

**Selection of salt- tolerant triticale (\times *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 CaCl₂ 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^+ discrimination ratio which explained 61.7% of the total grain yield variation in linear regression analysis was higher in most of triticales compared with wheat genotypes. Correlation analysis indicated that awn length had the highest correlation with grain yield. Acute angle between grain yield and K^+/Na^+ discrimination 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^+ discrimination 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: Genetic variation, K^+/Na^+ , Salt tolerance, Triticale

1 **Introduction**

2 Salinity is a major problem in arid and semi-arid areas of the world. Data show that 800 million
3 hectares, equivalent to 6% of the land is affected by salinity worldwide [1]. Salinity in
4 agricultural lands affects crop plants growth [2]. In Iran, about 20% of arable lands (equivalent to
5 34 million hectares) are affected by salinity of which 8.5 million hectares suffer from severe
6 salinity [3].

7 Triticale is mainly grown for feed grain and biomass production for thatching straw and
8 common human utilization. Regardless of the advantages, the early history of triticale as the first
9 man-made crop plant constituted mainly of a botanical curiosity of no agronomic value. It took
10 nearly a hundred years of research for botanical and genetic characteristics to evolve triticale into
11 a crop [4, 5]. A comprehensive review of triticale response and adaptation shows that it can
12 tolerate some of abiotic stresses better than small grain cereals [6]. A recent research by Motzo et
13 al. [7] did not confirm a yield reduction in triticale due to late season drought stress, in
14 comparison with durum wheat. According to USDA Salinity lab, the salinity tolerance of triticale
15 is basically better than that of wheat and might even approach that of barley [8]. In a field study
16 [9], triticale cultivars were cultivated over a range of saline soils and the threshold for yield
17 reduction under salinity stress was higher (7.3 dS m^{-1}) than that of cited in USDA Salinity lab
18 data (6.1 dS m^{-1}). The potential advantage of triticale over wheat in biomass and grain yield was
19 confirmed in the study of Estrada-Campuzano et al. [10].

20 An increase in salt concentrations affects nearly all physiological processes of plants
21 including photosynthesis, protein synthesis, energy metabolism and lipid metabolism [11].
22 Salinity due to osmotic effects on different metabolic activity induces water deficiency leading to
23 oxidative stress by increasing the reactive oxygen species (ROS) production comprising of

1 superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH). Oxidative stress may
2 cause cell damage through oxidation of lipids, proteins and nucleic acids [11, 12]. To overcome
3 the effects of salinity- induced oxidative stress, plants use antioxidants like superoxide dismutase
4 (SOD) [12]. Other factors comprising of ion toxicity, osmotic stress and nutrient imbalance are
5 associated with deleterious effect of salinity on plant growth and productivity. Therefore,
6 understanding mechanisms such as Na^+ exclusion, K^+/Na^+ discrimination and osmotic
7 adjustment is essential to improve salt tolerance in crop plants. Accumulation of K^+ or higher
8 K^+/Na^+ may be correlated with salt tolerance in crop plants [13]. Low Na^+ accumulation and
9 high K^+/Na^+ discrimination have been found to be strongly associated with enhanced salinity
10 tolerance in bread and durum wheats [14, 15]. Likewise, relationship between low Na^+
11 accumulation and salt tolerance has been found in barley [16, 17, 18].

12 Triticale seems to be an alternative to other small grain cereals, particularly wheat and
13 **barley**, for cultivation under unfavorable conditions or in the low-input agricultural systems.
14 Existence of appropriate genetic variation is a prerequisite for the improvement of any character,
15 through selection and breeding. Fortunately, diversity in salt tolerance at the intra specific level
16 has been found in triticale [19]. Triticale constitutes also a valuable genetic resource for
17 transferring genes of interest from rye into wheat, particularly those related to biotic and abiotic
18 stresses [20]. **Estimation** of genetic variation parameters and heritability of the adaptive-salt
19 tolerance traits are useful in making decisions for breeding triticale to become as important as
20 wheat or even more at a global scale. **For** better understanding the selection efficiency of salt-
21 tolerance in breeding programs, genotypic and phenotypic variations and heritability of traits is
22 very important. Hence, **in the present study**, we have focused on the response of some
23 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 4 levels of salinity were employed. Combinations of 1:1 ratio of NaCl and CaCl₂ 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 hypochlorite 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 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

1

2 **Ion measurement**

3 Na⁺ and K⁺ ions were measured four weeks after the application of salinity. Leaves of 5 plants in
4 each pot were collected and oven-dried at 70°C for 48 hr and were milled to a fine powder. The
5 samples were placed in a crucible and ashed by transferring to a furnace at 500°C for 2 h. An
6 amount of 5 ml HCl (2N) was added to each crucible and mixed thoroughly. Then, boiling
7 distilled water was added to the mixture and then filtered in a 50 ml volumetric flask.
8 Concentrations (mg/g of dry mater, DM) of Na⁺ and K⁺ ions were measured using flame
9 photometry according to Hamada [21] procedure. K⁺/Na⁺ ratio was also calculated.

10 **Total protein and antioxidant enzymes assays**

11 Forty five days after the start of salt treatment application, the leaves of genotypes in each pot
12 were separated and placed in liquid nitrogen immediately. Samples were kept in a refrigerator at
13 -4°C until distillation of leaves. The Bradford [22] procedure was used to measure total protein
14 (Tpr) in mg/ml. Beauchamp and Fridovich [23] method was followed to quantify SOD activity
15 based on unit of enzyme.

16

17 **Assays for agronomic traits**

18 Forty five days after the onset of salt stress treatment which was coincident with post-heading
19 stage, a leaf area meter instrument (ΔT-Cambridge device, the UK) was used to measure total

1 leaf area (LFA) as cm² per plant. Days to maturity was estimated based on the number of days
2 from the first irrigation to physiological maturity when spikes turned yellow. Twenty days after
3 the first irrigation with salt solutions, on a sunny day between 11 am to 3 pm, the concentration
4 of chlorophyll in SPAD unit was spectrophotometrically read using a chlorophyll meter instrument
5 (Minolta, Japan). Awn length (AWL) in cm was recorded by a ruler at the harvesting stage.
6 Grain yield (14% humidity) as g per plant was also measured.

7 **Statistical Analysis**

8 Mean comparisons, estimation of correlation coefficients, and cluster analysis were performed
9 using SAS 9.4 software. Principal component (PC) analysis was performed based on variations
10 in K⁺, Na⁺, K⁺/Na⁺ discrimination, SOD, Tpr and SPAD units using the software Minitab 17.
11 Linear regression analysis was conducted to determine the relationship between K⁺/Na⁺
12 discrimination and variations in grain yield. Genotypic (GCV) and phenotypic (PCV)
13 coefficients of variation and the heritability of traits were estimated using equations described
14 below [24]:

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

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

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

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

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

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

1 where, σ_g^2 , σ_e^2 , σ_p^2 , MS_g , MS_{sg} , MS_e , \bar{X} , CV_g , CV_{ph} , h^2 , r and S represent genetic variance,
2 environmental variance, phenotypic variance, mean squares of genotype, mean squares of
3 genotype \times salinity, mean squared of error, total mean, genetic coefficient of variation,
4 phenotypic coefficient of variation, broad-sense heritability, number of replications, and number
5 of salt treatments, respectively.

6

7 Results and Discussion

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

Table 2. Mean of traits for triticale, 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 (1.920)	0.318 (0.143)	7.495 (13.783)	0.141 (0.046)	0.708 (1.038)	35.251 (27.267)	19.780 (27.173)	96.250 (98.000)	3.833 (4.000)	0.529 (1.017)
TRT809	1.982 (1.337)	0.475 (0.110)	7.392 (12.570)	0.160 (0.046)	0.831 (1.160)	37.700 (36.870)	22.145 (25.820)	93.500 (107.667)	4.667 (5.167)	0.576 (0.827)
TRT811	1.910 (1.480)	0.672 (0.077)	4.950 (17.410)	0.090 (0.068)	0.613 (1.405)	32.267 (24.833)	22.775 (29.123)	97.083 (104.667)	4.167 (4.500)	0.620 (0.873)

TRT813	2.079 (2.227)	0.431 (0.153)	6.832 (14.347)	0.095 (0.032)	0.722 (1.108)	37.237 (33.300)	21.978 (30.223)	102.333 (125.334)	3.500 (3.000)	0.711 (1.147)
TRT815	2.042 (2.273)	0.258 (0.193)	9.063 (11.550)	0.106 (0.056)	0.761 (1.371)	30.611 (30.533)	23.040 (20.023)	90.667 (101.667)	1.458 (2.000)	0.747 (0.990)
TRT817	2.117 (1.920)	0.569 (0.130)	4.309 (14.817)	0.074 (0.050)	0.609 (1.263)	39.909 (40.133)	25.374 (30.237)	99.417 (98.000)	3.708 (3.500)	0.471 (0.730)
TRT818	2.326 (2.347)	0.644 (0.140)	4.701 (16.980)	0.111 (0.023)	0.601 (1.188)	29.233 (29.000)	22.305 (40.510)	91.417 (104.667)	5.667 (5.500)	0.537 (0.493)
ET	2.418 (1.470)	0.857 (0.133)	5.056 (11.483)	0.078 (0.022)	0.632 (1.428)	38.733 (31.180)	27.028 (22.217)	100.917 (115.000)	3.833 (3.334)	0.478 (0.353)
Sanabad	2.467 (2.237)	0.798 (0.197)	4.958 (11.690)	0.309 (0.040)	0.636 (1.241)	38.255 (39.023)	25.729 (26.220)	104.667 (118.000)	4.500 (4.334)	0.531 (0.823)
Rye	3.083 (2.767)	1.577 (0.310)	2.618 (9.187)	0.130 (0.042)	0.719 (1.505)	7.630 (3.333)	-	-	-	-
Niknezhad	1.607 (1.400)	0.457 (0.077)	5.235 (19.200)	0.125 (0.067)	0.550 (1.259)	32.433 (32.400)	11.849 (19.473)	105.250 (121.667)	4.583 (4.334)	0.575 (0.783)
Shiraz	2.020 (2.273)	0.951 (0.133)	3.548 (16.947)	0.166 (0.027)	0.691 (1.466)	31.089 (36.070)	18.892 (29.553)	109.833 (124.000)	4.250 (4.167)	0.315 (1.040)
Navid	0.976 (1.217)	0.614 (0.123)	1.578 (9.960)	0.137 (0.010)	0.633 (1.408)	7.267 (9.000)	13.217 (15.067)	95.583 (115.000)	3.358 (4.500)	0.059 (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

1

2 Averaged over three salinity levels, triticale genotypes comprising of Sanabad (2.467), ET

3 (2.418), TRT818 (2.326) and TRT806 (2.240) accumulated the highest amount of K⁺ ion in their

4 leaves. Variation in Na⁺ accumulation (mg/g DM) ranged from 0.193 in TRT805 to 0.951 in

5 Shiraz under salinity stress. Concentration of Na⁺ in rye genotype varied between 0.31 in control

6 condition to 1.57 under salt treatments. K⁺/Na⁺ discrimination decreased significantly under salt

7 stress treatments. Reduction in K⁺/Na⁺ discrimination in response to salinity stress has been

8 observed in previous studies with triticale and wheat [14, 25]. In triticale, TRT805 (9.56) and

9 TRT815 (9.063) had the highest leaf K⁺/Na⁺ discrimination ratio under salt stress treatments. The

1 leaf K^+/Na^+ discrimination was lower in the rye genotype (2.61) compared to the commercial
2 wheat cultivars with the exception of Navid (1.578). High K^+/Na^+ discrimination ratio of triticale
3 was fully ascribed to the wheat genome [26]. Linear regression analysis showed that grain yield
4 increased as K^+/Na^+ discrimination increased and this ratio explained $R^2=61.7\%$ in grain yield
5 variations of studied genotypes (Fig. 1). It has been found that the high K^+/Na^+ discrimination is
6 a common physiological response to alleviate deleterious effects of salinity on growth cycle of
7 plants [8]. In some experiments on wheat, leaf Na^+ with increasing salinity increased, but in
8 tolerant cultivars, this increase was non-significant [27]. Under salinity stress condition, Na^+ and
9 K^+ cations transmit by a common protein thus Na^+ competes with K^+ to enter the cell [28]. The
10 high ratio of K^+/Na^+ can be achieved by excretion of Na^+ of the cell and its accumulation within
11 cells especially in vacuole [29]. Khan et al. [30] demonstrated that genotypes with high levels of
12 K^+/Na^+ and chlorophyll content, had higher tolerance to salinity.

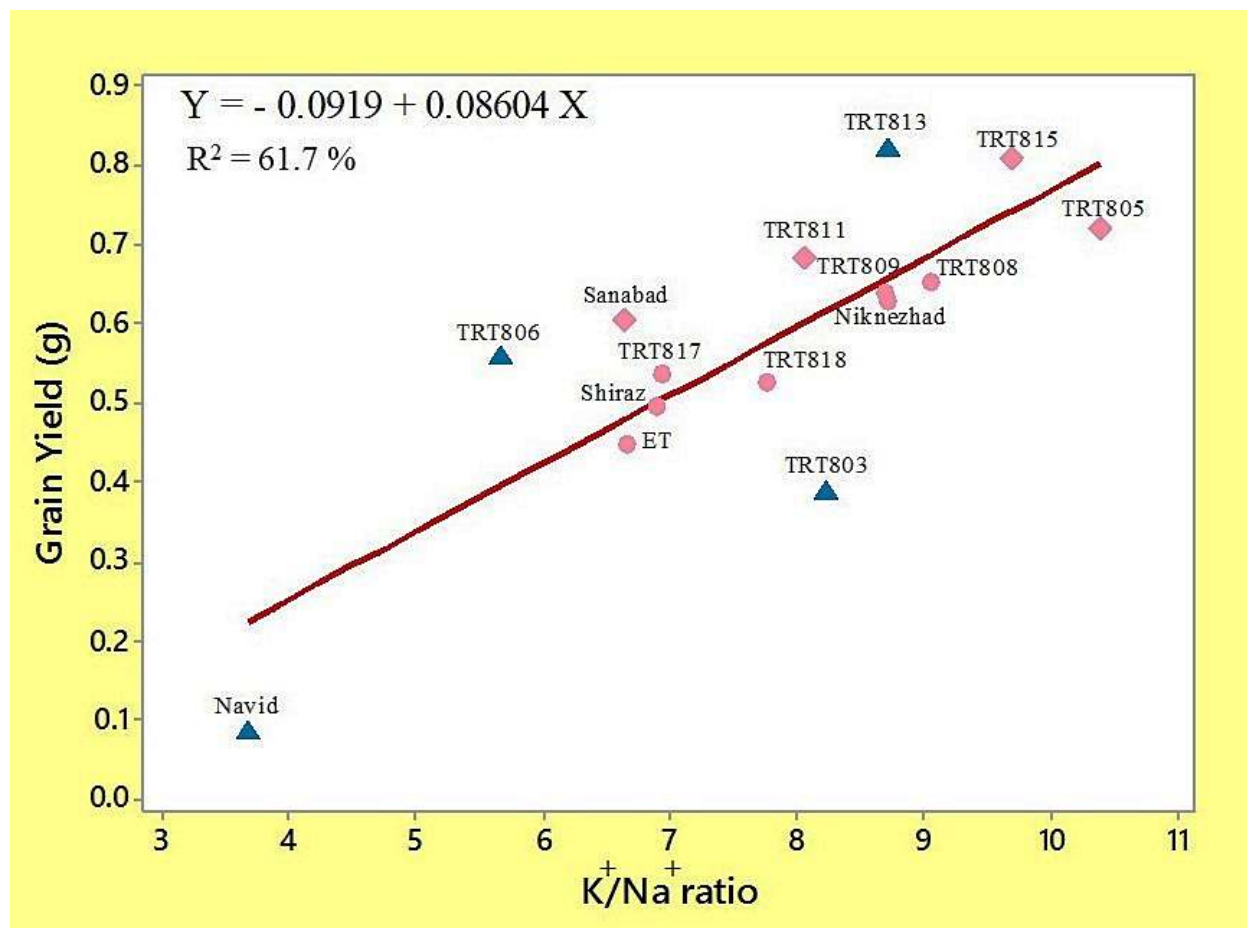


Figure 1. Relationship between K⁺/Na⁺ ratio and grain yield variation under salinity stress condition.

SOD activity was meaningfully increased under salinity stress treatments in all wheat, triticale and rye genotypes. An 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 the lowest (0.074) SOD activity. Tpr significantly decreased under salt stress treatments as compared to normal irrigation regime. A decrease in protein content in high concentrations of salt may be due to hydrolysis or

1 reduction in protein synthesis [31]. Tpr ranged from 1.038 mg/ml (TRT808) to 1.577 mg/ml
2 (TRT803) under normal irrigation and from 0.512 (TRT805) to 0.831 (TRT809) under saline
3 conditions. Accumulation of proteins under saline conditions may provide a storage form of
4 nitrogen that is re-utilized later in crop growth cycle [32]. Proteins may also play a role in
5 osmotic adjustment. They may be synthesized *de novo* in response to salt stress or may be
6 present constitutively at low concentration [33].

7 Navid (7.267) and rye (7.63) had the lowest SPAD unit under salt stress conditions.
8 Overall, variation in SPAD reads did not follow a clear pattern between salt stress and normal
9 irrigation conditions. Some genotypes had higher SPAD unit under saline conditions whereas in
10 some genotypes, plants irrigated with normal water showed higher values for SPAD reads.
11 Chlorophyll reduction under salinity conditions is due to low chlorophyll biosynthesis or its
12 destruction [34, 35]. In contrast to this result, in some studies, salt increases the amount of
13 chlorophyll [36, 37]. Leaf area (cm²) variation was between 20.023 and 40.51 in triticales
14 irrigated with normal water. Under salt stress conditions, LFA of triticales varied from 17.591 in
15 TRT806 to 29.259 in TRT803. Wheat cultivars had lower LFA compared with most of triticales
16 under salt stress condition with a variation between 11.849 and 18.892. Awn length in TRT803,
17 TRT806, TRT813, TRT817, TRT818, ET and Sanabad irrigated with saline solutions was higher
18 than AWL in triticales grown under normal conditions. For other triticales, no significant
19 decrease was found in AWL under salt stress conditions.

20 Number of days to maturity ranged between 90.6 and 104.6 for triticales under salinity
21 conditions. Day to maturity in wheat cultivars was from 95.5 to 105.2. This shows that triticales
22 genotypes matured earlier than wheat cultivars. Grain yield per plant (g) was significantly
23 decreased in all genotypes irrigated with saline solutions. Grain yield reduction under salinity

stress was also reported by Sadat Noori and Mc Neilly [38] and Poustini and Siosemardeh [39]. 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 [40], 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 [8]. In a study with doubled haploids and advanced recombinant lines, triticale genotypes significantly yielded higher than wheat commercial cultivars when irrigated with saline solutions [25]. In the present study, triticale genotypes had higher K^+/Na^+ discrimination ratio than wheat cultivars and higher K^+/Na^+ discrimination ratio had significant correlations with higher grain yield (Fig. 1). Few studies probed the basis of salinity tolerance in triticale. While molecular mapping of salinity tolerance is being pursued in wheat, no such work has yet been seen to-date for triticale. The high K^+/Na^+ discrimination ratio which is a common physiological salinity tolerance marker in crop plants was in triticale at least as high as in wheat and close to barley under saline hydroponics culture [8]. 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. Triticale produces more above-ground dry matter than other cereals under abiotic stress conditions [8]. It seems that the opinions on the hardiness of rye in terms of coping with soil and atmospheric stresses were derived from ample historical experience of farmers and

some research. A major physiological-genetic variable accounting for biomass and yield variations among crop plant genotypes has been linked to stomatal conductance and assimilation. Productive genotypes which also sustain growth and yield under stress and variable conditions express higher stomatal conductance and gas exchange.

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 read	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,* Letters in each row show significant differences between means using LSD (5%) test.

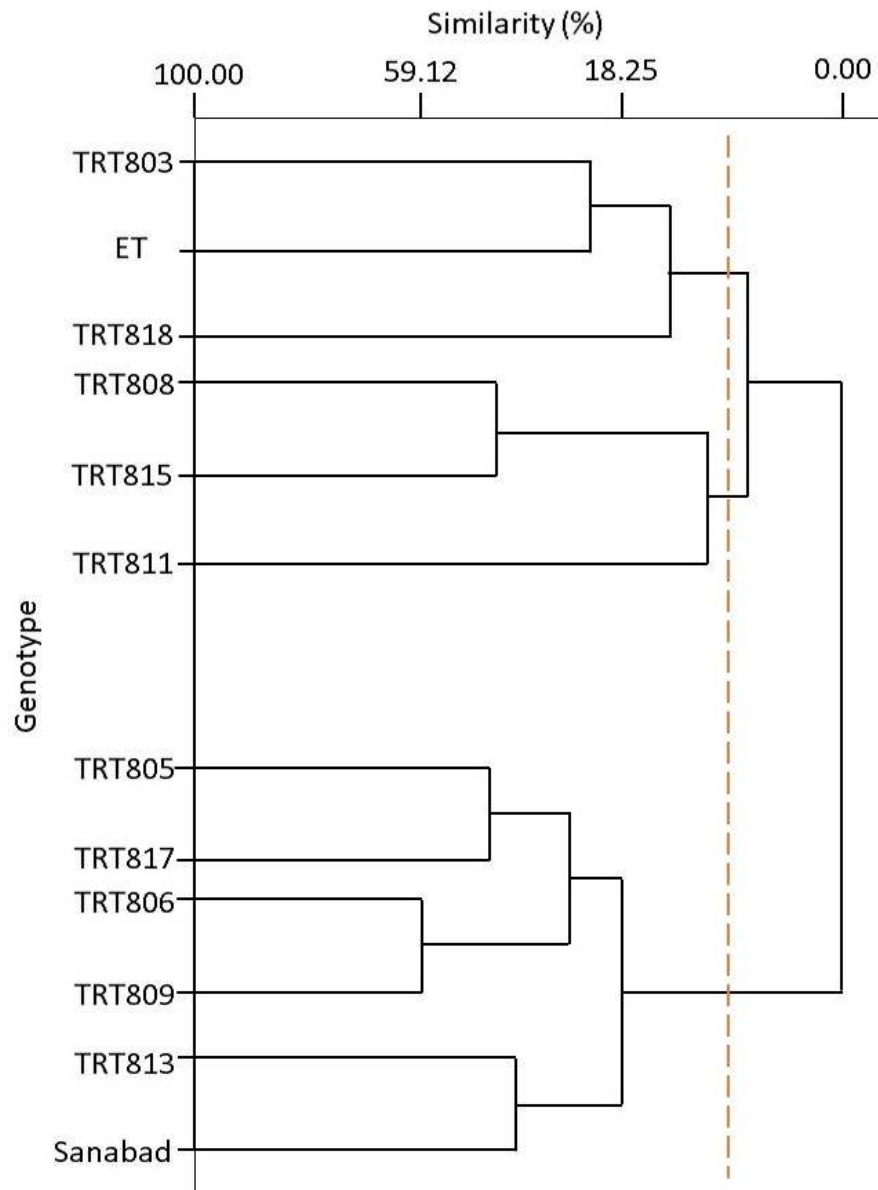


Figure 2. Tree dendrogram of triticale genotypes based on cluster analysis of traits under normal irrigation condition.

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

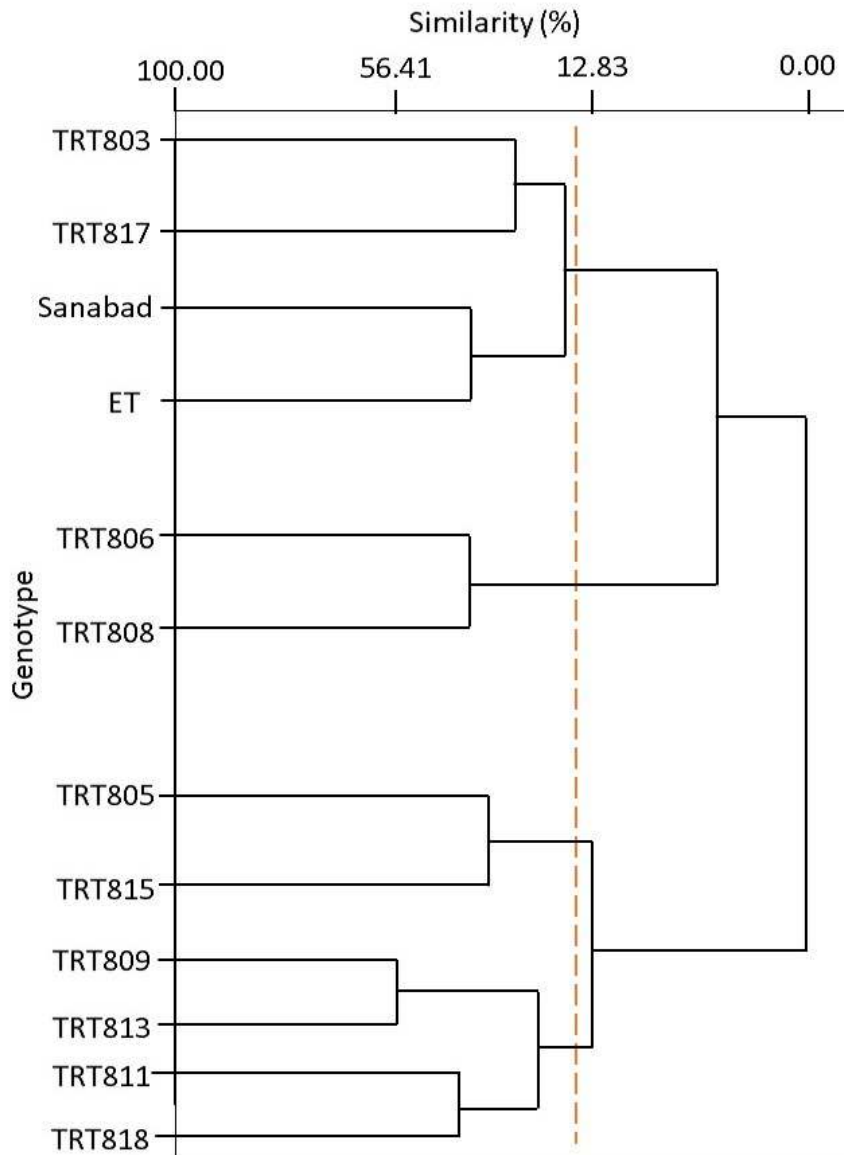


Figure 3. Tree dendrogram of triticale genotypes based on cluster analysis of traits under salt stress condition.

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^+ discrimination had positive correlation with leaf area ($r=0.65$) and grain yield ($r=0.34$). Correlation between K^+ and leaf area ($r=0.57$) shows that an increase in leaf area results in higher accumulation of K^+ ion

1 under salt stress condition. Correlation coefficients simply showed that total protein increased
2 when K^+ and SOD activity increased.

Table 5. Correlation coefficients between 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										-

3
4 In PC analysis, the first two PCs explained 49.7% of the variations of traits in genotypes
5 in dealing with salt stress (Fig. 4). The first PC was linked to K^+/Na^+ and grain yield variations in
6 S1 and S2 level of salinity. In contrast, second PC accounting for 18% of the variations of traits
7 and genotypes influenced by K^+ , Na^+ , SPAD, total protein, grain yield in S3 and SOD in S2
8 salinity levels. In bi-plot and PC analysis, the cosine of the angles between vectors shows the
9 extent of correlation between traits. The acute angles ($<90^\circ$) represent positive correlations,
10 whereas wide obtuse angles ($90^\circ <$) show a negative correlation. The intensity of the correlation
11 increases for the angles near 0° and 180° and the length of the vectors connecting traits to the
12 origin show the extent of variability [41]. In the present study, projection of genotypes on the
13 two detected PCs in bi-plot showed that the quadrant IV comprised of grain yield (under S1 and
14 S2) and K^+/Na^+ discrimination (under S1, S2 and S3) vectors with acute angels (Fig. 4). Thus,
15 genotypes (TRT805, TRT808, TRT809, TRT813 and TRT815) scattered between these vectors
16 had better performance under salt stress conditions. Vectors of K^+ under S1, S2 and S3 levels
17 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.

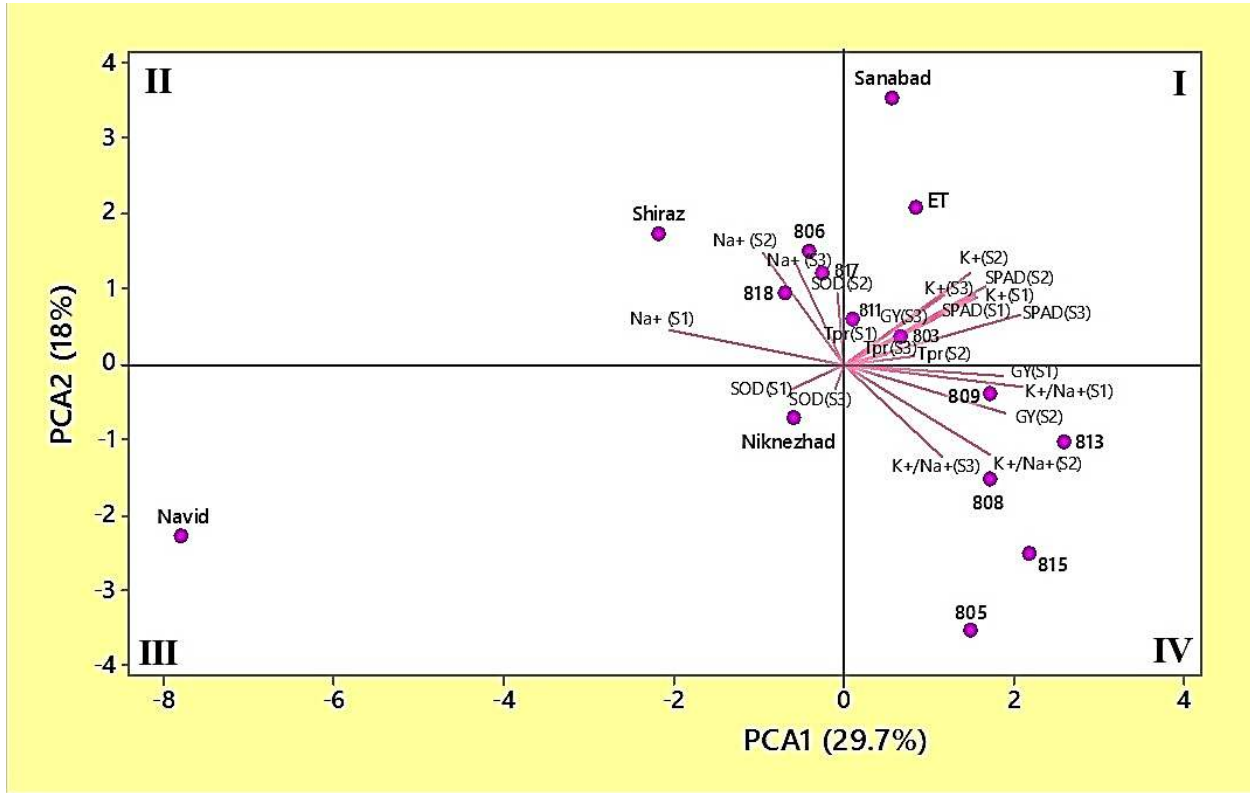


Figure 4. Projection of genotypes on the first (PCA1) and second (PCA2) principal components. GY: grain yield, SOD: superoxide dismutase, Tpr: total protein, SPAD: chlorophyll content, S1, S2 and S3: vectors of salt stress levels, respectively, for EC=7, 14 and 21 dS/m

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 to maturity time whereas a wide variation was observed for Na⁺ in response to salt stress conditions. GCV varied between 4.72% and 42.88%. After Na⁺, SPAD reads (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⁺ discrimination (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 (h²) 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^+ discrimination 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^+ discrimination 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 lead to higher protein accumulation. Acute angles 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 characteristics were more potent in their response to deal with salt stress and can be involved in breeding programs of triticales for saline conditions.

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