Original Research Article

Selection of salt- tolerant triticale (× *Triticosecale* Wittmack) and genetic variation assay for agronomic and physiological traits

Abstract

Salinity is a major limiting factor of growth and grain yield in most crop plants. In the present study, the response of 12 triticale genotypes, 3 wheat commercial cultivars and a rye genotype to four levels of salt stress (1:1 ratio of NaCl and $CaCl_2$ salts with EC=0, 7, 14 and 21 dS/m) were assayed in a pot experiment. Salt stress was applied at the four-leaf stage. Variations in two sets of agronomic and physiological traits were analyzed. Most of triticale genotypes had higher grain yield than wheat and rye genotypes under saline conditions. K^+/Na^+ discriminant ratio which explained 61.7% of grain yield variation in linear regression analysis was higher in most of triticales compared with wheat genotypes. Correlation analysis indicated that awn length had highest correlation with grain yield. Acute angel between grain yield and K^+/Na^+ discriminant vectors in principal components analysis showed tight association of these traits under salt stress levels. Calculation of genotypic coefficients of variation indicated the existence of higher genetic variation for K^+/Na^+ (19.82%), awn length (21.64%) and grain yield (26.55%) compared with maturity (4.72%) and total protein (4.63%). Cluster analysis results indicated that TRT808 and TRT806 joined in second group in tree dendrogram under salt stress conditions. This group had higher grain yield mean and K^+/Na^+ discriminant ratio and its genotypes matured earlier compared to other genotypes. Overall, it can be concluded that triticale performed better than wheat under different levels of salt stress. High genetic variation for some of salt stress- adaptive traits provides the opportunity for production of high-yielding triticales.

Keyword: Triticale, genetic variation, salt tolerance.

Introduction

Salinity is a major problem in arid and semi-arid areas of the world. Data show that 800 million hectares, equivalent to 6% of the land is affected by salinity worldwide (FAO, 2011). Salinity in agricultural lands is affects crop plants growth (Pakniyat *et al.*, 2003). In Iran, about 20% of arable lands (equivalent to 34 million hectares) are affected by salinity of which 8.5 million hectares suffer from severe salinity (Cheraghi *et al*., 2009).

Triticale is mainly used as feed grain and biomass production for thatching straw and common human utilization. Regardless of the advantages, the early history of triticale as the first man-made crop plant constituted mainly of a botanical curiosity of no agronomic value. It took nearly a hundred years of research for botanical and genetic characteristics to evolve triticale into a crop (Guedes-Pinto *et al*., 1996; Mergoum and Gómez-Macpherson 2004). A comprehensive review of triticale response and adaptation shows that it can tolerate some of abiotic stresses better than small grain cereals (Jessop 1996). A recent research by Motzo et al. (2013) did not confirm a yield reduction in triticale due to late season drought stress, in compassion with durum wheat. According to USDA Salinity lab, the salinity tolerance of triticale is basically better than that of wheat and might even approach that of barley (Blum, 2014). In a field study (Francois et al. 1988), triticale cultivars were cultivated over a range of saline soils and the threshold for yield reduction under salinity stress was higher (7.3dS m^{-1}) than that of cited in USDA Salinity lab data (6.1 dS m^{-1}). The potential advantage of triticale over wheat in biomass and grain yield was confirmed in the study of Estrada-Campuzano et al. (2012).

Increase in salt concentrations affects nearly all physiological processes of plants including photosynthesis, protein synthesis, energy metabolism and lipid metabolism (Parida and Das, 2005). Salinity due to osmotic effects on different metabolic activity induces water

deficiency leading to oxidative stress by increasing the reactive oxygen species (ROS) comprising of superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH). Oxidative stress may cause cell damage through oxidation of lipids, proteins and nucleic acids (Mudgal *et al.*, 2010; Parida and Das, 2005). To overcome the effects of salinity- induced oxidative stress, plants use antioxidants like superoxide dismutase (SOD) (Mudgal *et al.*, 2010). Other factors comprising of ion toxicity, osmotic stress and nutrient imbalance are associated with deleterious effect of salinity on plant growth and productivity. Therefore, understanding mechanisms such as Na^+ exclusion, K^+/Na^+ discrimination and osmotic adjustment is essential to improve salt tolerance in crop plants. Accumulation of K^+ or higher K^+/Na^+ may be correlated with salt tolerance in crop plants (Gorham *et al.*, 1990). Low Na^+ accumulation and high K^+/Na^+ discrimination have been found to be strongly associated with enhanced salinity tolerance in bread and durum wheats (Santa-Maria and Epstein, 2001; Houshmand *et al.*, 2005). Likewise, relationship between low Na^+ accumulation and salt tolerance has been found in barley (Forster *et al.*, 1994; Gorham *et al*, 1994; Pakniyat *et al.*, 1997).

Triticale seems to be an alternative to other small grain cereals, particularly wheat and barley for cultivation under unfavorable conditions or in the low-input agricultural systems. Existence of appropriate genetic variation is a prerequisite for the improvement of any character, through selection and breeding. Fortunately, diversity in salt tolerance at the intra specific level has been found in triticale (Norlyn and Epstein, 1984). Triticale constitutes also a valuable genetic resource for transferring genes of interest from rye into wheat, particularly those related to biotic and abiotic stresses (Vaillancourt *et al.*, 2008).Estimation of genetic variation parameters and heritability of the adaptive-salt tolerance traits are useful in making decisions for breeding triticale to become as important as wheat or better in a global scale. For better

understanding the selection efficiency of salt- tolerance in breeding programs, genotypic and phenotypic variations and heritability of traits is very important. Hence, in the present study we have focused on the response of some CIMMYT-derived triticale accessions, and commercial cultivars to different salinity levels based on variations in some agronomic and physiological traits. Genetic variation parameters and heritability of traits were also estimated.

Materials and Methods

Greenhouse experiment

The experiment was conducted at the greenhouse of the College of Agriculture, Shiraz University, Shiraz, Iran, during the 2011-2012 growth season. Characteristics of the soil are available in Table 1. The plant materials used in this study comprised of 12 triticale genotypes, three wheat cultivars and a rye genotype. In a factorial experiment based on completely randomized design, 16 genotypes and four levels of salinity. Combinations of 1:1 ratio of NaCl and $CaCl_2$ salts were used to prepare saline solutions with EC=7 dS/m (S1), 14 dS/m (S2) and 21 dS/m (S3) in three replications. Normal water (EC=1 dS/m) was used as control.

Before planting, the seeds of all genotypes were surface sterilized by 2.5% sodium hydrochloride solution for 15 min and rinsed 3 times with distilled water. The seeds were sown in pots containing 5.5 kg soil, peat and sand with the ratio of 1.5: 0.5: 2, respectively. A number of 10 seeds were sown that were thinned to five seedlings at the two-leaf stage of growth. For better establishment of seedlings, all pots were irrigated by normal water with EC 1 dS/m until the four- leaf stage. Forty days after sowing (four-leaf stage) saline solutions were applied to pots based on field capacity. Application of saline solutions was continued till end season of crop growth.

Table 1. Characteristics of the soil used for the pot experiment.

Soil parameter	data
Electrical conductivity (dS/m^{1})	1.0
pH	7.74
Clay (%)	35.5
Silt (%)	32.1
Sand (%)	32.0
Total N (%)	2.35
Organic C (%)	1.36
Available P (g/kg)	21.5
Available K^+ (g/kg)	68.0
Available Na (g/kg)	26.5

Ion measurement

Na^+ and K^+ ions were measured four weeks after the application of salinity. Leaves of 5 plants in each pot were collected and oven-dried at 70˚C for 48 hr and were milled to a fine powder. The samples were placed in a crucible and ashed by transferring to a furnace at 500˚C for 2 hr. An amount of 5 ml HCL (2N) was added to each crucible and mixed thoroughly. Then, boiling distilled water was added to the mixture and then filtered in a 50 ml volumetric flask. Concentrations (mg/g of dry mater, DM) of Na^+ and K^+ ions were measured using flame photometry according to Hamada (1994) procedure. K^+/Na^+ ratio was also calculated.

Total protein and antioxidant enzymes assays

Forty five days after the start of salt treatment application, the leaves of genotypes in each pot were separated and placed in liquid nitrogen immediately. Samples were kept in a refrigerator at -4˚C until distillation of leaves. The Bradford procedure (1976) was used to measure total protein (Tpr) in mg/ml. Beauchamp and Fridovich (1971) method was followed to quantify SOD activity based on unit of enzyme.

Assays for agronomic traits

Forty five days after the onset of salt stress treatment which was coincident with post-heading stage, a leaf area meter instrument (ΔT-Cambridge device, the UK) was used to measure total leaf area (LFA) as cm^2 per plant. Days to maturity was estimated based on the number of days from the first irrigation to physiological maturity when spikes turned yellow. Twenty days after the first irrigation with salt solutions, on a sunny day between 11 am to 3 pm, the concentration of chlorophyll in SPAD unit was spectrophometrically read using a chlorophyll meter instrument (Minolta, Japan). Awn length (AWL) in cm was recorded by a ruler at the harvesting stage. Grain yield (14% humidity) as g per plant was also measured.

Statistical Analysis

Mean comparisons, correlation coefficients, and cluster analysis were performed using SAS 9.4 computer program. Principal component (PC) analysis was performed based on variations in K^+, Na^+, K^+/Na^+ discriminat, SOD, Tpr and SPAD units using the software Minitab 17. Linear regression analysis was conducted to determine the relationship between K^+/Na^+ discrimination and variations in grain yield. Genotypic (GCV) and phenotypic (PCV) coefficients of variation and the heritability of traits were estimated using equations described below (Falconer *et al*., 1996):

$$\sigma_g^2 = \frac{MS_g - MS_{sg}}{rs}$$

$$\sigma_e^2 = \frac{Ms_e}{r}$$

$$\sigma_P^2 = \sigma_e^2 + \sigma_g^2$$

$$CV_g = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

$$CV_{Ph} = \frac{\sqrt{\sigma_P^2}}{\bar{X}} \times 100$$

$$h^2 = \frac{\sigma_g^2}{\sigma_P^2}$$

where, $\sigma_g^2, \sigma_e^2, \sigma_P^2, MS_g, MS_{sg}, MS_e, \bar{X}, CV_g, CV_{Ph}, h^2$, r and S represent genetic variance, environmental variance, phenotypic variance, mean squares of genotype, mean squares of genotype × salinity, mean squared of error, total mean, genetic coefficient of variation, phenotypic coefficient of variation, broad-sense heritability, number of replications, and number of salt treatments, respectively.

Results and Discussion

The means for ion concentrations, agronomic traits and Tpr are presented in Table 2. Results showed that all genotypes with the exception of TRT805, TRT813, TRT815, Shiraz, Navid, and rye, accumulated higher K^+ (mg/g DM) under salt treatments compared to normal irrigation regime.

Table 2. Mean of traits for trtiticale, wheat and rye genotypes under normal irrigation condition (in parenthesis) and salinity stress.

Genotype	K^+ (mg/g DM)	Na^+ (mg/g DM)	K^+/Na^+ (mg/g DM)	SOD	Tot. protein (mg/ml)	SPAD	Leaf area (cm^2)	Day to maturity	Awn length (cm)	Grain yield per plant (g)
TRT803	2.114 (1.568)	0.450 (0.107)	5.978 (15.000)	0.134 (0.028)	0.650 (1.577)	43.356 (34.967)	29.259 (32.107)	103.583 (111.330)	4.125 (3.334)	0.309 (0.623)
TRT805	1.788 (2.097)	0.193 (0.163)	9.560 (12.897)	0.111 (0.042)	0.512 (1.163)	29.945 (35.510)	23.383 (26.503)	91.750 (98.000)	4.042 (4.334)	0.669 (0.873)
TRT806	2.240 (1.773)	0.680 (0.160)	3.859 (11.087)	0.164 (0.048)	0.679 (1.366)	37.456 (37.867)	17.591 (28.563)	102.500 (107.667)	4.125 (3.833)	0.511 (0.690)

TRT808	2.165	0.318	7.495	0.141	0.708	35.251	19.780	96.250	3.833	0.529
	(1.920)	(0.143)	(13.783)	(0.046)	(1.038)	(27.267)	(27.173)	(98.000)	(4.000)	(1.017)
TRT809	1.982	0.475	7.392	0.160	0.831	37.700	22.145	93.500	4.667	0.576
	(1.337)	(0.110)	(12.570)	(0.046)	(1.160)	(36.870)	(25.820)	(107.667)	(5.167)	(0.827)
TRT811	1.910	0.672	4.950	0.090	0.613	32.267	22.775	97.083	4.167	0.620
	(1.480)	(0.077)	(17.410)	(0.068)	(1.405)	(24.833)	(29.123)	(104.667)	(4.500)	(0.873)
TRT813	2.079	0.431	6.832	0.095	0.722	37.237	21.978	102.333	3.500	0.711
	(2.227)	(0.153)	(14.347)	(0.032)	(1.108)	(33.300)	(30.223)	(125.334)	(3.000)	(1.147)
TRT815	2.042	0.258	9.063	0.106	0.761	30.611	23.040	90.667	1.458	0.747
	(2.273)	(0.193)	(11.550)	(0.056)	(1.371)	(30.533)	(20.023)	(101.667)	(2.000)	(0.990)
TRT817	2.117	0.569	4.309	0.074	0.609	39.909	25.374	99.417	3.708	0.471
	(1.920)	(0.130)	(14.817)	(0.050)	(1.263)	(40.133)	(30.237)	(98.000)	(3.500)	(0.730)
TRT818	2.326	0.644	4.701	0.111	0.601	29.233	22.305	91.417	5.667	0.537
	(2.347)	(0.140)	(16.980)	(0.023)	(1.188)	(29.000)	(40.510)	(104.667)	(5.500)	(0.493)
ET	2.418	0.857	5.056	0.078	0.632	38.733	27.028	100.917	3.833	0.478
	(1.470)	(0.133)	(11.483)	(0.022)	(1.428)	(31.180)	(22.217)	(115.000)	(3.334)	(0.353)
Sanabad	2.467	0.798	4.958	0.309	0.636	38.255	25.729	104.667	4.500	0.531
	(2.237)	(0.197)	(11.690)	(0.040)	(1.241)	(39.023)	(26.220)	(118.000)	(4.334)	(0.823)
Rye	3.083	1.577	2.618	0.130	0.719	7.630	-	-	-	-
	(2.767)	(0.310)	(9.187)	(0.042)	(1.505)	(3.333)				
Niknezhad	1.607	0.457	5.235	0.125	0.550	32.433	11.849	105.250	4.583	0.575
	(1.400)	(0.077)	(19.200)	(0.067)	(1.259)	(32.400)	(19.473)	(121.667)	(4.334)	(0.783)
Shiraz	2.020	0.951	3.548	0.166	0.691	31.089	18.892	109.833	4.250	0.315
	(2.273)	(0.133)	(16.947)	(0.027)	(1.466)	(36.070)	(29.553)	(124.000)	(4.167)	(1.040)
Navid	0.976	0.614	1.578	0.137	0.633	7.267	13.217	95.583	3.358	0.059
	(1.217)	(0.123)	(9.960)	(0.010)	(1.408)	(9.000)	(15.067)	(115.000)	(4.500)	(0.160)
$LSD_{0.05}$	0.717	0.214	0.114	0.039	0.266	8.410	10.331	8.71	0.85	0.100

Differences higher than LSD (least significant differences) values are significant at 5% probability level in each column

Averaged over three salinity levels, triticale genotypes comprising of Sanabad (2.467), ET (2.418), TRT818 (2.326) and TRT806 (2.240) accumulated the highest amount of K^+ ion in their leaves. Variation in Na^+ accumulation (mg/g DM) ranged from 0.193 in TRT805 to 0.951 in Shiraz under salinity stress. Concentration of Na^+ in rye genotype varied between 0.31 in control condition to 1.57 under salt treatments. $K^{+/}Na^+$ discriminant decreased significantly under salt stress treatments. Reduction in K^+/Na^+ discriminant in response to salinity stress has been observed in previous studies with triticale and wheat (Houshmand et al. 2005; Salehi and Arzani 2014). In triticale, TRT805 (9.56) and TRT815 (9.063) had the highest leaf $K^{+/}Na^+$ discriminant ratio under salt stress treatments. The leaf K^+/Na^+ discriminant was lower in the rye genotype (2.61) compared to the commercial wheat cultivars with the exception of Navid (1.578). High

$K^{+/}Na^{+}$ discriminant ratio of triticale was fully ascribed to the wheat genome (Morant-Manceau et al. 2004). Linear regression analysis showed that grain yield increased as K^{+}/Na^{+} discriminant increased and this ratio explained R^2=61.7% in grain yield variations of studied genotypes (Fig. 1). It has been found that the high K^{+}/Na^{+} discriminant is a common physiological response to alleviate deleterious effects of salinity on growth cycle of plants (Blum, 2014). In some experiments on wheat, leaf Na^{+} with increasing salinity increased, but in tolerant cultivars this increase was non-significant (Sairam *et al*, 2002). Under salinity stress condition, Na^{+} and K^{+} cations transmit by a common protein thus Na^{+} competes with K^{+} to enter the cell (Parvaiz and Satyawati, 2008). The high ratio of K^{+}/Na^{+} can be excreted out Na^{+} of the cell and its accumulation within cells especially in vacuole (Blumwald *et al*, 2004). Khan et al. (1994) demonstrated that genotypes with high levels of K^{+}/Na^{+} and chlorophyll content, had higher tolerance to salinity.

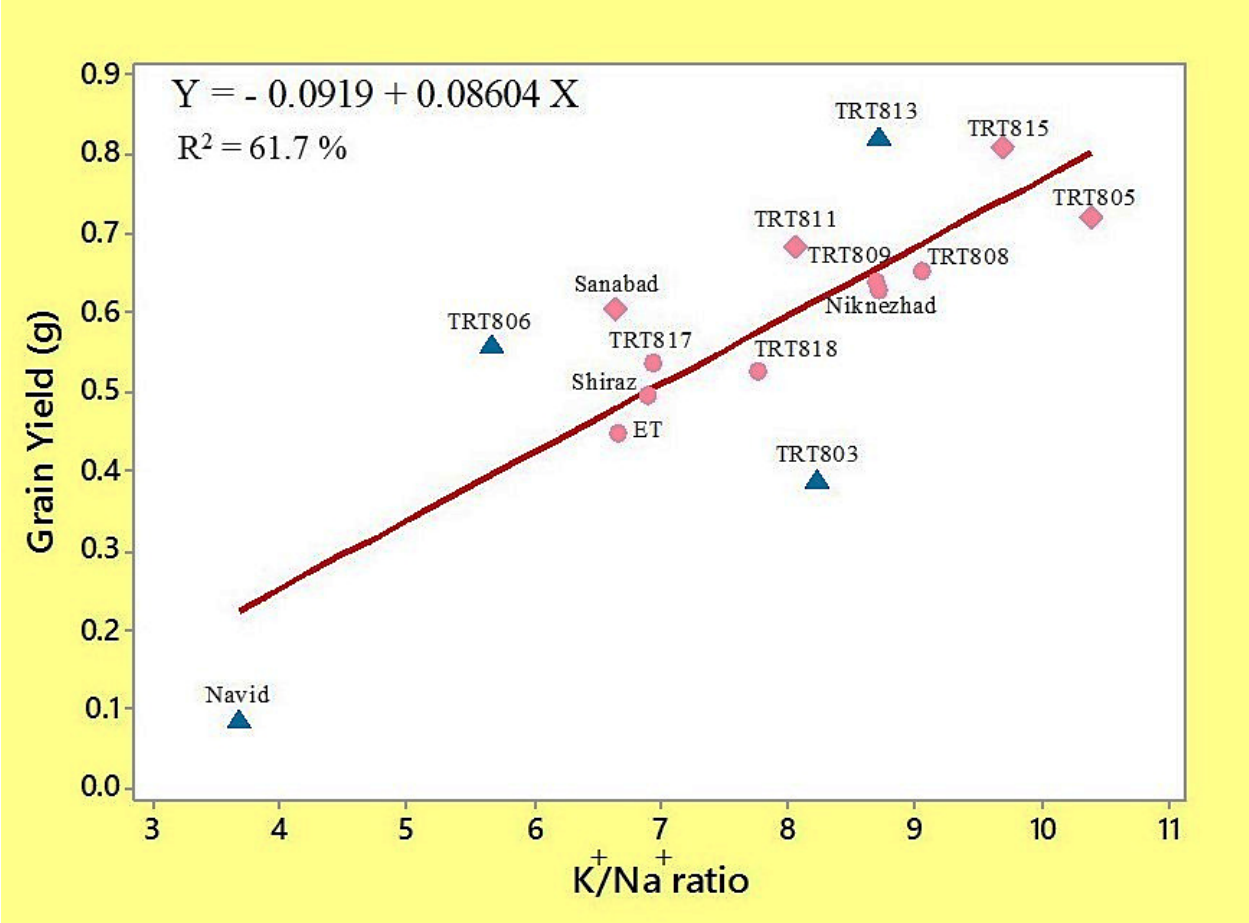

Figure 1. Relationship between K^+/Na^+ ratio and grain yield variation under salinity stress condition.

SOD activity was meaningfully increased under salinity stress treatments in all wheat, triticale and rye genotypes. Increase in antioxidants is a reaction to detoxify deleterious free radicals induced by abiotic stresses. In triticale, SOD activity in unit of enzyme ranged from 0.022 in ET to 0.068 in TRT811 under normal irrigation conditions. Under salinity stress conditions, Sanabad (triticale) had the highest (0.309) and TRT817 (triticale) showed lowest (0.074) SOD activity. Tpr significantly decreased under salt stress treatments as compared to normal irrigation regime. Decrease in protein content in high concentrations of salt may be due to hydrolysis or reduction

in protein synthesis (Cherian and Reddy, 2003). Tpr ranged from 1.038 mg/ml (TRT808) to 1.577 mg/ml (TRT803) under normal irrigation and from 0.512 (TRT805) to 0.831 (TRT809) under saline conditions. Accumulation of proteins under saline conditions may provide a storage form of nitrogen that is re-utilized later in crop growth cycle (Singh et al. 1987). Proteins may also play a role in osmotic adjustment. They may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentration (Pareek-Singla and Grover 1997).

Navid (7.267) and rye (7.63) had the lowest SPAD unit under salt stress conditions. Overall, variation of SPAD unit did not follow a clear pattern between salt stress and normal irrigation conditions. Some genotypes had higher SPAD unit under saline conditions whereas in some genotypes, plants irrigated with normal water showed higher values for SPAD unit. Chlorophyll reduction under salinity conditions is due to low chlorophyll biosynthesis or its destruction (Yokoi *et al*, 2002; Zhao *et al*, 2007). In contrast to this result, in some studies salt increases the amount of chlorophyll (Kaya *et al*, 2002; Han and Lee, 2005). Leaf area (cm^2) variation was between 20.023 and 40.51 in triticales irrigated with normal water. Under salt stress conditions LFA of triticales varied from 17.591 in TRT806 to 29.259 in TRT803. Wheat cultivars had lower LFA compared with most of triticales under salt stress condition with a variation between 11.849 and 18.892. Awn length in TRT803, TRT806, TRT813, TRT817, TRT818, ET and Sanabad irrigated with saline solutions was higher than AWL in triticales grew under normal conditions. For other triticales, no significant decrease was found in AWL under salt stress conditions.

Number of day to maturity ranged between 90.6 and 104.6 for triticales under salinity conditions. Day to maturity in wheat cultivars was from 95.5 to 105.2. This shows that triticale genotypes matured earlier than wheat cultivars. Grain yield per plant (g) was significantly

decreased in all genotypes irrigated with saline solutions. Grain yield reduction under salinity stress was also reported by Sadat Noori and Mc Neilly (2003) and Poustini and Siosemardeh (2004). Grain yield variation was from 0.059 g to 0.575 g in wheat and from 0.309 to 0.747 g in triticale genotypes irrigated with saline solutions. Grain yield as a genetically complex trait is broadly influenced by environmental stresses such as salinity. Rye genotype did not complete its growth and no seed was produced. Among triticales, TRT815 (0.747 g) and TRT813 (0.711 g) had the highest grain yield over salt stress treatments. TRT813 had the highest grain yield (1.147 g) under normal irrigation conditions. Most triticale genotypes had higher grain yield than wheat cultivars under normal irrigation conditions. Oettler (2005), also pointed out the superiority of triticale to wheat under stress conditions. The potential yield of cereals i.e. wheat or triticale, has an important impact on its performance under moderate stress (Blum, 2014). The potential advantage of triticale over wheat is the higher biomass of triticale due to greater radiation-use efficiency (RUE) derived from greater radiation interception by the triticale canopy. In a study with doubled haploids and advanced recombinant lines, triticale genotypes significantly yielded higher than wheat commercial cultivars when irrigated with saline solutions (Salehi and Arzani, 2014).

Cluster analysis of genotypes was performed based on variations in days to maturity, LFA, ion concentrations, SPAD unit and grain yield for both normal irrigation and salinity stress conditions. Under normal irrigation regime, genotypes were classified into three main groups (Fig. 2) Group 3 comprising of TRT805, TRT817, TRT809, TRT813 and Sanabad had the highest grain yield. These genotypes matured earlier than genotypes in groups 1 and 2 (Table 3).

Table 3. Mean of traits for groups of genotypes identified in cluster analysis under normal irrigation regime.

Trait	Group		
	1	**2**	**3**
Day to maturity (day)	110.333 a	109.111 a	101.445 a
Total leaf area (cm^2)	31.611 a	27.928 a	25.44 a
K^+(mg/g DM)	0.127 a	0.152 a	0.138 a
Na^+(mg/g DM)	1.794 a	1.932 a	10891 a
K^+/Na^+	14.488 a	12.901 a	14.248 a
SPAD unit	31.716 b	37.117 a	27.544 b
Grain yield per plant (g)	0.49 b	0.848 a	0.96 a

DM: dry matter

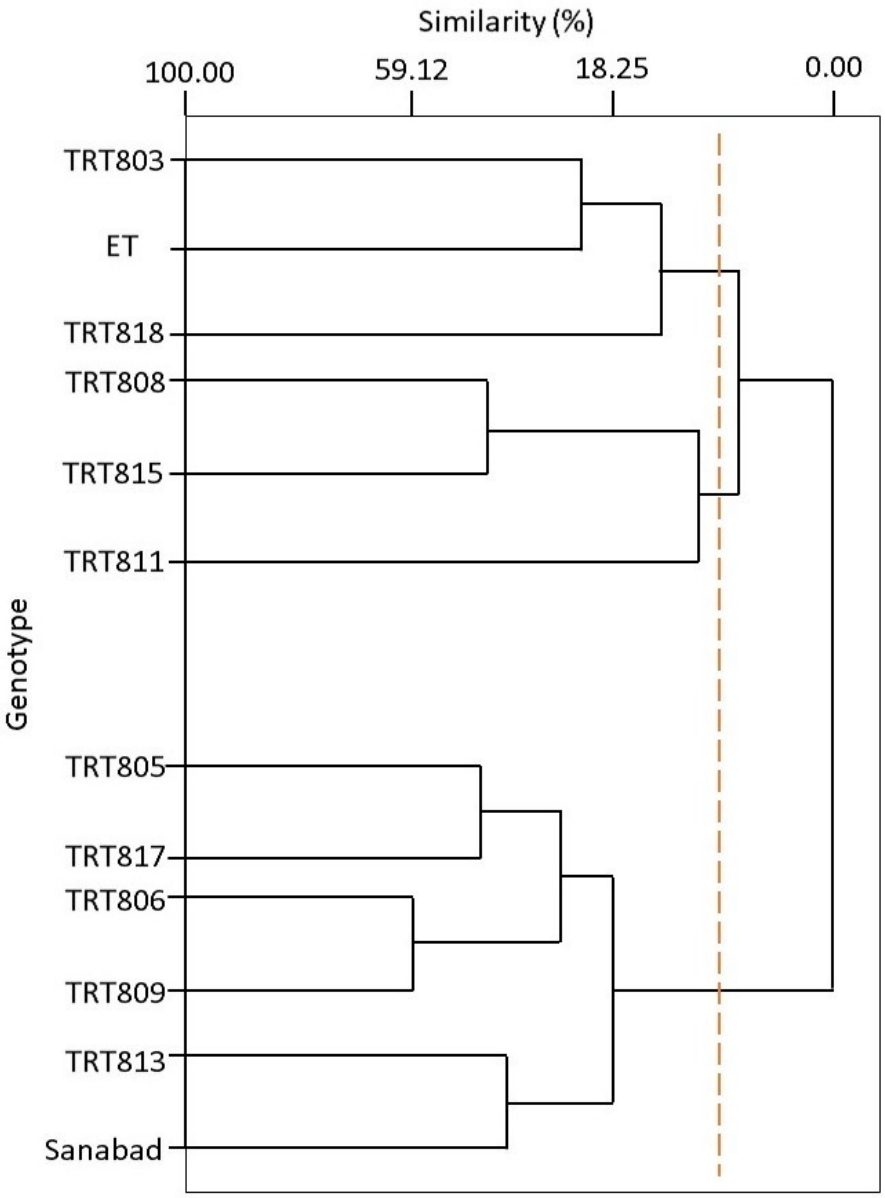

Figure 2. Tree dendrogram of triticale genotypes based on cluster analysis of traits under normal irrigation condition.

K^+/Na^+ discriminant ratio for group 3 was higher than the ratio in group 2 and non-significantly lower than the ratio in group 1. Thus, genotypes of group 3 especially TRT808, TRT811 and TRT815 had better performance than other genotypes under normal irrigation conditions. Four main groups were identified based on average data under salinity stress (Fig. 3). Group 2 comprising of TRT806 and TRT808 had the highest mean for grain yield and K^+/Na^+ discriminant ratio. These two genotypes matured significantly earlier than genotypes in other groups (Table 4). Genotypes of group 2 accumulated lowest Na+ in average. It can be concluded that genotypes of group 2 can be used in breeding programs or cross hybridization for transferring genes of salt stress tolerance in triticale. Group 4 ranked in second for grain yield, K^+/Na^+ discriminant and early maturity.

Table 4. Mean of traits compared between clusters of genotypes identified in cluster analysis under salt stress condition.

Trait	Group			
	1	2	3	4
Day to maturity (day)	99.333 a	88.333 b	98.222 a	91.25 b
Total leaf area	26.847 a	23.211 b	18.681 c	22.301 b
K^+	2.279 a	1.915 a	2.203 a	2.074 a
Na^+	0.668 a	0.226 b	0.499 ab	0.556 ab
K^+/Na^+	5.075 b	9.312 a	5.677 b	5.969 b
SPAD unit	40. 61 a	30.278 c	36.354 ab	34.109 bc
Grain yield per plant (g)	0.447 c	0.708 a	0.52 bc	0.611 ab

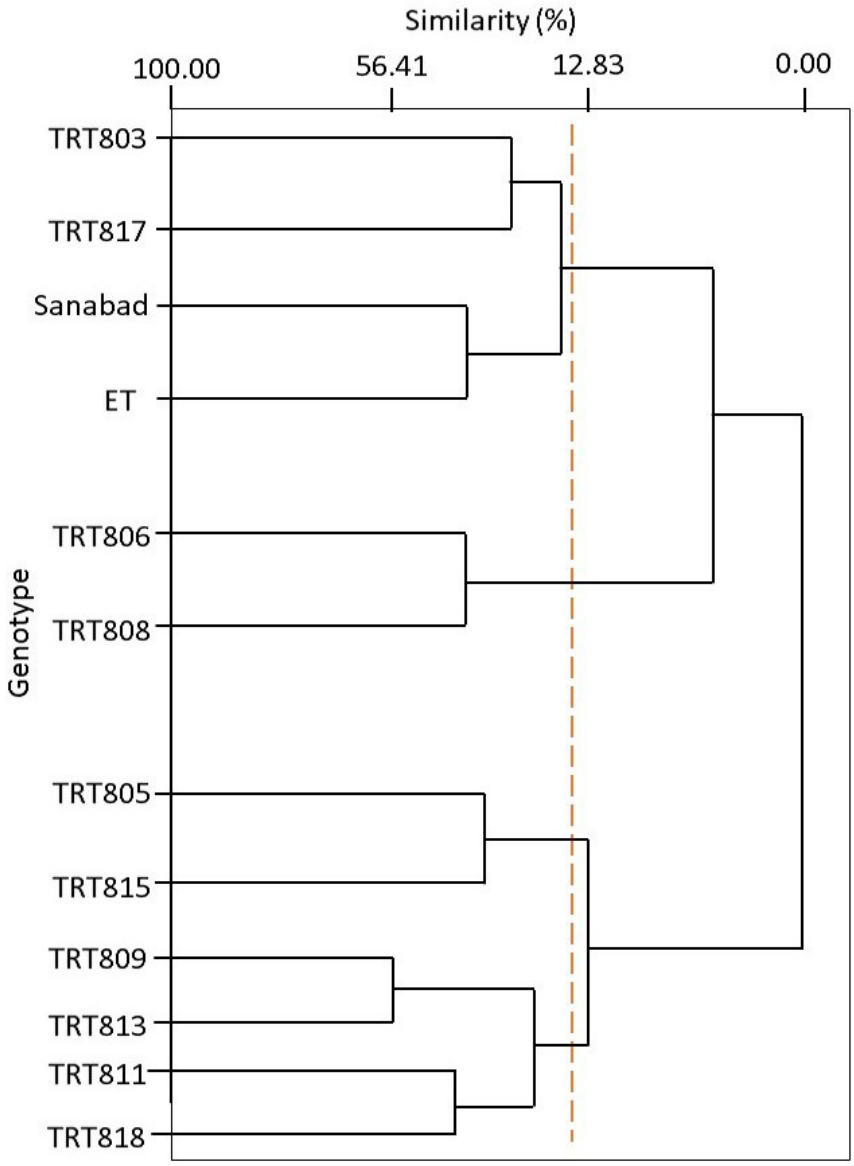

Figure 3. Tree dendrogram of triticale genotypes based on cluster analysis of traits under salt stress condition.

Correlation analysis indicated that grain yield had highest correlation (r=0.36) with awn length which shows the importance of awn in salt tolerance (Table 5). K^+/Na^+ discriminant had positive correlation with leaf area (r=0.65) and grain yield (r=0.34). Correlation of K^+ and leaf area (r=0.57) shows that increase in leaf area results in higher accumulation of K^+ ion under salt stress

condition. Correlation coefficients simply showed that total protein increased when K^+ and SOD activity increased.

Table 5. Correlation coefficients of traits under salinity stress condition.

	K^+	Na^+	K^+/Na^+	SOD	Tpr	SPAD	LFA	DMA	AWL	GY
1- K^+	-	0.54	0.14	0.25	0.33	0.39	0.57	-0.28	0.18	0.15
2- Na^+		-	0.71	0.16	0.27	0.08	0.65	0.003	0.01	0.34
3- K^+/Na^+			-	0.12	0.04	-0.61	0.27	-0.01	-0.16	0.29
4- SOD				-	0.47	-0.003	0.20	-0.04	0.04	-0.08
5- Tpr					-	0.24	0.32	-0.20	0.26	-0.15
6- SPAD						-	0.39	0.06	0.23	-0.10
7- LFA							-	0.15	0.24	0.25
8- DMA								-	-0.07	-0.17
9- AWL									-	0.36
10-Grain yield										-

In PC analysis, the first two PCs explained 49.7% of the variations of traits in genotypes in dealing with salt stress (Fig. 4). The first PC was linked to K^+/Na^+ and grain yield variations in S1 and S2 level of salinity. In contrast, second PC accounting for 18% of the variations of traits and genotypes influenced by K^+, Na^+, SPAD, total protein, grain yield in S3 and SOD in S2 salinity levels. In bi-plot and PC analysis, the cosine of the angles between vectors shows the extent of correlation between traits. The acute angles (<90˚) represent positive correlations, whereas wide obtuse angles (90˚<) show negative correlation. The intensity of the correlation increases for the angles near 0˚ and 180˚ and the length of the vectors connecting traits to the origin show the extent of variability (Tahmasebi et al. 2014). In the present study, projection of genotypes on the two detected PCs in bi-plot showed that the quadrant IV comprised of grain yield (under S1 and S2) and K^+/Na^+ discriminant (under S1, S2 and S3) vectors with acute angels (Fig. 4). Thus, genotypes (TRT805, TRT808, TRT809, TRT813 and TRT815) scattered between these vectors had better performance under salt stress conditions. Vectors of K^+ under S1, S2 and S3 levels had narrow angels with the vector of grain yield under S3 which shows the importance

of K^+ accumulation in variation of grain yield under salt stress conditions. Ordination of vectors of Na^+ ion in quadrant II and III indicated that wheat cultivars were more sensitive to the adverse effects of salinity compared with triticale genotypes.

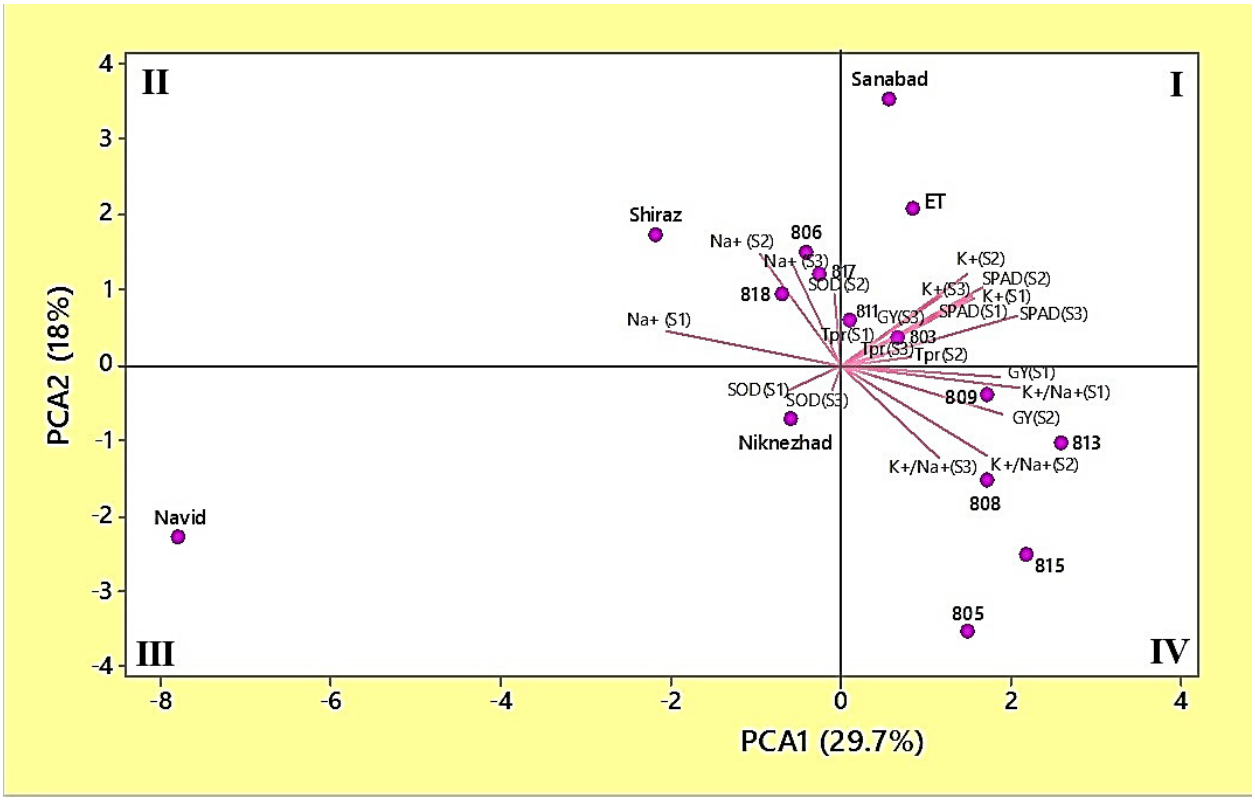

Figure 4. Projection of genotypes on the first (PCA1) and second (PCA2) principal components. GY: grain yield, SOD: superoxide dismutase, Tpr: total protein, SPAD: chlorophyll content, S1, S2 and S3: vectors of salt stress levels respectively for EC=7, 14 and 21 dS/m

Estimations of heritability, PCV and GCV under salt stress condition are presented in Table 6. PCV ranged from 12.19% (for day to maturity) to 50.28% (for Na^+). These figures show that studied genotypes were restricted for maturity time whereas wide variation was observed for Na^+ in response to salt stress conditions. GCV varied between 4.72% and 42.88%. After Na^+, SPAD unit (31.45%) and grain yield (26.55%) had the highest genetic variation in genotypes dealing with salt stress condition. GCV for awn length (21.64%) ranked fourth after Na+, SPAD and grain yield. The importance of awn is linked to its capacity in photosynthesis under abiotic stresses. Heritability estimates ranged from 14.96% for day to maturity to 85.85% for grain yield. High heritability which is associated with higher contribution of genetic variance in phenotypic variation shows higher efficiency for selection of high-yielding genotypes in breeding programs especially for production of salt tolerant cultivars. In the present study, relatively low heritability of K^+/Na^+ discriminant (29.07%) may associate with non-additive components comprising of environmental effects. For such traits with low heritability, selections may be performed via correlated traits with higher heritability.

Table 6 Genotypic and (GCV) and phenotypic (PCV) coefficients of variation and heritability (h^2) of traits in triticale genotypes.

Traits				$GCV_{(\%)}$	$PCV_{(\%)}$	h^2 (%)
K^+	0.148	0.197	0.345	18.920	28.870	42.960
Na^+	0.047	0.018	0.064	42.880	50.280	72.720
K^+/ Na^+	1.106	1.080	7.612	19.820	36.760	29.070
SOD	0.0004	0.0006	0.001	19.240	30.890	38.790
Total protein	0.001	0.0271	0.029	4.630	20.580	5.060
SPAD	97.199	27.095	124.294	31.450	35.560	78.200
Leaf area	12.956	40.841	53.797	15.700	31.990	24.080
Day to maturity	21.796	123.905	145.702	4.720	12.190	14.960
Awn length	0.742	1.186	1.928	21.640	34.880	38.500
Grain yield per plant	0.023	0.004	0.027	26.550	28.650	85.850

Conclusion

In the present study, the responses of triticale, wheat and rye genotypes to three levels of salt stress (7, 14 and 21 dS/m) were assayed in a pot experiment. Variation in two sets of traits comprising of agronomic (grain yield, awn length, leaf area and maturity) and physiological (K^+, Na^+, K^+/Na^+, chlorophyll content in SPAD unit, superoxide dismutase activity and total protein) traits were investigated in genotypes irrigated with saline solutions and normal water. Grain yield decreased significantly in all genotypes irrigated with saline solutions. Among triticales TRT813 and TRT815 had the highest grain yield under salinity stress conditions. Most of triticale genotypes had higher grain yield than wheat and rye genotypes under both conditions. K^+/Na^+ discriminant ratio which explained 61.7% of grain yield variation in linear regression analysis was higher in most of triticales compared with wheat genotypes. Cluster analysis of genotypes using variations in some of agronomic and physiological traits indicated that TRT808 and TRT806 joined in the second group in tree dendrogram under salt stress conditions. These genotypes had relatively high grain yield and K^+/Na^+ discriminant ratio and matured earlier compared to genotypes of other groups. Correlation analysis indicated that awn length had the highest correlation with grain yield which shows the role of awn in salt stress tolerance. Correlation coefficients also revealed that higher K^+ and SOD activity end to accumulation of higher protein accumulation. Acute angels between the grain yield and K^+/Na^+ vectors in PC analysis indicated strong association of these traits under 7, 14 and 21 dS/m salt stress levels. Calculation of genetic variance showed the existence of genetic variation for grain yield, K^+ accumulation, leaf area and awn length in studied genotypes. Heritability of grain yield was relatively high which shows efficiency of selection of high-yielding genotypes under salt stress conditions. Overall, it can be concluded that triticale genotypes performed better than wheat

cultivars and were genetically variable in response to different levels of salt stress. High genetic variations for some of salt stress- adaptive traits provide opportunities for production of high-yielding triticales. For such purpose, TRT806, TRT808, TRT815 with higher grain yield, K^+/Na^+ ratio or early maturity characteristic were more potent in their response to deal with salt stress and can be involved in breeding programs of triticale for saline conditions.

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