grown under salinity stress and normal field conditions.

Traits	x + c (mg/g fw)		Chl a+b/x+c		Proline (mg g fw)		Grain yield (kg h^{-1})	
Genotype	Non stress	Stress	Non stress	Stress	Non stress	Stress	Non stress	Stress
F_8 lines								
1	0.21gh	0.18g	4.27a	3.10a-d	5.21d	6j	9469a	5125a
2	0.24d-h	0.19efg	3.46d-g	2.99a-e	6.55c	9.39g	8043ab	4387a-d
3	0.27de	0.21d-g	2.87hi	2.65d-g	6.54c	11.39de	5706efg	3341d-g
4	0.22fgh	0.20efg	3.65cde	2.65d-g	3.93fgh	13.41c	8117ab	4365a-d
5	0.24d-h	0.19fg	4abc	3.32abc	4.76de	9.79g	7043b-e	3101efg
6	0.23e-h	0.23a-e	3.45c-g	2.80c-f	8.16a	19.48a	4909fgh	3971b-e
7	0.28cd	0.27a	3.08ghi	2.42fg	2.81kl	15.68b	6339c-f	4384a-d
8	0.28bcd	0.22c-f	2.84i	2.49efg	5.34d	12.09d	7269bcd	3517def
9	0.32bc	0.24a-d	3.28d-i	2.51efg	3.06jkl	6.10j	7749bc	5075ab
DH lines								
1	0.23fgh	0.23b-e	3.46d-g	2.63d-g	7.21b	10.61f	7522bcd	3832cde
2	0.20h	0.14h	3.38d-h	2.67d-g	4.10fg	7.61i	8136ab	2680fg
3	0.26def	0.21d-g	4.20ab	2.20g	2.87kl	7.66i	5312fgh	2651fg
4	0.32b	0.25abc	3.13f-i	2.94a-f	2.74l	4.25k	7061b-e	3800cde
5	0.25d-h	0.19efg	3.74bcd	3.41ab	4.46ef	6.13j	6115def	3539def
6	0.26def	0.23a-e	3.25d-i	3.02a-e	3.75ghi	11.18ef	7763bc	4176a-e
7	0.27de	0.19fg	3.52c-g	3.42ab	2.91kl	6.43j	7069b-e	3835cde
8	0.22fgh	0.23a-e	3.69b-e	2.88b-f	3.43h-k	8.56h	8069ab	4875abc
9	0.39a	0.26abc	2.86i	2.63d-g	6.60bc	15.12b	7565bc	4979ab
Roshan	0.24d-h	0.18gh	3.61c-f	3.50a	3.22i-l	13.72c	3869h	2317g
Kavir	0.26def	0.27ab	3.21e-i	2.52efg	3.58g-j	6.79j	4333gh	2235g
LSD	0.04	0.04	0.51	0.56	0.65	0.80	1449	1111

Means followed by the same letter within a column are not significantly different ($LSD_{0.05}$).

Free proline content of the leaves was significantly affected by genotype and salinity (Table 1). Salinity stress caused an increase in proline content of the genotypes (Table 3). Several plant species, including halophytes, accumulate high proline levels in response to the osmotic stress, as a tolerance mechanism to high salinity and water deficit (Delauney and Verma, 1993). F_8 lines had higher proline content than DH lines under both growth conditions (Table 3). High level of proline enables a plant to maintain low water potential and, thus, buffering the immediate effect of water storages within the organism. There was a negative correlation between grain yield loss due to salinity and proline content under salt-stressed conditions ($r= -0.55^{*}$). Grain yield of the genotypes was significantly reduced by salinity stress (Table 3). Under salt stress conditions, the F_8 line number 1, 2, 4, 7, 9, DH line number 6, 8 and 9 produced higher grain yield and ranked as the superior genotypes. F_8 line number 1, 2, 4 and DH line number 8 were superior under both environmental conditions, if the grain yield is considered. However, the latter group has usually been preferred because they characterized the breeding potential of high yield stability.

Conclusions

In this study, three variables salinity stress, plant species and genotypes within species were hypothesized to affect physiological traits and grain yield and have been empirically substantiated. The strong relationship between the leaf K^+/Na^+ ratio and the grain yield production under saline field conditions implies the key role of the ionic mechanism, which effectively discriminate between potassium and sodium ions in triticale. On the other hand, the non-association between Ca^{2+}/Na^+ ratio and salinity tolerance in our study may display a lower turn-over of calcium than potassium in the ionic balance. The comparable performance of triticale lines non-deliberately selected triticale line with that of wheat cultivars, either selected for salinity tolerance or drought tolerance, further underlies the adaptability of triticale to saline environment as well as the possibility of improving salinity tolerance in triticale to tolerate a higher level of salinity.

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