Evaluation of some triticale genotypes under different salt concentrations at germination and early seedling stage (#84031)

First submission

Guidance from your Editor

Please submit by **27 Apr 2023** for the benefit of the authors (and your token reward) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files
Download and review all files from the materials page.

8 Figure file(s)
9 Table file(s)
1 Raw data file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	***Example***
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Evaluation of some triticale genotypes under different salt concentrations at germination and early seedling stage

Ebrahim Ramadan [1] , **Haytham A. Freeg** [1] , **Nagwa Shalaby** [1] , **Mosa S. Rizk** [1] , **Jun Ma** [2] , **Wenhua Du** [2] , **Omar M. Ibrahim** [3] , **Ick-Hyun Jo** [Corresp., 4] , **Khairiah Alwutayd** [5] , **Hamada AbdElgawad** [6] , **Amira El-Tahan** [Corresp. 3]

[1] Agricultural research center, Egypt, Kafr Elshiekh, Egypt

[2] Gansu Agricultural University, Gansu, China

[3] city of scientific research and technological applications, Alexandria, Egypt

[4] Dankook University, Cheonan, Republic of Korea

[5] Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia

[6] Beni-Suef University, Beni-Suef, Egypt

Corresponding Authors: Ick-Hyun Jo, Amira El-Tahan
Email address: ntron@dankook.ac.kr, aeltahan@srtacity.sci.eg

The current investigation aims to study the effect of salinity on triticale genotypes at germination and early seedling stage. Nine triticale genotypes were used. Six salt concentrations i.e. control, 40, 80, 120, 160 and 200 mM NaCl were applied. Results indicated that increasing salinity concentrations negatively affected the studied traits. The genotypes Zhongsi 10841048, C6, C23, and C25**,** had better performance for germination rate, germination vigor index, germination percentage, mean daily germination, and relative salt injury. Highly significant positive correlations were revealed among the traits, including germination rate, germination vigor index, germination percentage, mean daily germination, seedling vigor index, and root length**,** indicating the importance of these traits for the selection of salt tolerance genotypes at the germination stage. PCA was able to group the most desirable genotypes into two clusters.

Evaluation of some triticale genotypes under different salt concentrations at germination and early seedling stage

Ebrahim Ramadan[1], Haytham A. Freeg[1], Nagwa Shalaby[1], Mosa S. Rizk[1], Jun Ma[2], Wenhua Du[2], Omar M. Ibrahim[3], Ick-Hyun Jo[4], Khairiah Alwutayd[5], Hamada AbdElgawad[6], Amira M. El-Tahan[3*]

[1]Field Crops Research Institute, Agricultural Research Center, Egypt

[2]College of Grassland Science, Gansu Agricultural University (GASU), P. R. China

[3]Plant Production Department, Arid Lands Cultivation Research Institute, the City of Scientific Research and Technological Applications, SRTA-City. Borg El Arab, Alexandria, Egypt.

[4]Department of Crop Science and Biotechnology, Dankook University, Cheonan 31116, Republic of Korea

[5]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia.

[6]Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Beni Suef, Egypt.

Correspondence: Amira M. El-Tahan e-mail: aeltahan@srtacity.sci.eg

Ick-Hyun Jo e-mail: ntron@dankook.ac.kr

Abstract

The current investigation aims to study the effect of salinity on triticale genotypes at germination and early seedling stage. Nine triticale genotypes were used. Six salt concentrations i.e. control, 40, 80, 120, 160 and 200 mM NaCl were applied. Results indicated that increasing salinity concentrations negatively affected the studied traits. The genotypes Zhongsi 10841048, C6, C23, and C25**,** had better performance for germination rate, germination vigor index, germination percentage, mean daily germination, and relative salt injury. Highly significant positive correlations were revealed among the traits, including germination rate, germination vigor index, germination percentage, mean daily germination, seedling vigor index, and root length**,** indicating the importance of these traits for the selection of salt tolerance genotypes at the germination stage. PCA was able to group the most desirable genotypes into two clusters.

Keywords: salinity, triticale, genotypes, seedling stage, PCA

1. Introduction

Triticale (x *Triticosecale* Wittmack) is a cereal crop that belongs to the grass family Poaceae. It is derived from the hybridization between wheat (genus Triticum) and rye (genus Secale). It possessed the ability to grow in poorer soils than rye and productivity and grain quality from wheat (Cooper, 1985). It has a strong fibrous root system and a high ability to grow efficiently in poor soils. It is also known for higher yields on marginal lands, a good source of protein, and tolerance to drought and biotic stresses (Hill 1990 and Cantale et al., 2016). There are two types of triticale, i.e., hexaploid and octoploid triticale (Bushuk and Larter, 1980).

It is estimated that the global population will be more than 9 billion in 2050 (Godfray et al., 2010); this increasing human population will require more food from more than double the production of crops (Ray et al., 2013). Population increase from one side and reduction in land available for cultivation from another are two threats to agricultural sustainability (Shahbaz and Ashraf, 2013).

Salinity is one of the abiotic stresses that limit cereals and other crops' production. Salinity affects about one billion hectares of global land, causing a loss in crop production (Saade et al., 2016). Currently, about 20% of the total cultivated area and 33% of irrigated agricultural regions of the world are affected by salinity. Furthermore, the salinized areas are increasing at a rate of 10% annually for various reasons, i.e., low precipitation, high evaporation, irrigation with saline water, and poor cultural practices. 50% of the arable land or more will probably be salinized by the year 2050 (Pitman and Läuchli, 2002; Jamil et al., 2011). In arid and semi-arid regions, salinity is one of the most important environmental factors affecting germination uniformity (Demir et al., 2003). Comparing plant growth phases, germination and seedling growth phases, and the cultivars' response to salt. (Ghoulam and Fares 2001)

Triticale is identified to be a salt-tolerant species. Triticale was reported as a moderate halophyte with a high salinity threshold (Grieve et al., 2012). The plant growth doesn't show significant differences with increasing salinity even up to 10 dSm^{-1} (Ozturk et al., 2018). Kotuby-Amacher et al., (2000) reported that the salinity threshold differed among the studied species in a study to compare the salinity tolerance in triticale with other cereals. In general, triticale tolerated salinity at a higher threshold of 6.1 dSm^{-1} ECe in comparison to corn (2.7 dSm^{-1}), Rye (5.9 dSm^{-1}), and wheat (4.7 dSm^{-1}). When soil salinity is up to 7.3 dSm^{-1}, it didn't affect the relative grain yield of triticale genotypes. Each unit increase in soil salinity above 7.3 dSm^{-1}

reduced the grain yield of triticale by 2.8%, placing triticale in the salt-tolerant category (Francois et al., 1988).

Seed germination determines the seedling vigor and the plant's future growth, so that this stage can be described as a susceptible stage for plant growth (Hakim et al., 2010). Better plant growth and establishment in saline soil are determined by the salt tolerance of cultivated genotypes in early growth stages (Keshavarizi et al., 2012). In general, increasing salinity negatively affects all traits associated with germination and early seedling growth of the plants exposed to salt stress. Salinity can influence the germination process of seeds either by altering osmotic potential that lower water uptake or by ionic toxicity effects of specific ions such as Na^+ and Cl^- ions which are related to the embryonic damage and reduced and inhibited seed germination, shoot elongation and plant growth (Sosa et al., 2005, Munns and Tester 2008 and Farooq et al., 2015). The effect of salinity differs among different varieties, depending on the salinity stress applied (Jamil et al., 2006, Mbinda and Kimtai, 2019). Though triticale is generally considered tolerant to salt stress, cultivars are slightly less salt tolerant at the germination stage than they became after the three-leaf growth stage (Francois et al. (1988). The current investigation aimed to study the effect of different salt concentrations on triticale genotypes at germination and early seedling stage

2. Materials and methods

Nine triticale genotypes were used in the current study names and characteristics are listed in **Table (1)**. The experiment was conducted at Gansu Agricultural University, P. R. China. Seeds of the studied genotypes were sterilized using Sodium Hypochlorite (1%) for half an hour and washed using distilled water three times. After that, fifty seeds of each genotype were germinated on Whatman No.1 filter paper in 9 cm Petri dishes. Germination was conducted under six salinity concentrations i.e. 0.0 mM, 40 mM, 80 mM, 120 mM, 160 mM and 200 mM NaCl. The seeds were allowed to germinate at 20 ± 1 ^{o}C in the dark (16 h) and light (8 h) for 7 days (Warham *et al.*, 1995). Seeds were irrigated and washed twice daily by test solution and the paper was altered once every 2 days to prevent salt accumulation (Rehman et al., 1996). After two days of planting, germinated seeds were counted, and the seed was considered to have germinated when the emerging radicle elongated to 1 mm. Germination percentage was recorded every 24 h for 5 days. After 7 days of planting, data were collected on shoot length (SL) (cm), root length (RL) (cm), shoot fresh weight (SFW) (mg), root fresh weight (RFW) (mg), shoot dry weight (SDW) (mg), root dry weight (RDW) (mg) and root/shoot dry weight ratio (RSR). Dry matter was measured after drying samples at 70 ^oC for 72 h in an oven. Germination traits were measured as follows:

Germination rate $(\boldsymbol{GR}) = \sum_{i=1}^{n} S_i / D_i$ (**Maguire, 1962**) (1)

Where, S_i is the germinated seeds per counting, D_i represents seed numbers until n^{th} day, and n is the number of the countings.

Germination vigor index $(\boldsymbol{GVI}) = \sum_{i=1}^{k} n_i / t_i$ (**Maguire, 1962**) (2)

Where, n_i is the percentage of seeds germinated on the n^{th} day, and t_i is the number of days counted from the start of the experiment (*i*) to the last day on which seeds germinated (*k*). Higher values represent a more rapid rate of germination.

Germination percentage (GP%) = (Seeds germinated / Total seeds) × 100 (**Manmathan and Lapitan, 2013).** (3)

Mean daily germination (MDG) = Final germination percentage/number of days to final germination (4)

Mean germination time (MGT) = $\sum(T_i N_i)/\sum N_i$ (**Kankarla *et al.*, 2020**) (5)

Where, N_i is the number of the newly germinated seeds in times of T_i

The energy of germination (GE) = Percentage of the germinated seeds 4 days after planting / Total number of seeds tested **(Ruan et al. 2002)**. (6)

Relative salt injury (RSI) = (Germination percentage of the control – Germination percentage of the treatment) /Germination percentage of the control (7)

Seedling vigor index (SVI) = (Average shoot length + Average root length) x Germination percentage (**Abdul-Baki and Anderson 1973**) (8)

Statistical analysis

The experiment was carried out in a factorial, completely randomized design (CRD) (where Factor-1 was genotyped including nine levels and Factor-2 was salt stress treatments including six levels) with three replications and 50 seeds in each replicate. Data were analyzed by 2-way analysis of variance using the using SAS statistical software, version 9.2. The comparison of the means was done using Duncan's multiple range test ($P < 0.05$). The correlation coefficient was carried out using SPSS version 16. Principal Component Analysis (PCA) was conducted using Statistical Package PAST (Hammer et al., 2001) to visualize the differences among the studied genotypes for various stress-related traits.

3. Results and discussion

ANOVA analysis:

Mean squares of treatments, genotypes, and the interaction between them for all studied traits are found in **Table 2.** All estimates showed significant differences for all variance components except for mean germination time, where the mean square was non-significant for genotypes and significant for the interaction. This result indicated the presence of a high amount of variation among the studied genotypes under different salt stress treatments.

Mean performance of genotypes

For germination traits, data in **Table 3** showed that the four triticale genotypes Zhongsi 1084 1048, C6, C23, and C25 scored the highest mean values for germination rate (3.93, 3.26, 3.14, and 3.19%, respectively), germination vigor index (28.83, 24.98, 23.3 and 23.92 respectively), germination percentage (79.15, 63.32, 63.1 and 62.64%, respectively), mean daily germination (11.31, 9.05, 9.01 and 8.95, respectively) and germination energy (49.26, 49.09, 44.62 and 46.17%, respectively). On the other hand, triticale genotypes Gannong No.2 and Shida No.1 exhibited the lowest mean values for germination rate (1.85 and 1.91, respectively), germination vigor index (13.76 and 14.64, respectively), germination percentage (38.49 and 39.06%, respectively) and meant daily germination (5.5 and 5.58, respectively). Meanwhile, Shida No.1 and C16 revealed the lowest values for germination energy (39.02 and 39.42, respectively. Genotypes C6, Gannong No.2, and C25 revealed the lowest values for mean germination time (2.85, 2.94, and 2.96 days, respectively), while C16, C23, and Gannong No.2 exhibited the highest values (3.29, 3.28 and 3.26 days, respectively). The lowest relative salt injury was observed for Zhongsi 1084, C6, and C23 (0.19, 0.31, and 0.38, respectively), while the highest injury happened for Shida No.1 and C36 (0.64 and 0.58, respectively). Genotypes Zhongsi 1084, C6 scored 39 and 18.1% higher than the general mean for seedling vigor index; meanwhile, Gannong No.2 revealed 41 % less than the general mean.

For seedling traits, among the studied genotypes, C6 and Zhongsi 1084 scored 12.4% and 9.1% higher than the overall mean performance for shoot length, while Gannong No.2 and C16 revealed 16.7 and 6.1% less than the overall mean performance. Regarding root length, Zhongsi 1084, C6, and C23 scored the highest mean performance with 17.8, 16.2, and 11.3% over the overall mean value. Meanwhile, genotypes C36 and Gannong No.2 showed the lowest mean performance with 16.8 and 12.4% less than the overall mean value. Concerning root/shoot ratio, the highest ratios were observed in C23 and Gannong No.2; meanwhile, the lowest ratio was obtained in C36. The highest increase over the overall mean for fresh shoot weight was observed in C6 (15.9%) and Gannong No.4 (12.1%), while the highest decrease was observed in both triticale genotypes Gannong No.2 (13%), C16 (10%) and C25 (9.7%). For fresh root weight, the highest mean values were revealed by C6 and Gannong No.4, which had 32.1 and 20.4%, respectively, more than the general mean. Meanwhile, genotypes C25, C16 and C36 had the lowest mean values compared with the general mean, with 13.1, 11.7 and 11% decrease, respectively. Regarding shoot dry weight, the highest mean values were revealed by C6 with 12.9% increase over the general mean; meanwhile, both genotypes Gannong No.2 and C16 had the lowest mean values compared with the general mean with 13.1 and 8.2% decrease, respectively. Regarding root dry weight, both genotypes C6 and Gannong No.4 scored 24.5 and 20.5% higher than the general mean. Meanwhile, C23, C25, and Gannong No.2 recorded 13.5, 12.6, and 11.1 % less than the general mean.

These results showed that the response for salinity differed among the studied genotypes. Genotypes Zhongsi 1084, C6, C23, and C25 were the most desirable genotypes for germination properties under salinity. Meanwhile, C6 and Gannong No.4 were the best for seedling traits. On the other hand, Gannong No.2 and Shida No.1 were the most affected genotypes by salinity for germination traits, while Gannong No.2 was the most affected regarding seedling traits. These results indicated that the effect of salinity on triticale at germination and early seedling stage varied between the different genotypes. According to Shannon (1997), soil salinity's effect on plants is associated with their growth stage. Seed germination and seedling establishment are the most salt-sensitive stages of the plant (Ashraf and Foolad, 2005). The effect of NaCl on seed germination of triticale was studied by Atak et al., (2006), who reported that the delay in germination was mainly due to high Na^+ accumulation in the seeds rather than osmotic stress in triticale cultivars. Kandil et al. (2012) studied the impact of salt stress under different salinity levels of NaCl on eleven bread wheat varieties (*Triticum aestivum* L.). They reported that wheat cultivars significantly varied in means of the final germination percentage, germination rate, seedling vigor index, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight.

The effects of salt treatments

Table (4) showed that the germination rate ranged between 1.42 and 4.4. The highest rates were observed in the control and 40 mM, which had the same value—beyond 40 mM germination rate gradually reduced along with the increased NaCl concentration. The reduction percentage increased from 41% under 80 mM to 67.9% under 200 mM treatment. For germination vigor index, significant differences were observed while increasing the salinity level; mean values ranged from 10.28 to 33.54 over the different treatments, the highest value was scored by 40 mM treatment while the lowest value was exhibited for 200 mM treatment, no significant differences were observed between control and 40 mM treatments, and the highest reduction percentages of 60, 62 and 69.2% were observed in the treatments 120, 160 and 200 mM NaCl. Regarding germination percentage, no significant differences were observed between control and 40 mM NaCl treatments. Significant differences were recorded while the NaCl concentration increased from 80 mM to 200 mM, and the germination percentage reduced by 39.8% at 80 mM concentration. The highest percentage of 88.04% was exhibited for 40 mM treatment, while the lowest percentage of 28.29% was recorded for 200 mM treatment. The highest values for mean daily germination were observed in both treatments, 40 mM NaCl and control (12.58 and 12.50, respectively); the lowest value was exhibited in the 200 mM treatment. The reduction percentage increased from 39.8 to 67.4%, while the NaCl concentration increased from 80 to 200 mM. The number of days required for germination increased from 2.48 days at the control to 4.09 days at 120 mM NaCl treatment. Beyond 120 mM concentration, the number of days for germination decreased gradually along with the increasing NaCl concentration. No significant differences were observed among 40, 80, 160, and 200 mM treatments. Germination energy decreased from 48.76% at the control to 35.96% at 120 mM NaCl. Beyond 120 mM concentration, germination energy increased gradually, and it reached 51.35% at 200 mM. No significant differences were exhibited among control, 160 mM, and 200 mM treatments. The relative salt injury was negative at 40 mM NaCl and increased significantly with the increasing salt concentration. It increased from 39.82% under 80 mM NaCl to 67.44% under 200 mM NaCl treatment. Seedling vigor index decreased dramatically along with increased salt concentration, and significant differences were observed among all applied treatments. The reduction percentage ranged from 27.2% at 40 mM NaCl to 95.6% at 200 mM NaCl.

As Table (4) showed, both shoot length and root length reduced significantly with increasing salt stress for seedling traits. The highest mean values were recorded under control, while the lowest mean values were recorded under 200 mM NaCl. Regarding shoot length, mean values varied from 9.83 cm to 1.77 cm and the reduction percentage ranged from 27.2% (40 mM NaCl) to 82% (200 mM NaCl). Means of the root length varied from 6.57 cm to 0.48 cm, and the reduction percentage ranged from 32.4% at 40 mM NaCl to 92.7% at 200 mM NaCl treatment. Root/shoot ratio decreased gradually from 0.67 at control to 0.3 at 200 mM NaCl. No significant differences have existed between 120 and 160 mM treatments. More than 50% reduction was recorded compared to control at 200 mM concentration. Shoot fresh weight and shoot dry weight were significantly affected by the salt stress. Comparing control, the reduction percentage ranged from 13.6 to 75.4% regarding fresh weight and from 10.3 to 68.1% regarding dry weight, while the NaCl concentration increased from 40 to 200 mM. Root fresh weight and root dry weight were also significantly reduced by salinity. While the NaCl concentration increased from 40 to 200 mM the reduction percentage ranged from 18.4 to 69% for root fresh weight and from 14.5 to 55.6% for root dry weight. The obtained results agree with Akgun *et al.,* (2011), who studied the effects of different salt concentrations (EC = 3.9, 6.1, 8.3, 10.5, 14.9, 19.3, 25.0 dSm^{-1}) on germination and seedling traits of triticale. They reported that germination rate, shoot and root length, and dry weights of green parts and roots decreased considerably with increased salt concentration. Kandil et al. (2012) and Atri et al. (2018) reported that along with the increasing salt concentration, the average germination and seedling growth traits reduced gradually. Francois et al. (1988) reported that when soil water salinity was up to 11.6 dSm^{-1}, there was no significant effect on the final germination percentage of triticale; however, salt levels greater than 6.0 dSm^{-1} delayed seed germination. They also reported that the final germination could be reduced by 17%, increasing salinity levels up to 20.5 dSm^{-1}.

Interaction effects

The mean performance of the studied genotypes as affected by salt treatments is found in **Figures 1** and **2**. The highest values of germination rate, germination vigor index and germination percentage were observed for Zhongsi 1084 under salt concentrations from 40 to 200 mM NaCl, while the lowest values were observed for Shida No.1 under salt concentrations from 80 to 200 mM NaCl. For mean daily germination, Zhongsi 1084 was the best genotype under salt concentrations from 40 to 200 mM NaCl, while Shida No.1 was the most affected under high salt concentrations from 120 to 200 mM NaCl. The mean germination time ranged from 2.01 to 3.41 days at control, 2.7 to 3.31 at 40 mM, 2.58 to 3.96 at 80 mM NaCl, 3.23 to

4.59 at 120 mM, 2.68 to 3.53 days at 160 mM, and from 2.59 to 3.37 days at 200 Mm NaCl treatment. The lowest number of days under control and 120 Mm NaCl treatments was observed in genotype C6. With respect to germination energy, the best genotypes were Gannong No.4 under control (55.97%), Zhongsi 1084 under 40 and 120 mM NaCl treatments (52.84 and 48.03%, respectively), C6 under 80 mM treatment (46.06%), and Shida No.1 under 160 and 200 mM treatments (56.5 and 57.5%, respectively). Relative salt injury increased with the increase of salt concentration. The lowest percentage of injury was observed in Zhongsi 1084 (10.23, 24.18, 25.36, and 38% under 80, 120, 160, and 200 mM NaCl, respectively). Meanwhile, the highest percentage of injury was exhibited in Shida No.1 (57.57, 82.17, 87.38, and 87.21% under 80, 120, 160, and 200 mM NaCl, respectively). For seedling vigor index, the most desirable genotypes were both Zhongsi 1084 and Gannong No.4 under control, Zhongsi 1084 and C4 under 40 mM, 120 and 200 mM NaCl treatments, Zhongsi 1084 and C23 under 80 mM, and both Zhongsi 1084 and C25 under 160 mM, on the other hand, Shida No.1 was the most affected genotype under high salt concentration.

Concerning the shoot length, Zhongsi 1084 scored the highest mean values under control and 40 mM NaCl treatments, but it revealed the lowest values under 160 and 200 mM NaCl treatments. C6 scored the highest values under 80 and 120 mM NaCl treatments, while C16 scored the highest values under 160 and 200 mM NaCl treatments. The lowest means under control, 40, 80, and 120 mM, were exhibited for Gannong No.2. The means of root length ranged between 8.57 and 5.13 cm at control, 5.45 and 3.61 at 40 mM, and decreased gradually to be ranged between 0.97 and 0.62 cm at 160 mM and between 0.61 and 0.29 cm at 200 Mm NaCl treatment. The root/shoot ratio decreased by increasing salt concentrations. The ratios ranged from 0.79 to 0.53 under control and from 0.42 to 0.20 under 200 mM NaCl treatment. Shida No. 1 revealed the highest ratios under 160 and 200 mM NaCl, while C16 revealed the lowest ratios. Both C6 and Shida No1 were the best genotypes regarding shoot fresh weight under control, 40 and 80 NaCl concentrations, while both Gannong No.4 and C6 were the best under 120, 160, and 200 mM NaCl treatments. The genotypes C6 and Gannong No.4 were the best for root fresh weight under control, 40, 80, and 120 mM NaCl concentrations, while C6 and Gannong No.2 were the best under 160 and 200 mM NaCl treatments. Concerning shoot dry weight, the highest mean values were scored by Shida No.1 and C6 under control, 40 mM and 80 mM treatments, C6 under 120 and 160 mM treatments, and Gannong No.4 under 200 mM. In contrast, the lowest mean values under high salt concentrations were exhibited by Zhongsi 1084 and C36. Gannong No.4 and C6 were the most desirable genotypes under control, 40 Mm, 80 mM and 120 mM salt treatments for root dry weight. Under 160 and 200 mM salt treatments, C6 was the best, while C23 was the most affected genotype. Saboora et al., 2006 reported that different salinity concentrations caused considerable effects on germination percentage, germination rate, total dry weight, and all seedling traits in all studied genotypes. Similar results for the interaction between salt stress and genotypes have been reported by Kandil et al. (2012).

Phenotypic correlation

Phenotypic correlation coefficients among the studied traits are found in **Table** (**5**). The highest positive correlation (r = 1.00) was observed between germination percentage and mean daily germination. Highly significant positive correlations were recorded among the parameters germination rate, germination vigor index, germination percentage, mean daily germination, seedling vigor index, and root length. Significant positive correlations were recorded among the traits root length, shoot fresh weight, root fresh weight, and shoot dry weight. Seedling vigor index was highly significant positive correlated with root length. Germination vigor index was significantly positively correlated with germination energy and shoot length. Significant positive correlations were observed between germination energy, seedling vigor index and shoot length, and between shoot length and root length. Positive but non-significant correlation coefficients were recorded between the germination rate, germination vigor index, germination percentage, mean germination time, germination energy, and seedling vigor index from one side and the seedling traits root/shoot ratio, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight from the other side.

On the other hand, highly significant negative correlations were recorded between relative salt injury and each of germination rate, germination vigor index, germination percentage, and mean daily germination. Significant negative correlations were recorded between mean germination time and root dry weight and between relative salt injury and both seedling vigor index and root length. Similar results were obtained by Alom *et al.* (2016), who reported that the salt tolerance index for seedling dry weight of wheat genotypes after 10 days of irrigation with saline water (15 dSm^{-1}) was significantly positively correlated with the salt tolerance index for germination rate, germination vigor index, shoot length, and root length which indicated that these parameters could be used as selection criteria for screening wheat genotypes against salt stress. Aflaki *et al.* (2017) studied the effect of salinity on germination of wheat genotypes; they found that mean daily germination recorded the highest correlation value with germination percentage.

Principal Component Analysis (PCA)

Principal component analysis (PCA) is a multi-variable statistical analysis that reduces the dimension of high-dimension data, in which fewer eigenvectors can explain the information of multivariate data as possible (Shlens 2005). In the current study PCA analysis (**Fig. 3**) classified the studied genotypes into four clusters based on their mean performance under NaCl treatments. The first cluster was found in the 1st quadrant. It included triticale genotypes C6 and Gannong No.4. Both genotypes scored the highest values for the seedling traits shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, high values for root length, seedling vigor index, mean daily germination and germination vigor index. The second cluster was found in the 2nd quadrant and included Zhongsi 1084, C23, and C25. Those genotypes scored high mean values for germination rate, germination vigor index, germination percentage, mean daily germination, seedling vigor index, shoot length, and root length and revealed the low relative salt injury. The third cluster was found in the 3rd quadrant and included both Gannong No.2 and C16, while the fourth cluster was found in the 4th quadrant and included both Shida No.1 and C36. The genotypes in the third and the fourth clusters had the lowest mean values for germination rate, germination vigor index, germination percentage, mean daily germination, seedling vigor index, and root length. These results suggested considerable genetic variability for salt tolerance in the studied triticale genotypes. PCA analysis was able to classify different genotypes of wheat and soya bean into three groups, i.e., salt tolerant, moderately salt tolerant, and salt susceptible, based on the performance of these genotypes under different salt concentrations at the early seedling stage (Saboora et al., 2006 and Shelke et al., 2017).

Salinity stress tolerance

As a quantitative measure, stress indices can quantify a crop's stress response. They are easily useable than raw data due to their direct interpretation. Many indices of abiotic tolerance have been proposed (Table 6) for estimating abiotic stress tolerant genotypes using a mathematical equation that describes the relationship between growth under stress and control conditions. The abiotic stress indices are classified into two types; the first type contains indices with maximum values indicating high-stress tolerance, while the other type includes other indices with minimum values indicating high-stress tolerance. Using these indices, we can identify the tolerant and sensitive genotypes and their stability (Parvaze et al., 2018).

The results in Tables (7 & 8) reveal that GannongNo.4 was the most tolerant genotype with an average rank (AR) equal to 2.12 (Figure 4); however, Zhongsi1084 was the least tolerant genotype (AR = 8.04). Both GannongNo.2 and C25 were moderately tolerant as their average rankings were 4.29 and 4.62, respectively. When the values of the average rank increase, the tolerance of the genotypes decreases; as shown in Table 8, it was helpful to take the average of all ranks of the different abiotic stress indices due to their different results.

<u>Cluster analysis:</u>

To cluster the genotypes under both control and salinity stress, cluster analysis was performed using R software version 4.1.0, 2021. Euclidian metric as a distance measure was used to measure dissimilarity among the genotypes, and Ward's algorithm (Ward, 1963) was applied for grouping the genotypes. Shoot fresh weight (SFW) and Root fresh weight (RFW) were used to construct a distance matrix and generate the tanglegram showing dissimilarity among all the genotypes under control and the highest saline treatment (200 mM), as shown in Figure 5. Before conducting the analysis, the data were standardized due to their different scale by subtracting the mean from each value and dividing by the standard deviation. The cubic cluster criterion (Milligan and Cooper, 1983) was used to ensure whether clusters existed. Fuzzy C-means as a soft clustering algorithm (Bezdek, 1974; 1981) was used to detect if overlapping existed between clusters. The fuzzy C-means method shows that low overlap existed between clusters, so hard clustering methods were applied to construct the Tanglegram (Figure 5). Six hard clustering methods were compared using an agglomerative coefficient to choose the most accurate method for clustering the data. They were average, generalized average, single, and weighted.

Complete, and ward. The valued of agglomerative coefficients were 0.76, 0.81, 0.53, 0.77, 0.85, and 0.88 respectively, under control. Where, under 200 mM, they were 0.68, 0.72, 0.55, 0.73, 0.77, and 0.81 respectively. These results reveal that the Ward method had the highest coefficient compared to the other five methods under control and the highest saline treatment. So, the Ward method was chosen to conduct the cluster analysis. To identify the optimum number of clusters in the data, 30 internal validation indices were, and voting among them was done to determine the optimum number of clusters in the data (Charrad et al., 2014). As shown in figure 5, all the genotypes were separated into two clusters under control and highest saline treatment, with the average of the studied traits shown in Table 9. The structure of the clusters changed markedly when the genotypes were subjected to the highest saline treatment except for the genotypes (Gannong No.4 and C6), which migrated from cluster 1 under control to cluster 2 under the highest saline treatment because they were more tolerant than the other members of their cluster.

Heatmap Figures (7 &8) show the relationship between the genotypes and the studied traits based on standardized (scaled) data using a color scale under control and the highest saline treatment. The red color in the heatmap represents high values of the traits, while the blue color represents low values. Before drawing the heatmap, the data were standardized by subtracting the mean from each value and dividing by the standard deviation. The genotype C6 was the highest in SFW and SDW under control, while the genotype Gannong No.4 was the highest in SFW and SDW under the highest salinity treatment (200 mM). These results demonstrated that Gannong No. 4 was the most tolerant genotype. The lowest genotype in SFW and SDW under control was C16, while Zhongsi 1084 was the lowest in SFW and C26 was the lowest in SDW under 200 mM. Concerning GP, the genotypes Gannong No.4 and Gannong No. 2 were the highest and the lowest, respectively, under control.

On the other hand, the genotypes Zhongsi 1084 and Shida No.1 were the highest and the lowest, respectively, under 200 mM. The genotype Zhongsi 1084 had higher values of germination traits under the highest salinity treatment; however, it had the lowest values of SFW, RFW, SL, and RSI. Gannong No.4 had the higher value of germination traits under control. From the heatmap, there seemed to be no association between germination traits and the tolerance of the genotypes except for MGT, which appears to be negatively associated with the tolerance of the genotypes.

Conclusion

In the previous results, the mean performance of most studied traits decreased gradually by increasing salt concentration relative to salt injury. Mean germination time increased by increasing NaCl up to 120 mM, then decreased by increasing NaCl concentration. Non-significant differences were observed under control and 40 mM treatments for the traits germination rate, germination vigor index, germination percentage, and mean daily germination. Genotypes Zhongsi 1084, C6, C23, and C25, scored the best performance for germination rate, germination vigor index, germination percentage, mean daily germination, germination energy, relative salt injury, seedling vigor index, and root length or most of these traits. Highly significant positive correlations were revealed among the traits germination rate, germination vigor index, germination percentage, mean daily germination, seedling vigor index, and root length. C6 and Gannong No.4 were the best genotypes for seedling traits shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight. PCA was able to divide the studied genotypes into four clusters. The most desirable genotypes were gathered into clusters 1 and 2, while the other genotypes were grouped into clusters 3 and 4.

Contributions

All authors have contributed equally to the research and analysis of the various results sections within the review. All have corrected and modified the different versions of the manuscript as prepared by the corresponding and senior authors. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding: This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R402), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia

References

Abdul-Baki A. A. and J. D. Anderson. 1973. Vigor determination in soybean by multiple criteria. Crop Sci. 13: 630-33. http://dx.doi.org/10.2135/cropsci1973.0011183X001300060013x

Aflaki, F., M. Sedghi, A. Pazuki and M. Pessarakli. 2017. Investigation of seed germination indices for early selection of salinity tolerant genotypes: A case study in wheat. Emir J Food Agric. 29(3): 222-226 doi: 10.9755/ejfa.2016-12-1940

Akgun, I., B. Kara and D. Altinda. 2011. Effect of salinity (NaCl) on germination, seedling growth and nutrient uptake of different triticale genotypes. Turkish J. Field Crop. 16(2): 225-232

Alom, R., M. A. Hasan, M. R. Islam and Q. F. Wang. 2016. Germination characters and early seedling growth of wheat (*Triticum aestivum* L.) genotypes under salt stress conditions. J. Crop Sci. Biotechnol. 19: 383–392. https://doi.org/10.1007/s12892-016-0052-1

Atak, M., M. D. Kaya, G. Kaya, Y. Cikili and C. Y. Ciftci. 2006. Effects of NaCl on the germination, seedling growth and water uptake of Triticale. Turk J Agric For. 30: 39-47

Bezdek, J.C. (1974). Cluster validity with fuzzy sets. J. Cybernetics, 3: 58-73. doi:10.1080/01969727308546047.

Bezdek, J.C. (1981). Pattern Recognition with Fuzzy Objective Function Algorithms; Plenum Press: New York, NY, USA.

Bidinger, F. R., V. Mahalakshmi, and G. D. Rao. 1987. Assessment of drought resistance in pearl millet (*Pennisetum americanum* (L.) Leeke). II. Estimation of genotype response to stress. *Australian Journal of Agricultural Research* 38: 49–59.

Bouslama, M., Schapaugh, W.T. (1984). Stress tolerance in soybean. Part 1: Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci*., 24**:** 933–937.

Bushuk, W. and E. N. Larter. 1980. Triticale: production, chemistry, and technology. *Advances in Cereal Science and Technology*. 3: 115–157

Cantale, C., F. Petrazzuolo, A. Correnti, A. Farneti, F. Felici, A. Latini and P. Galeffi. 2016. Triticale for Bioenergy Production. Agric. Agric. Sci. Procedia. 8: 609–616.

Charrad, M., Ghazzali, N., Boiteau, V., and Niknafs, A. (2014). NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. *Journal of Statistical Software*, *61*(6), 1–36. https://doi.org/10.18637/jss.v061.i06

Choukan R., Taherkhani T., Ghannadha M. R., Khodarahmi M. (2006). Evaluation of drought tolerance in grain maize inbred lines using drought tolerance indices. *Iranian Journal of Agricultural Science,* **8:** 79-89.

Cooper, K.V. 1985. The Australian Triticale Cookery Book, Adelaide, South Australia, Savvas Publishing.

Demir, I., K. M. Mavi and G. Okcu. 2003. Effect of salt stress on germination and seedling growth in serially harvested aubergine (*Solanum melongena* L.) seeds during development. Isr. J. Plant Sci. 51: 125-131.

Farooq, M., M. Hussain, A. Wakeel and K. H. Siddique. 2015. Salt stress in maize: effects, resistance mechanisms, and management. A review, Agron. Sust. Develop. 35: 461–481.

Farshadfar, E. and Shukla J. (2003). Screening drought tolerance criteria in maize. *Acta Agronomica Hungarica*., 50: 411-416.

Fernandez, G. C. J. 1992. Effective selection criteria for assessing plant stress tolerance. *In* C. G. Kuo [ed.], Adaptation of food crops to temperature and water stress, 257–270. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Fischer, R. A., and Wood, J. T. (1979). Drought resistance in spring wheat cultivars. III. Yield associations with morpho-physiological traits. *Australian Journal of Agricultural Research*, 30(6), 1001-1020.

Francois, L. E., T. J. Donovan, E. V. Maas and G. L. Rubenthaler. 1988. Effect of salinity on grain yield and quality, vegetative growth, and germination of triticale. Agron. J. 80:642-647.

Gavuzzi, P., F. Rizza, M. Palumbo, R. G. Campaline, G. L. Ricciardi, and B. Borghi. 1997. Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Canadian Journal of Plant Science* 77: 523–531.

Ghoulam, C. and K. Fares. 2001. Effect of salinity on seed germination and early seedling growth of sugar beat (*Beta vulgaris* L.). Seed Sci. Technol. 29: 357-364.

Godfray H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir and C. Toulmin. 2010. Food security: the challenge of feeding 9 billion people. Sci. 327: 812–818.

Grieve, C. M., S. R. Grattan and E. V. Maas. 2012. Plant Salt Tolerance. In Agricultural Salinity Assessment and Management (W. W. Wallender and K. K. Tanji, eds). ASCE Manuals and Reports on Engineering Practice No. 71, 2nd edition. 405-459. American Society of Civil Engineers (ASCE). Reston, VA

Hakim, M. A., A. S. Juraimi, M. Begum, M. M. Hanafi, M. R. Ismail and A. Selamat. 2010. Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). Afr. J. Biotechnol. 9: 1911–1918.

Hill, G. M. 1990. Quality: Triticale in animal nutrition. In Proceedings of the 2nd Int. Triticale Symp, Passo Fundo, Rio Grande do Sul, Brazil, 1–5 October; pp. 422–427.

Jamil, A., S. Riaz, M. Ashraf and M. R. Foolad (2011). Gene expression profiling of plants under salt stress. Crit. Rev. Plant Sci. 30(5):435–458.

Jamil, M., D. B. Lee, K. Y. Jung, M. Ashraf, S. C. Lee and E. S. Rha. 2006. Effect of salt (NaCl) stress on germination and early seedling growth of four Vegetable species. J. Cent. Eur. Agric. 7: 273–282.

Kankarla, V., M. K. Shukla, G. A. Picchioni, D. VanLeeuwen and B. J. Schutte. **2020**. Germination and emergence responses of alfalfa, triticale and quinoa irrigated with Brackish groundwater and desalination concentrate. Agron J. 10(4): 549.

Keshavarizi, B. and H. Mohammed. 2012. Studying the effects of different levels of salinity which caused by NaCl on early and germination of *Lctuca sativa* L. seedling. J. Stress Physiol. Bioch. 8: 203–208.

Kotuby-Amacher, J., R. Koenig and B. Kitchen. 2000. Salinity and plant tolerance. Utah State University: Cooperative Extension.

Maguire, J. D. 1962. Speed of germination-aid selection and evaluation for seedling emergence and vigor. Crop Sci. 2: 176-177.

Mbinda, W. and M. Kimtai. 2019. Evaluation of morphological and biochemical characteristics of Sorghum [*Sorghum bicolor* [L.] Moench] varieties in response salinity stress. Annu. Res. Rev. Biol. 1–9.

Milligan, G. W. and M. C. Cooper. 1983. "An Examination of Procedures for Determining the Number of Clusters in a Data Set" *College of Administrative Science Working Paper Series* 83-51. Columbus: The Ohio State University.

Moosavi, S., Yazdi Samdi B., Naghavi M., Zali A., Dashti H. and Pourshabazi A. (2008). Introduction of new indices to identify relative drought tolerant and resistant genotypes of wheat. *Desert*, 12: 165-178.

Moradi, H., Akbari, G. A., Khorasani, S. K., and Ramshini, H. A. (2012). Evaluation of drought tolerance in corn (*Zea mays* L.) new hybrids with using stress tolerance indices. *European Journal of Sustainable Development*, **1**(3), 543-560.

Ozturk, O. F., M. K. Shukla, B. Stringam, G. A. Picchioni and C. Gard. 2018. Irrigation with brackish water changes evapotranspiration, growth and ion uptake of halophytes. Agric. Water Manag. 195: 142–153.

Parvaze A. Sofi, K. Rehman, Asmat Ara and Musharib Gull. (2018). Stress tolerance indices based on yield, phenology and biomass partitioning: A review. Agricultural Reviews, 39(4): 292-299.

Pitman, M. G. and A. Läuchli. 2002. Global Impact of Salinity and Agricultural Ecosystems. In: Läuchli A., Lüttge U. (eds) *Salinity: Environment - Plants - Molecules*. Springer, Dordrecht. https://doi.org/10.1007/0-306-48155-3_1

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

R. Munns and M. Tester. 2008. Mechanisms of salinity tolerance, Annu. Rev. Plant Biol. 59: 651–681.

Ramýrez, P. and Kelly, J. D. (1998). Traits related to drought resistance in common bean. *Euphytica*, 99**:** 127–136.

Ray, D. K., N. D. Mueller, P. C. West and J. A. Foley. 2013. Yield trends are insufficient to double global crop production by 2050. PLoS One. Jun 19;8(6):e66428. doi: 10.1371/journal.pone.0066428. PMID: 23840465; PMCID: PMC3686737.

Rosielle, A. A., and J. Hamblin. 1981. Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Science* 21: 943–946.

Ruan, S., Q. Xue and K. Tylkowska. 2002. The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soils. Seed Sci Technol. 30: 61–67.

Saade, S., A. Maurer, M. Shahid, H. Oakey, S. M. Schmöckel, S. Negrão and M. Tester. 2016. Yield related salinity tolerance traits identified in a nested association mapping (NAM) population of wild barley. Sci. Rep. 6: 32586

Saboora, A., K. Kiarostami, F. Behroozbayati and S. Hajihashemi. 2006. Salinity (NaCl) tolerance of wheat genotypes at germination and early seedling growth. Pak. J. Biol. Sci. 9: 2009-2021. DOI: 10.3923/pjbs.2006.2009.2021

Schneider, K. A., Rosales-Serna, R., Ibarra-Perez, F., Cazares-Enriquez, B., Acosta-Gallegos, J. A., *et al*. (1997). Improving common bean performance under drought stress. *Crop Science*, **37**(1), 43-50.

Shahbaz, M. and M. Ashraf. 2013. Improving salinity tolerance in cereals. Crit. Rev. Plant Sci. 32:237–249.

Shelke, D. B., M. Pandey, G. C. Nikalje, B. N. Zaware, P. Suprasanna and T. D. Nikam. 2017. Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes. Plant Physiol. Biochem. 118:519-528. doi:10.1016/j.plaphy.2017.07.013

Shlens, J. 2005. A tutorial on principal component analysis. Systems Neurobiology Laboratory, Salk Institute for Biological Studies.

Sosa, L., A. Llanes, H. Reinoso, M. Reginato and V. Luna (2005). Osmotic and Specific Ion Effects on the Germination of *Prosopis strombulifera*. Annals of Botany. 96: 261–267

Sosa, L., A. Llanes, H. Reinoso, M. Reginato and V. Luna. 2005. Osmotic and specific ion effects on the germination of *Prosopis strombulifera*. Ann Bot. 96(2):261-7. doi: 10.1093/aob/mci173. Epub 2005 May 31. PMID: 15928009; PMCID: PMC4246873.

Ward J.H., Jr. (1963). Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc., 58: 236–244.

Warham, E. J., L. D. Butler and B. C. Sutton. 1995. Seed testing of maize and wheat: A Laboratory Guide. CIMMYT/CAB International, Mexico, D.F./Wallingford.

Figure caption

Figure 1. Mean performance of germination traits as affected by the interaction between genotypes and salt treatments (mM NaCl)

Figure 2. Mean performance of seedling traits as affected by the interaction between genotypes and salt treatments (mM NaCl)

Figure 3. Two dimensional ordination of nine studied genotypes based on the overall mean performance under salt treatments

Figure 4. Tolerance of genotypes according to the average rank of 22 abiotic stress indices (small number of average ranks means tolerant)

Figure 5. Tanglegram showing results of cluster analysis based on Euclidian coefficient and Ward method under normal and water stress conditions.

Figure 6. Pearson correlation matrix among the studied traits

Figure 7. Heatmap of the relationship between genotypes and the studied traits under control

Figure 8. Heatmap of the relationship between genotypes and the studied traits under 200 mM

Figure 1

Mean performance of germination traits as affected by the interaction between genotypes and salt treatments (mM NaCl)

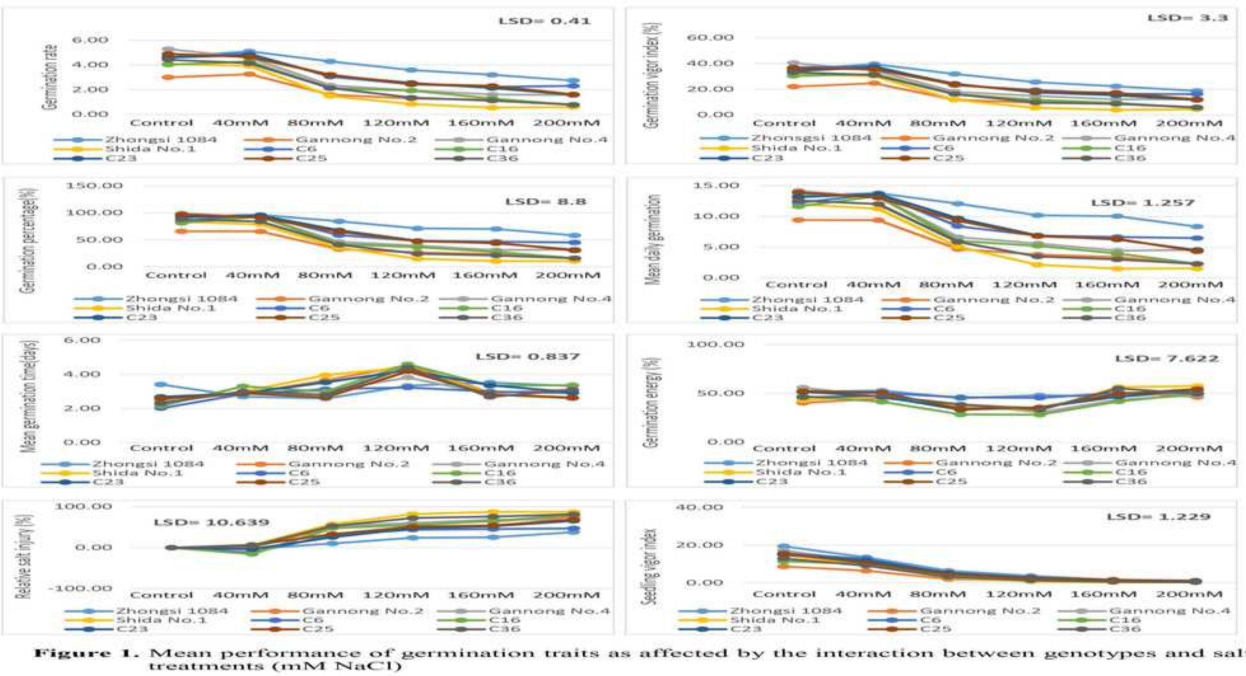

Figure 1. Mean performance of germination traits as affected by the interaction between genotypes and salt treatments (mM NaCl)

Figure 2

Mean performance of seedling traits as affected by the interaction between genotypes and salt treatments (mM NaCl)

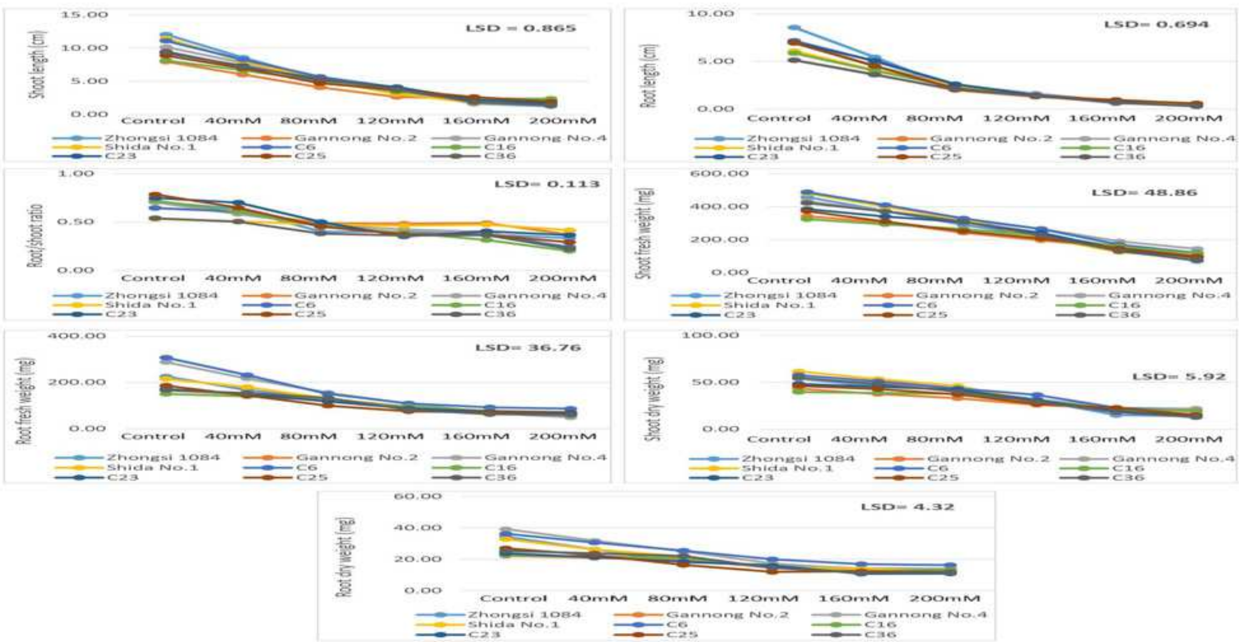

Figure 2. Mean performance of seedling traits as affected by the interaction between genotypes and salt treatments (mM NaCl)

Figure 3

Two dimensional ordination of nine studied genotypes based on the overall mean performance under salt treatments

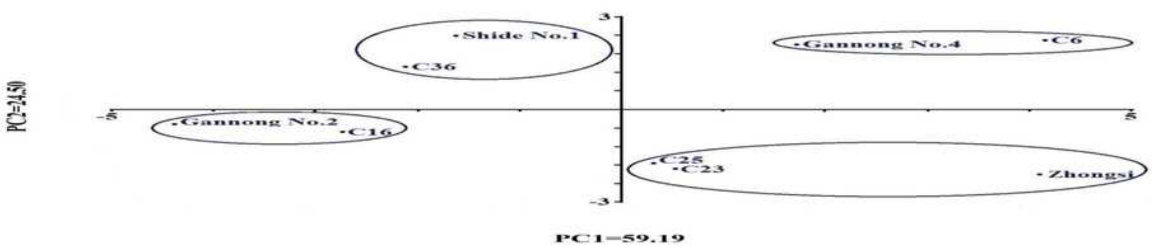

Figure 3. Two dimensional ordination of nine studied genotypes based on the overall mean performance under salt treatments

Figure 4

Tolerance of genotypes according to the average rank of 22 abiotic stress indices (small number of average ranks means tolerant)

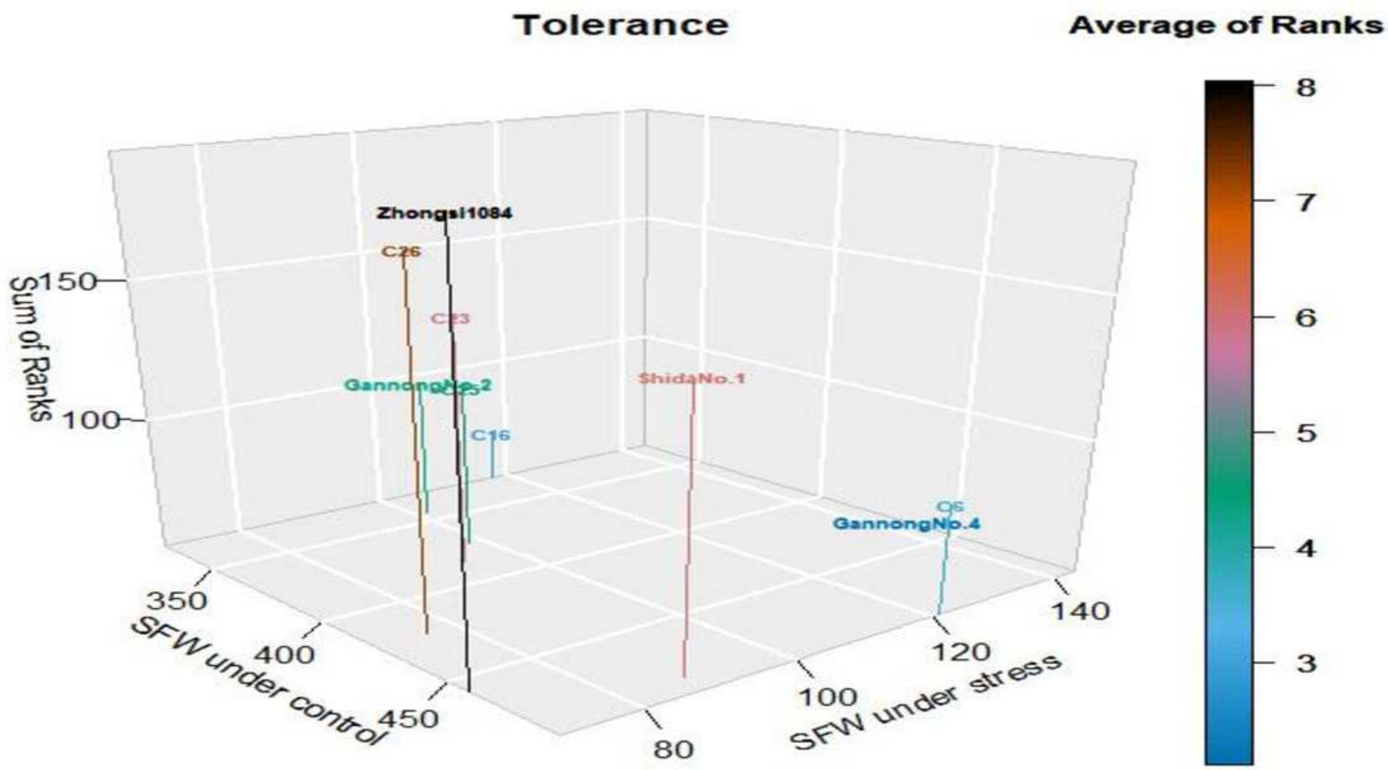

Figure 5

Tanglegram showing results of cluster analysis based on Euclidian coefficient and Ward method under normal and water stress conditions.

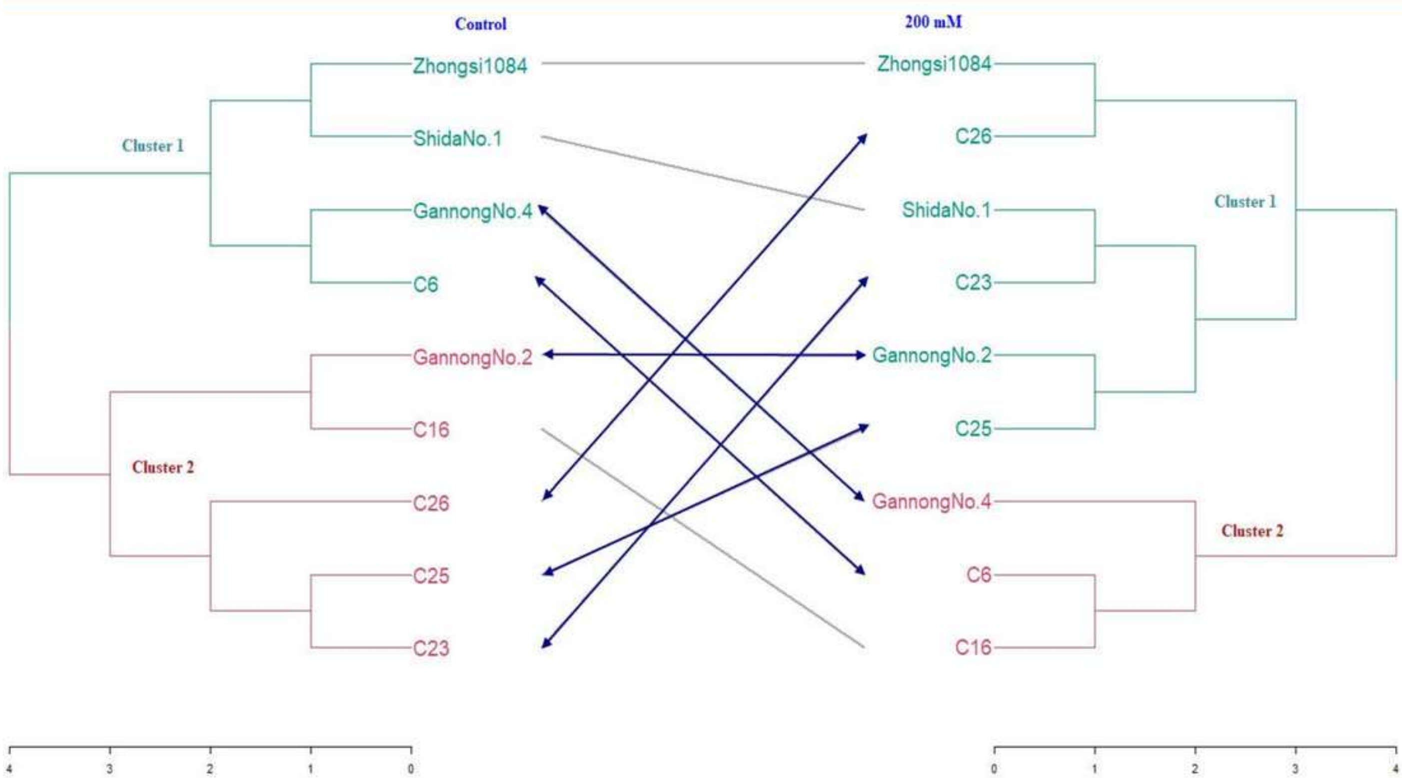

Figure 6

Pearson correlation matrix among the studied traits

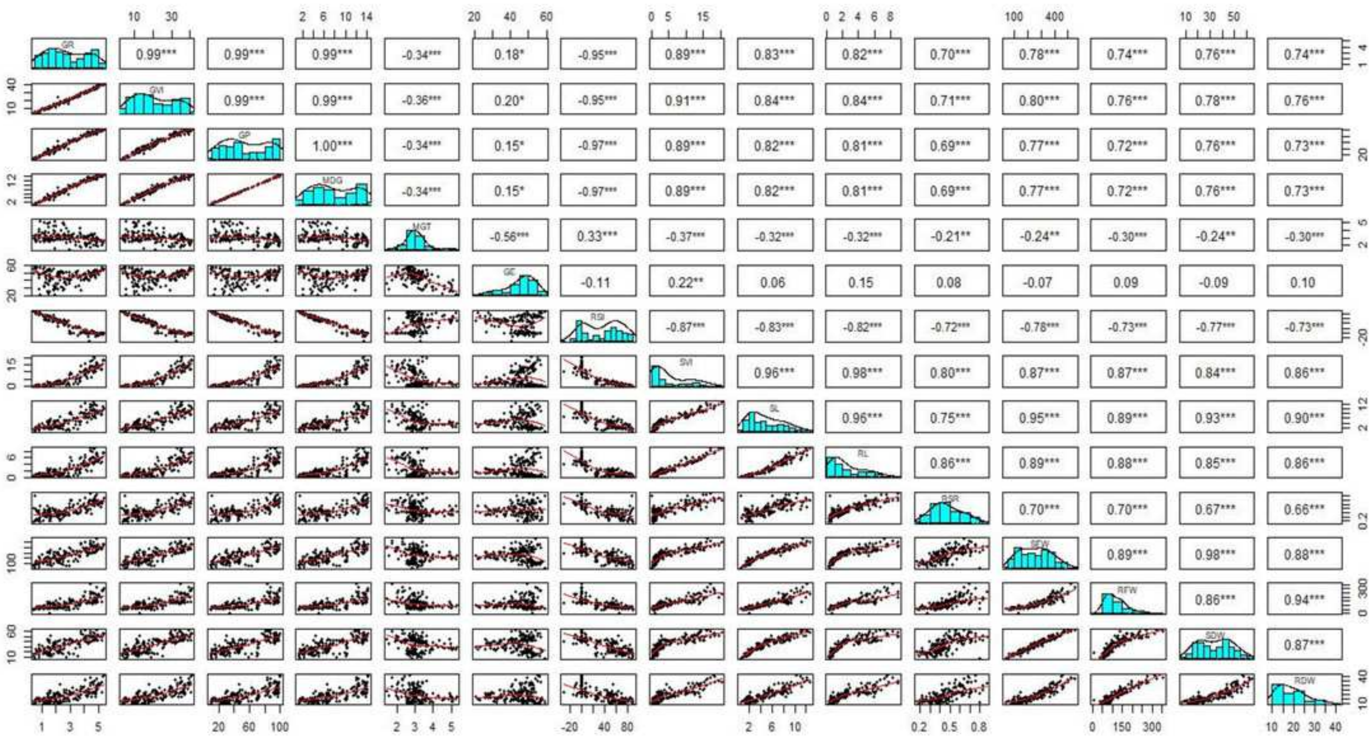

Figure 7

Heatmap of the relationship between genotypes and the studied traits under control

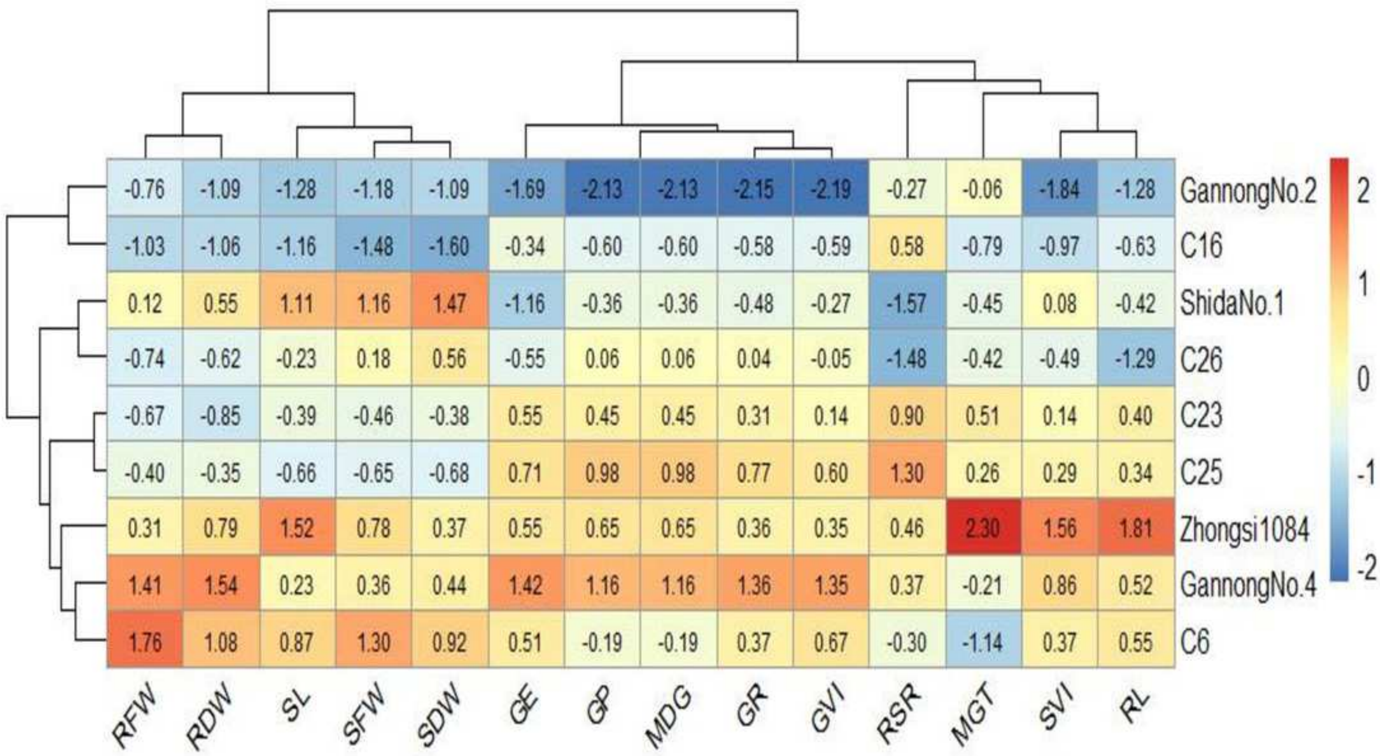

Figure 8

Heatmap of the relationship between genotypes and the studied traits under 200 mM

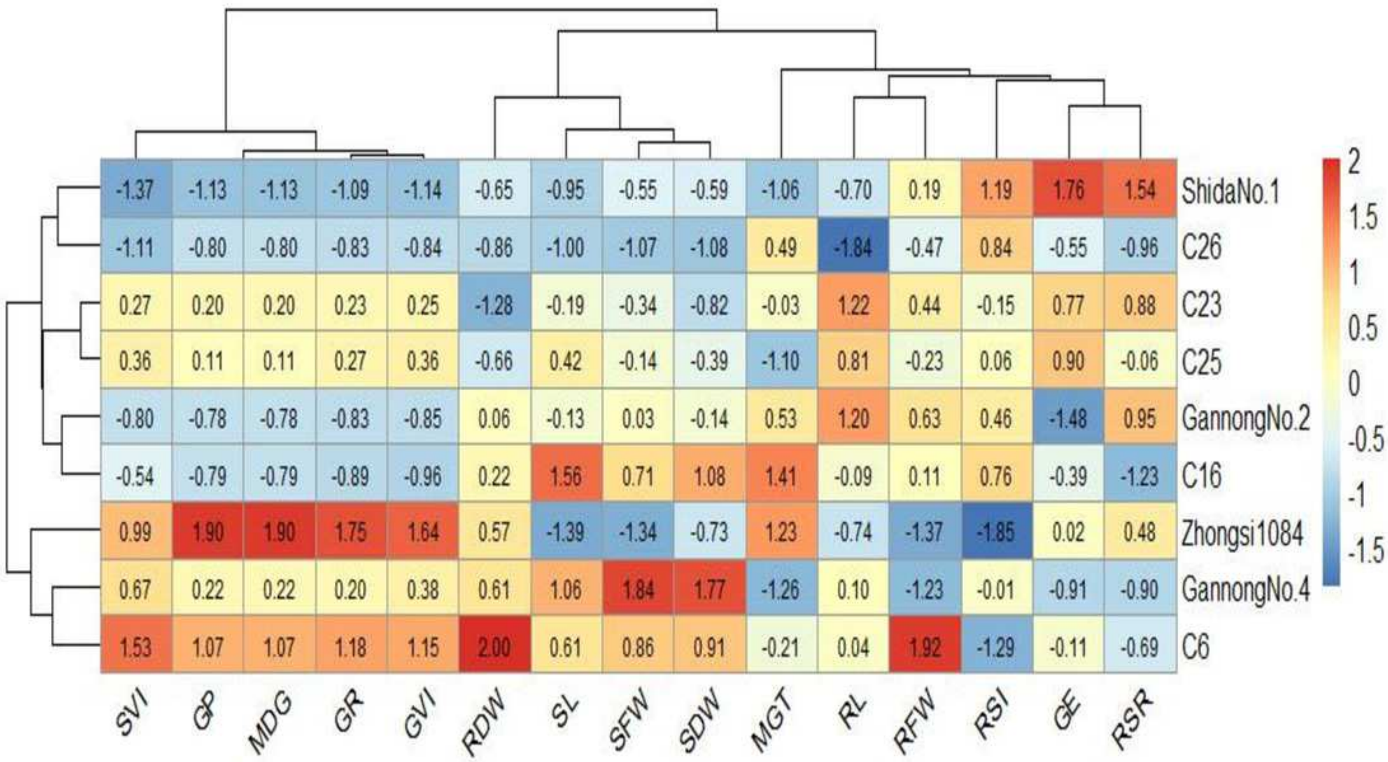

Table 1(on next page)

List of studied genotypes, names and characteristics

Table 1. List of studied genotypes, names and characteristics

number	Genotypes names
1	Zhongsi 1084 (Chinese Triticale Cultivar)
2	Gannong No.2 (Chinese Triticale Cultivar)
3	Gannong No.4 (Chinese Triticale Cultivar)
4	Shida No.1 (Chinese Triticale Cultivar)
5	C6 (Triticale line bred by GSAU*)
6	C16 (Triticale line bred by GSAU)
7	C23 (Triticale line bred by GSAU)
8	C25 (Triticale line bred by GSAU)
9	C36 (Triticale line bred by GSAU)

Where GSAU means Gansu Agricultural University of P.R. China

Table 2(on next page)

Mean square estimates for the studied parameters

Table 2. Mean square estimates for the studied parameters

SOV	Treat.	Gen.	Treat. X Gen.	Error
d.f	5	8	40	108
Germination rate	47.51**	8.59**	0.365**	0.06
Germin. vigor index	2891.46**	470.32**	18.675**	4.16
Germin. (%)	18758.28**	3197.63**	170.011**	29.56
Mean daily germination	382.77**	65.25**	3.475**	0.60
Mean germination time (days)	7.67**	0.48ns	0.413*	0.27
Germination energy (%)	1164.17**	168.13**	55.973**	22.18
Relative salt injury	25066.84**	2360.53**	243.20**	43.22
Seedling vigor index	881.77**	30.83**	4.481**	0.58
Shoot length (cm)	268.10**	3.44**	1.43**	0.29
Root length (cm)	152.64**	1.90**	0.67**	0.18
Root / shoot ratio	0.54**	0.02**	0.01**	0.01
Shoot fresh weight (mg)	382627.79**	11844.21**	2341.06**	911.49
Root fresh weight (mg)	88069.30**	6957.79**	1506.71**	515.80
Shoot dry weight (mg)	5271.16**	171.06**	38.94**	13.39
Root dry weight (mg)	1217.71**	143.46**	18.28**	7.11

Where ** means highly significant differences exited at the 0.01 level, * means significant differences exited at the 0.05 level and ns means no significant differences exited

Table 3(on next page)

The overall mean performance of different studied triticale genotypes under six salt treatments

Table 3. The overall mean performance of different studied triticale genotypes under six salt treatments

Traits	Genotypes	Zhongsi 1084	Gannong No.2	Gannong No.4	Shida No.1	C6	C16	C23	C25	C36	Mean
Germination traits	Germination rate	3.93a	1.85e	2.89c	1.91e	3.26b	2.40d	3.14b	3.19b	2.33d	2.77
	Germin. vigor index	28.83a	13.76f	22.02d	14.64f	24.98b	17.50e	23.30cd	23.92bc	17.44e	20.71
	Germin. (%)	79.15a	38.49f	56.65c	39.06f	63.32b	49.52d	63.10b	62.64b	45.74e	55.3
	Mean daily germin.	11.31a	5.50f	8.09c	5.58f	9.05b	7.07d	9.01b	8.95b	6.53e	7.9
	Mean germin. time (days)	3.14ab	3.26a	2.94ab	3.2ab	2.85b	3.29a	3.28a	2.96ab	3.05ab	3.11
	Germin. energy	49.26a	42.66b	43.78b	45.43b	49.09a	39.42c	44.62b	46.17ab	44.93b	45.04
	Relative salt injury	0.19	0.50	0.51	0.64	0.31	0.47	0.38	0.43	0.58	0.45
	Seedling vigor index	7.57a	3.22e	6.03bc	4.68d	6.44b	4.59d	6.13bc	5.85c	4.51d	5.45
Germination traits	Shoot length (cm)	5.42ab	4.14f	5.27abc	5.16bcd	5.58a	4.67e	4.97cde	4.80de	4.85de	4.99
	Root length (cm)	3.11a	2.31de	2.86ab	2.55cd	3.07ab	2.46cde	2.94ab	2.75bc	2.20e	2.69
	Root / shoot ratio	0.48ab	0.51a	0.47ab	0.48ab	0.45b	0.45b	0.51a	0.49ab	0.40c	0.47
	Shoot fresh weight (mg)	258.99c	223.25e	287.44ab	271.74bc	297.25a	230.84de	251.52cd	231.62de	255.94c	256.51
	Root fresh weight (mg)	122.03bc	114.36bc	148.09a	124.95b	162.56a	108.62bc	111.40bc	105.83c	109.49bc	123.04
	Shoot dry weight (mg)	33.53b	29.65c	37.18a	37.78a	38.52a	31.32bc	33.33b	31.86bc	33.99b	34.13
	Root dry weight (mg)	20.57b	17.32c	23.48a	20.25b	24.26a	17.55c	16.85c	17.04c	18.10c	19.49

Values followed by the different letter(s) are significantly different from each other by Duncan's multiple range test at 5% level of probability

11

Table 4 (on next page)

The overall mean performance of the six salt treatments

Table 4. The overall mean performance of the six salt treatments

Traits	Treatments	Control	40 mM	80 mM	120 mM	160 mM	200 mM	Mean
Germination traits	Germination rate	4.40a	4.40a	2.60b	2.04c	1.74d	1.42e	2.77
	Germin. vigor index	33.39a	33.54a	19.65b	14.69c	12.71d	10.28e	20.71
	Germin. (%)	87.50a	88.04a	52.66b	39.63c	35.47d	28.49e	55.30
	Mean daily germin.	12.50a	12.58a	7.52b	5.66c	5.07d	4.07e	7.90
	Mean germin. time (days)	2.48c	2.94b	3.15b	4.09a	3.03b	2.96b	3.11
	Germin. energy (%)	48.76ab	47.61b	37.51c	35.96c	49.05ab	51.35a	45.04
	Relative salt injury	0.00e	-0.62e	39.82d	54.71c	59.47b	67.44a	36.80
	Seedling vigor index	14.46a	10.53b	3.96c	2.04d	1.06e	0.64f	5.45
Germination traits	Shoot length (cm)	9.83a	7.42b	5.14c	3.60d	2.15e	1.77f	4.99
	Root length (cm)	6.57a	4.44b	2.32c	1.50d	0.85e	0.48f	2.69
	Root / shoot ratio	0.67a	0.60b	0.45c	0.40d	0.40d	0.30e	0.47
	Shoot fresh weight (mg)	411.88a	355.74b	298.72c	233.47d	130.17e	101.20f	255.20
	Root fresh weight (mg)	208.23a	169.93b	128.82c	89.86d	69.42e	64.56e	121.80
	Shoot dry weight (mg)	51.09a	45.82b	40.50c	30.28d	20.75e	16.32f	34.13
	Root dry weight (mg)	29.27a	25.04b	20.89c	15.54d	13.20e	13.01e	19.49

Values followed by the different letter(s) are significantly different from each other by Duncan's multiple range test at 5% level of probability

Table 5(on next page)

Phenotypic correlation coefficients among the studied traits

Table 5. Phenotypic correlation coefficients among the studied traits

Traits	GR	GVI	GP	MDG	MGT	GE	RSI	SVI	SL	RL
GVI	0.997**									
GP	0.996**	0.988**								
MDG	0.996**	0.988**	1.000**							
MGT	-0.4	-0.462	-0.333	-0.333						
GEN	0.652	0.681*	0.600	0.600	-0.569					
RSI	-0.881**	-0.864**	-0.900**	-0.900**	0.200	-0.566				
SVI	0.952**	0.960**	0.944**	0.944**	-0.432	0.700*	-0.769*			
SL	0.642	0.677*	0.614	0.614	-0.567	0.708*	-0.432	0.823**		
RL	0.868**	0.886**	0.864**	0.864**	-0.382	0.648	-0.771*	0.928**	0.777*	
RSR	0.203	0.200	0.240	0.240	0.330	0.060	-0.272	0.200	-0.100	0.430
SFW	0.292	0.348	0.246	0.246	-0.627	0.542	-0.106	0.506	0.869**	0.536
RFW	0.251	0.308	0.209	0.209	-0.654	0.445	-0.218	0.388	0.694*	0.533
SDW	0.156	0.214	0.109	0.109	-0.572	0.492	0.065	0.409	0.835**	0.445
RDW	0.303	0.354	0.264	0.265	-0.672*	0.488	-0.212	0.467	0.782*	0.535

Where: GR, germination rat; GVI, germination vigor index; GP, germination percentage; MDG, mean daily germination; MGT, mean germination time; GE, germination energy; RSI, relative salt injury; SVI, seedling vigor index; SL, shoot length; RL, root length; RSR, root/shoot ratio; SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight, RDW, root dry weight; **, highly significant differences exited at the 0.01 level; * , significant differences exited at the 0.05 level.

Table 6(on next page)

Abiotic stress screening indices

Table 6: Abiotic stress screening indices

Index	Formula	Reference
Indices with maximum values corresponding to more tolerant		
Mean productivity (MP) Geometric mean productivity (GMP) Harmonic mean (HM) Stress Tolerance Index (STI) Yield index (YI) Modified stress tolerance index-I (MSTI1) Modified stress tolerance index- II (MSTI2) Yield stability index (YSI) Relative stress index (RSI) Drought index (DI) Stress/non-stress productivity index (SNPI) Relative efficiency index (REI) Mean relative performance (MRP) Golden mean (Gm)	$(Y_S + Y_{NS}) / 2$ $(Y_{NS})^{(1/2)} * Y_S$ $2 * (Y_S * Y_{NS}) / (Y_S + Y_{NS})$ $(Y_S * Y_{NS}) / (Y_{NS \cdot m})^2$ $Y_S / Y_{S \cdot m}$ $((Y_{NS})^2 / (Y_{NS.m})^2) *((Y_S * Y_{NS}) / (Y_{NS \cdot m})^2)$ $((Y_S)^2 / (Y_{S \cdot m})^2) *((Y_S * Y_{NS}) / (Y_{NS \cdot m})^2)$ Y_S / Y_{NS} $(Y_S / Y_{NS}) / (Y_{S \cdot m} / Y_{NS.m})$ $(Y_S*(Y_S / Y_{NS})) / Y_{S.m}$ $((Y_{NS}+Y_S) / (Y_{NS}-Y_S))^{(1/3)} * (Y_{NS}* Y_S *Y_S)^{(1/3)}$ $(Y_S*Y_{NS})/(Y_{S \cdot m}*Y_{NS.m})$ $(Y_S / Y_{S \cdot m}) + (Y_{NS} / Y_{NS.m})$ $(Y_{NS} + Y_S) / (Y_{NS} - Y_S)$	Rosielle and Hamblin (1981) Fernandez (1992) Bidinger et al. (1987) Fernandez (1992) Gavuzzi et al. (1997) Farshadfar and Shukla (2003) Farshadfar and Shukla (2003) Bouslama and Schapaugh (1984) Fischer and Wood (1979) Bidinger *et al.* (1987) Moosavi *et al.* (2008) Ramirez and Kelly (1998) Ramirez and Kelly (1998) Moradi *et al.* (2012)
Indices with minimum values corresponding to more tolerant		
Tolerance index (TOL) Stress Susceptibility Index (SSI) Stress Susceptibility Percentage Index (SSPI) Yield Reduction (YR) Abiotic Stress Tolerance Index (ATI) Mean Productivity Index (MPI) Schnieders Stress Susceptibility Index (SSSI) Sensitivity Drought Index (SDI)	$Y_{NS} – Y_S$ $(1 - (Y_S / Y_{NS})) / (1 - (Y_{S \cdot m} / Y_{NS.m}))$ $(Y_{NS} - Ys) / (2 * Y_{NS.m})$ $1- (Ys / Y_{NS})$ $((Y_{NS} - Ys) / (Y_{NS.m} / Y_{S \cdot m})) * (Y_{NS} * Ys)^{(1/2)}$ $(Y_{NS} - Ys)/2$ $1-(Ys / Y_{NS}) - (1- (Y_{S \cdot m} / Y_{NS.m}))$ $(Y_{NS} -Ys)/ Y_{NS}$	Rosielle and Hamblin (1981) Schnieder *et al.* ((1997) Moosavi *et al.* (2008) Choukan *et al.* (2006) Moosavi *et al.* (2008) Rosielle and Hamblin (1981) Schnieder *et al.* ((1997) Farshadfar and Javadina (2011)

Table 7(on next page)

Values of 22 abiotic stress screening indices based on shoot fresh weight under stress (Ys) and control (Yc).

Table 7: Values of 22 abiotic stress screening indices based on shoot fresh weight under stress (Ys) and control (Yc).

Genotype	Yns	Ys	MP	GMP	HM	STI	YI	MSTI1	MSTI2	YSI	RSI	DI
Zhongsi1084	457.67	70.00	263.83	1497.52	121.43	0.19	0.69	0.23	0.09	0.15	0.62	0.11
GannongNo.2	342.25	101.87	222.06	1884.53	157.00	0.21	1.01	0.14	0.21	0.30	1.21	0.30
GannongNo.4	433.00	144.00	288.50	2996.45	216.12	0.37	1.42	0.41	0.74	0.33	1.35	0.47
ShidaNo.1	480.33	88.33	284.33	1935.96	149.22	0.25	0.87	0.34	0.19	0.18	0.75	0.16
C6	488.33	121.17	304.75	2677.57	194.16	0.35	1.20	0.49	0.50	0.25	1.01	0.30
C16	324.67	117.80	221.23	2122.58	172.88	0.23	1.16	0.14	0.31	0.36	1.48	0.42
C23	385.00	93.34	239.17	1831.40	150.25	0.21	0.92	0.19	0.18	0.24	0.99	0.22
C25	373.33	97.97	235.65	1892.90	155.21	0.22	0.97	0.18	0.20	0.26	1.07	0.25
C26	422.33	76.33	249.33	1568.71	129.30	0.19	0.75	0.20	0.11	0.18	0.74	0.14

Cont.

Genotype	SNPI	REI	MRP	GM	TOL	SSI	SSPI	YR	ATI	MPI	SSSI	SDI
Zhongsi1084	145.06	0.77	1.80	1.36	387.67	1.12	0.47	0.85	17048.80	193.83	0.09	0.85
GannongNo.2	187.21	0.84	1.84	1.85	240.38	0.93	0.29	0.70	11028.18	120.19	-0.05	0.70
GannongNo.4	261.72	1.50	2.47	2.00	289.00	0.88	0.35	0.67	17731.07	144.50	-0.09	0.67
ShidaNo.1	175.84	1.02	2.04	1.45	392.00	1.08	0.48	0.82	19839.54	196.00	0.06	0.82
C6	228.31	1.42	2.38	1.66	367.17	1.00	0.45	0.75	21944.46	183.58	0.00	0.75
C16	212.80	0.92	1.95	2.14	206.87	0.84	0.25	0.64	9940.17	103.43	-0.12	0.64
C23	176.52	0.86	1.86	1.64	291.66	1.00	0.35	0.76	13584.70	145.83	0.00	0.76
C25	183.04	0.88	1.87	1.71	275.37	0.98	0.33	0.74	12939.30	137.68	-0.02	0.74
C26	152.50	0.77	1.78	1.44	346.00	1.09	0.42	0.82	15264.15	173.00	0.06	0.82

\

Table 8(on next page)

Rank of genotypes by 22 abiotic stress indices and shoot fresh weight under stress (Ys) and control (Yc) as well as their average rank (AR).

Table 8: Rank of genotypes by 22 abiotic stress indices and shoot fresh weight under stress (Ys) and control (Yc) as well as their average rank (AR).

Genotype	Yns	Ys	MP	GMP	HM	STI	YI	MSTI1	MSTI2	YSI	RSI	DI
GannongNo.4	4	1	2	1	1	1	1	2	1	2	2	1
C16	9	3	9	3	3	4	3	9	3	1	1	2
C6	1	2	1	2	2	2	2	1	2	5	5	4
GannongNo.2	8	4	8	6	4	7	4	8	4	3	3	3
C25	7	5	7	5	5	5	5	7	5	4	4	5
C23	6	6	6	7	6	6	6	6	7	6	6	6
ShidaNo.1	2	7	3	4	7	3	7	3	6	7	7	7
C26	5	8	5	8	8	8	8	5	8	8	8	8
Zhongsi1084	3	9	4	9	9	9	9	4	9	9	9	9

Cont.

Genotype	SNPI	REI	MRP	GM	TOL	SSI	SSPI	YR	ATI	MPI	SSSI	SDI	AR
GannongNo.4	1	1	1	2	4	2	4	2	7	4	2	2	2.12
C16	3	4	4	1	1	1	1	1	1	1	1	1	2.92
C6	2	2	2	5	7	5	7	5	9	7	5	5	3.75
GannongNo.2	4	7	7	3	2	3	2	3	2	2	3	3	4.29
C25	5	5	5	4	3	4	3	4	3	3	4	4	4.62
C23	6	6	6	6	5	6	5	6	4	5	6	6	5.88
ShidaNo.1	7	3	3	7	9	7	9	7	8	9	7	7	6.08
C26	8	8	9	8	6	8	6	8	5	6	8	8	7.29
Zhongsi1084	9	9	8	9	8	9	8	9	6	8	9	9	8.04

Table 9 (on next page)

Average of the studied traits for the 2 clusters under normal and water stress conditions

Table (9). Average of the studied traits for the 2 clusters under normal and water stress conditions

TRT	Group.1	GR	GVI	GP	MDG	MGT	GE	RSI	SVI	SL	RL	RSR	SFW	RFW	SDW	RDW
Control	1	4.28	32.15	86.89	12.41	2.62	47.42	0.00	14.32	9.84	6.49	0.66	410.15	188.29	51.37	27.59
	2	4.65	35.86	88.71	12.67	2.19	51.46	0.00	14.73	9.80	6.73	0.69	415.33	248.11	50.53	32.63
200 mM	1	1.35	9.79	27.17	3.88	2.96	52.17	69.46	0.55	1.56	0.48	0.33	87.97	63.06	14.31	12.24
	2	1.54	11.26	31.13	4.45	2.95	49.70	65.02	0.81	2.19	0.48	0.22	127.66	67.56	20.34	14.54