Response of nine triticale genotypes to different salt concentrations at the germination and early seedling stages (#84031)

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Response of nine triticale genotypes to different salt concentrations at the germination and early seedling stages

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Type of the Paper (Article Salinity stress poses a major challenge to agricultural productivity worldwide, and understanding their responses at early growth stage is vital for devising strategies to cope with this stress. The cereal crop triticale (× *Triticosecale* Wittmack) is developed by crossing wheat (*Triticum* spp.) and rye (*Secale cereale*) plants. Based on its growth stages, it shows differential sensitivity to salinity stress. Therefore, to improve triticale productivity, this study investigated the salinity stress tolerance of different salt-tolerant triticale genotypes aiming to cultivate them on saline soils. To this end, salinity stress impact on nine triticale genotypes, i.e., Zhongsi 1084, Gannong No. 2, Gannong No. 4, Shida No. 1, C6, C16, C23, C25 and C36 at germination and early seedling stages was evaluated. Each genotype was subjected to six treatments inducing control, 40, 80, 120, 160 and 200 mM NaCl treatments to study their effect on seedling and termination traits of the nine genotypes. Compared to the overall mean seedling vigor index, the seedling vigor index was higher in the genotypes Zhongsi 1084 and C6 (39% and 18.1%, respectively) and lower in Gannong No.2 (41%). Increasing NaCl concentrations negatively affected germination and seedling traits. Compared to other genotypes, Zhongsi 1084 had the highest mean germination rate, germination vigor index, germination percentage, mean daily germination and germination energy. It also showed the lowest relative salt injury. The relative salt injury was higher in the genotype Shida No .1 than those in Gannong No. 2, Gannong No. 4, Shida No. 1, C16, and C36 genotypes. All genotypes exhibited desirable mean germination time except for line C6. High significant positive correlations were observed among germination rate, germination vigor index,

germination percentage, mean daily germination, seedling vigor index and root length. Principal component analysis (PCA) grouped the most desirable genotypes into two clusters. Our study indicated the importance of these traits for salt-tolerant triticale genotypes selection at the germination stage. Moreover, these primary results can be used for additional breeding programs.

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Abstract

Salinity stress poses a major challenge to agricultural productivity worldwide, and understanding their responses at early growth stage is vital for devising strategies to cope with this stress. The cereal crop triticale (× *Triticosecale* Wittmack) is developed by crossing wheat (*Triticum* spp.) and rye (*Secale cereale*) plants. Based on its growth stages, it shows differential sensitivity to salinity stress. Therefore, to improve triticale productivity, this study investigated the salinity stress tolerance of different salt-tolerant triticale genotypes aiming to cultivate them on saline soils. To this end, salinity stress impact on nine triticale

genotypes, i.e., Zhongsi 1084, Gannong No. 2, Gannong No. 4, Shida No. 1, C6, C16, C23, C25 and C36 at germination and early seedling stages was evaluated. Each genotype was subjected to six treatments inducing control, 40, 80, 120, 160 and 200 mM NaCl treatments to study their effect on seedling and termination traits of the nine genotypes. Compared to the overall mean seedling vigor index, the seedling vigor index was higher in the genotypes Zhongsi 1084 and C6 (39% and 18.1%, respectively) and lower in Gannong No.2 (41%). Increasing NaCl concentrations negatively affected germination and seedling traits. Compared to other genotypes, Zhongsi 1084 had the highest mean germination rate, germination vigor index, germination percentage, mean daily germination and germination energy. It also showed the lowest relative salt injury. The relative salt injury was higher in the genotype Shida No .1 than those in Gannong No. 2, Gannong No. 4, Shida No. 1, C16, and C36 genotypes. All genotypes exhibited desirable mean germination time except for line C6. High significant positive correlations were observed among germination rate, germination vigor index, germination percentage, mean daily germination, seedling vigor index and root length. Principal component analysis (PCA) grouped the most desirable genotypes into two clusters. Our study indicated the importance of these traits for salt-tolerant triticale genotypes selection at the germination stage. Moreover, these primary results can be used for additional breeding programs.

Keywords: germination rate; relative salt injury; salinity; seedling stage; seedling vigor index; triticale.

Introduction

Cereals are an important source of food for both human and animal consumption and nutrition (Barati and Bijanzadeh, 2021). Among cereals, triticale (*Triticosecale* Wittmack) is an important cereal crop that belongs to the grass family Poaceae and was developed by hybridizing wheat (*Triticum* spp.) and rye (*Secale cereale*) (Yang et al. 2023). Two types of triticale have been developed, i.e., hexaploid and octoploid (Kang et al., 2016, Alatrash et al., 2022). Triticale is rich in protein (Cantale et al., 2016; Hill, 1990). Therefore, it is a good food source and feed for cattle, particularly in grazed, stored forage, silage and green fodder (Zhao et al., 2022). Moreover, it

The increase in population and the reduction in arable-land area are the two major threats to agricultural sustainability (Shahbaz and Ashraf, 2013). In this context, the global population is estimated to be more than 9.8 billion in 2050 (United Nations, 2023). Thus, the food demand will be more than double the crop production (Van Dijk et al., 2021). To this end, using important crops such as triticale in crop rotation will help minimize soil pests, reduce nutrient levels through leaching and increase crop production (Cao et al., 2022). Additionally, the widespread triticale root system contributes to the grain's soil-particle-binding effect (Demirbas and Balkan, 2020).

Abiotic stresses are the most significant factors limiting crop development and productivity (Zhao et al., 2020). Salinity stress represents the most serious threat to agricultural production, particularly in arid and semi-arid regions where soil nutrient and organic matter levels contribute to physical instability (Zhao et al., 2020). It affects approximately one billion hectares of global land worldwide, thus affecting crop production (Saade et al., 2016). Furthermore, increasing salinity stress negatively affects all traits of plants associated with germination and early seedling growth. Salinity-induced toxic ions like Na^+ and Cl^- affect seed germination by changing osmotic potential, lowering water uptake, causing embryonic damage, and reducing seed germination, shoot elongation, and plant growth (Farooq et al., 2015; Munns and Tester, 2008; Sosa et al., 2005). Approximately 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 by a rate of 10% annually for several reasons, including low precipitation, high evaporation, irrigation using saline water, and poor cultural practices. Moreover, approximately 50% of arable land will probably be salinized by 2050 (Jamil et al., 2011; Barati and Bijanzadeh, 2021). In arid and semi-arid regions, salinity is one of the most important environmental factors affecting uniformity in seed germination (Deng et al., 2020). Germination is a crucial stage in the development of a plant, as it influences the early growth of the seedling and its relationship with the environment and its productivity (Mbarki et al. 2020). Salinity stress induced plant growth inhibition dependent on salt concentration and duration of exposure (Guo et al., 2022). It reduces germination rate and capacity of glycophytes (Saddiq et al., 2021). This may explained by the increase in osmotic pressure of the soil solution (Ma et al., 2022). During germination, the effects of salinity

can manifest as osmotic (reversible) and deleterious (irreversible) effects (Mbarki et al. 2020). For the most majority of crops, seeds are the means by which sophisticated genetics are transferred to the production field. Specifically, rapid and synchronous seed germination and seedling growth are vital for the development of seedlings in the field and thus are crucial to crops production (Reed et al., 2022). Seed germination determines seedling vigor and plant growth. Therefore, this stage is considered a susceptible stage for plant growth (Hakim et al., 2010). Improvement in plant growth and establishment in saline soil are dependent on the salt-tolerating ability of the cultivated genotypes in early growth stages (Keshavarizi and Mohammed, 2012).

Resilience to abiotic stresses is the driving force behind the development of high-yielding and stable triticale cultivars, which in turn led to an increase in the amount of land used for triticale farming (Zhao et al., 2020). Compared to winter cereals, triticale can outproduce on low fertility soils. It has a more robust root system than wheat, barley or oats, allowing it to bond light soils and extract more nutrients (Saddiq et al., 2021). Additionally, triticale is tolerant of low pH (acidic soils), sodic soils and boron-rich soils.

Triticale is also a moderate halophyte with high salinity threshold and it is considered a salt-tolerant species (Grieve et al., 2012). It showed salinity tolerant even up to 10 dSm^{-1} (Ozturk et al., 2018). The salinity threshold of triticale EC (6.1 dSm^{-1}) is higher than that of corn (2.7 dSm^{-1}), rye (5.9 dSm^{-1}) and wheat (4.7 dSm^{-1}). Moreover, Kotuby-Amacher et al. (2000) reported that the salinity threshold differed among various triticale species compared to other cereals. However, the relative grain yield of triticale genotypes varies at 7.3 dSm^{-1} soil salinity. Each unit increase in soil salinity above 7.3 dSm^{-1} reduced triticale grain yield by 2.8%, placing triticale in the salt-tolerant category (Francois et al., 1988).

The establishment of salt-tolerant plants is still in its infancy and shedding the light on the of salinity tolerance mechanisms. Numerous plant species, varieties and halophytes have been studied for their salt tolerance mechanisms, which have proved to be complex (Mbarki et al. 2020). Utilizing more appropriate plant cultivars should increase productivity in salinity stressed marginal areas (Cao et al., 2022). Thus, for

the future of agriculture in arid and semiarid regions, genotypes selection with higher salt tolerance has become an absolute necessity (Golebiowska-Paluch and Dyda, 2023). Although triticale is considered as salinity tolerant crop, some genotypes are less tolerant at the germination stage particularly, after the three-leaf growth stage (Francois et al., 1988). There is insufficient information in the literature on the genotypes tolerance to salinity. Therefore, we aimed to determine salt stress tolerance of nine triticale genotypes at germination and early seedling stages. The objective was to select salt-tolerant genotypes that can be cultivated on saline soil or after salt irrigation. This indeed will improve crop productivity and provide traits that can be used for additional breeding programs.

Materials and methods

Plant genotypes and characteristics

Nine triticale genotypes were used in the current study, and their names and characteristics are listed in **Table 1**. "Zhongsi 1084", Gannong No.2', 'GannongNo.4' "Shida 1" and lines "C6, C16, C23, C25, C36" were bred by the College of Grassland Science, Gansu Agricultural University, China, using the traditional sexual hybridization techniques and a pedigree selection method (Ramadan, et al., 2023).

Study location

The experiment was conducted at Gansu Agricultural University, P. R. China. 36° 5' 26" north, 103° 41' 41" east.

Germination conditions

The seeds of the studied genotypes were sterilized using sodium hypochlorite (1%) for 30 min and washed thrice using distilled water. Next, 50 seeds of each genotype were germinated on Whatman No. 1 filter paper in 9-cm Petri dishes under the following six NaCl concentrations: control, 40 mM, 80 mM, 120 mM, 160 mM, and 200 mM. The seeds were allowed to germinate in an incubator at 20 ± 1°C under a 16/8-h dark/light cycle for 7 d (Warham et al., 1995); they were irrigated and washed twice daily using their corresponding treatment solution, and the filter papers were changed once every 2 d to prevent salt

accumulation. After 2 d of planting, the germinated seeds were counted; the seeds were considered to have been germinated when the emerging radicle was 1 mm in length. Germination percentage was evaluated every 24 h for 5 d.

Analysis of different germination and growth parameters

After 7 d of planting, 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) were measured. Dry weight was measured after drying the roots or shoots at 70°C for 72 h in an oven.

Germination traits were calculated as follows:

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

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

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

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 the 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)

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 d after planting/Total number of seeds tested (Ruan et al., 2002). (6)

153 **Relative salt injury (RSI)** = (Germination percentage of the control – Germination percentage of
154 the treatment)/Germination percentage of the control (7)
155 **Seedling vigor index (SVI) =** (Average shoot length + Average root length) × Germination
156 percentage (Abdul-Baki and Anderson, 1973) (8)
157
158
159 **Salinity stress tolerance**
160 As a quantitative measure, stress indices can quantify the stress responses of a crop. They are easier
161 to use and interpret than raw data. Many indices of abiotic stress tolerance have been proposed (**Table 2**)
162 for estimating abiotic stress tolerant genotypes using a mathematical equation that describes the relationship
163 between growth under stress and control conditions. The abiotic stress indices are classified into two types:
164 The first type contains indices with maximum values indicating high-stress tolerance, whereas the other
165 type includes other indices with minimum values indicating high-stress tolerance. Using these indices, the
166 tolerant and sensitive genotypes and their stability can be identified (Parvaze and Ahmed 2018).
167
168 **Statistical analysis**
169 The experiment was performed as per a factorial, completely randomized design (CRD) (where
170 Factor-1 was genotype including nine levels, and Factor-2 was salt stress treatments including six levels)
171 with three replicates and 50 seeds in each replicate. Two-way analysis of variance (ANOVA) was used for
172 data analysis using SAS statistical software, version 9.2. The means were compared using Duncan's
173 multiple range test ($P < 0.05$), and correlation coefficient was calculated using SPSS version 16. Principal
174 component analysis (PCA) was performed using the statistical package PAST (Hammer et al., 2001) to
175 visualize the differences in various stress-related traits among the nine genotypes.
176 To categorize the genotypes under both control and salinity stress treatments, cluster analysis was
177 performed using R software version 4.1.0, 2021 (R Core Team**,** 2021**)**. Euclidian metric as a distance

178 measure was used to measure dissimilarity among the genotypes, and Joe's algorithm (Joe and Ward, 1963)
179 was applied for grouping the genotypes.
180 Before conducting the analysis, the data were standardized due to their different scale by
181 subtracting the mean from each value and dividing the obtained value by the standard deviation. The cubic
182 cluster criterion (Milligan and Cooper, 1985) was used to ensure whether clusters existed. Fuzzy C-means
183 as a soft clustering algorithm (Bezdek, 1973, 1981) was used to detect if overlapping existed between
184 clusters. PCA is a multi-variable statistical analysis that reduces the dimensions of high-dimension data,
185 and fewer eigenvectors explain the multivariate data (Shlens, 2005).
186
187 **Results**
188 **Genotypes differentially responded to salinity stress.**
189 To study genotype specific responses to salt stress treatment, Analysis of variance (ANOVA) were
190 performed. Highly significant mean squares due to the genotypes and treatments, and genotypes ×
191 treatments were detected for all studied traits except for MGT, where the mean square was non-significant
192 for genotypes and significant for the interaction between treatments and genotypes **(Table 3)**. This result
193 indicated high variation among the studied genotypes under different salt stress treatments.
194
195 **Mean performance of different genotypes**
196 Analysis of germination traits (**Table 4)** revealed that the triticale genotype Zhongsi 1084 had the
197 highest mean GR, GVI, GP%, MDG and GE. In contrast, the genotypes Gannong No.2 and Shida No. 1
198 exhibited the lowest mean of GR, GVI, GP% and MDG. There was no significant difference between the
199 two cultivars. All genotypes exhibited the highest MGT except for line C6. The lowest RSI was observed
200 for Zhongsi 1084, whereas the highest RSI was observed in case of Shida No.1. The SVI of genotypes
201 Zhongsi 1084 and C6 was 39% and 18.1%, respectively. This was higher than the overall mean SVI,
202 whereas that of Gannong No.2 was 41% less than this mean.

Analysis of seedling traits revealed that the mean SL of C6 and Zhongsi 1084 was 12.4% and 9.1% higher than the overall mean of SL, respectively. Whereas the means SL of Gannong No. 2 and C16 were 16.7% and 6.1% lower than the overall mean of SL, respectively. Moreover, the mean RL of Zhongsi 1084, C6, and C23 was the highest. They recorded 17.8%, 16.2%, and 11.3% higher than the overall mean value, respectively. The mean RL of genotypes C36 and Gannong No. 2 was the lowest i.e., 16.8% and 12.4% lesser than the overall mean value, respectively. The highest RSRs were observed for genotypes C23 and Gannong No. 2, whereas the lowest ratio was observed for genotype C36. The highest increase in SFW compared with the overall mean SFW was observed for genotypes C6 (15.9%) and Gannong No. 4 (12.1%). Whereas the highest decrease was observed in Gannong No. 2 (13%), C16 (10%), and C25 (9.7%). For RFW, the highest mean values were exhibited by C6 and Gannong No. 4. They recorded 32.1% and 20.4% more than the general mean, respectively. Meanwhile, genotypes C25, C16 and C36 exhibited the lowest mean values i.e., they were 13.1%, 11.7%, and 11% lower than the overall mean, respectively. Genotype C6 had the highest mean SDW i.e., 12.9% higher than the general mean. Meanwhile, both genotypes Gannong No. 2 and C16 had the lowest mean SDW values, exhibiting 13.1% and 8.2% decreases compared to the general mean, respectively. Moreover, both genotypes C6 and Gannong No. 4 had the highest mean RDW values. They were 24.5% and 20.5% higher than the general mean. The mean RDW values of genotypes C23, C25 and Gannong No. 2 were 13.5%, 12.6%, and 11.1% was lower than the general mean.

Differential effect of salt treatments on germination

The GR of different triticale genotypes under varying salt concentrations were 1.42–4.4% (**Table 5**). The highest GR was observed in the control and 40 mM NaCl treated groups. GR gradually also reduced with increasing NaCl concentrations. GR reduced by 41% and 67.9% in the 80 mM and 200 mM NaCl treated groups, respectively. GVI was significantly different under different salinity levels, whereas mean values of different treatments were 10.28–33.54. The highest value was observed in the 40 mM NaCl treated group i.e., the lowest value was observed in the 200 mM NaCl group. Moreover, significant differences were not observed between the control and 40 mM NaCl groups. The highest reduction in GVI was observed, where 60%, 62%, and 69.2 were observed under 120, 160, and 200 mM NaCl treatments,

229 respectively. However, the significant differences were not observed in GP% between control and 40 mM
230 NaCl treated groups. In contrast, significant differences in GP% were observed as NaCl concentration
231 increased from 80 mM to 200 mM and the GP% reduced by 39.8% in the 80 mM NaCl group. The highest
232 GP% (88.04%) was observed for the 40 mM NaCl treated group, whereas the lowest GP% (28.29%) was
233 observed in the 200 mM NaCl treated group. The highest MDG was observed in both control and 40 mM
234 NaCl groups (12.50 and 12.58, respectively) and the lowest value was observed in the 200 mM NaCl treated
235 group. The reduction % in MDG increased from 39.8% to 67.4% as NaCl concentration increased from 80
236 mM to 200 mM. The number of days required for germination increased from 2.48 day in the control group
237 to 4.09 day in the 120 mM NaCl treated group. In groups treated with NaCl concentration >120 mM, the
238 number of days for germination gradually decreased with increasing NaCl concentrations. However,
239 significant differences were not observed among 40, 80, 160, and 200 mM NaCl groups. GE decreased
240 from 48.76% in the control group to 35.96% in the 120 mM NaCl group. However, in groups with NaCl
241 concentration > 120 mM, GE gradually increased and it was 51.35% at 200 mM NaCl. However, significant
242 differences in GE were not observed between the control, and 160 mM and 200 mM NaCl treated groups.
243 The RSI was negative in the 40 mM NaCl treated group and increased significantly with increasing salt
244 concentration. It increased from 39.82% under 80 mM NaCl treatment to 67.44% under 200 mM NaCl
245 treatment. SVI decreased with increasing salt concentrations, where SVI was reduced by 27.2% in the 40
246 mM NaCl treated group and by 95.6% in the 200 mM NaCl treated group.
247
248 Both SL and RL reduced significantly with increasing salt stress (**Table 5**). The highest mean
249 values were observed in the control group, whereas the lowest mean values were recorded in the 200 mM
250 NaCl group. Mean SL varied from 9.83 cm to 1.77 cm, and the reduction in SL ranged from 27.2% (40 mM
251 NaCl) to 82% (200 mM NaCl). Mean RL varied from 6.57 cm to 0.48 cm, and the reduction in RL ranged
252 from 32.4% (40 mM NaCl) to 92.7% (200 mM NaCl). RSR gradually decreased from 0.67 in the control
253 group to 0.3 in the 200 mM NaCl treated group; however, significant differences were not observed between
254 the 120 and 160 mM NaCl treated groups. In the 200 mM NaCl group, >50% reduction in RSR was

observed compared to that of the control group. SFW and SDW were significantly affected by salt stress. Compared to that of the control group, reduction in SFW and SDW was 13.6–75.4% and 10.3–68.1% under increased NaCl concentration from 40 mM to 200 mM. Moreover, RFW and RDW were significantly reduced by salinity, where increasing NaCl concentration increased from 40 mM to 200 mM reduced the RFW and RDW values by18.4–69% and 14.5–55.6%, respectively.

Interaction effects

The mean performance of the different triticale genotypes under salt stress (**Figures 1 A, B** and **2 A, B)**. The highest GR, GVI, and GP% were observed for Zhongsi 1084 under 40–200 mM NaCl treatments, whereas the lowest values were observed for Shida No.1 under 80–200 mM NaCl treatments. Zhongsi 1084 exhibited the best MDG under 40–200 mM NaCl treatments, whereas Shida No. 1 was the most affected under high salt concentrations (120–200 mM NaCl). MGT was 2.01–3.41, 2.7–3.31, 2.58–3.96, 3.23–4.59, 2.68–3.53 and 2.59–3.37 days for control, and 40 mM, 80 mM, 120 mM, 160 mM, and 200 mM NaCl treated groups, respectively. The lowest number of days under control and 120 mM NaCl treatments was observed in genotype C6. Gannong No. 4 exhibited the best GE under control treatment (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 mM and 200 mM NaCl treatments (56.5 and 57.5%, respectively). RSI increased with increasing salt concentrations. The lowest percentage of injury was observed in Zhongsi 1084 i.e., 10.23, 24.18, 25.36 and 38% under 80, 120, 160, and 200 mM NaCl treatments, respectively. Meanwhile, the highest percentage of injury was observed in Shida No. 1 (57.57, 82.17, 87.38, and 87.21% under 80, 120, 160, and 200 mM NaCl treatments, respectively). For SVI, the most desirable genotypes were Zhongsi 1084 and Gannong No. 4 under control treatment; Zhongsi 1084 and C6 under 40 mM, 120, and 200 mM NaCl treatments; Zhongsi 1084 and C23 under 80 mM NaCl treatments; and both Zhongsi 1084 and C25 under 160 mM NaCl treatments. In contrast, Shida No. 1 was the most affected genotype under high salt concentrations.

Furthermore, Zhongsi 1084 had the highest mean SL under control and 40 mM NaCl treatments, but the lowest mean SL under 160 and 200 mM NaCl treatments. C6 had the mean highest SL under 80

and 120 mM NaCl treatments. C16 also had the highest mean SL under 160 and 200 mM NaCl treatments. The lowest mean SL under control and 40 mM, 80 mM, and 120 mM NaCl treatments were exhibited by Gannong No. 2. The mean RL was 8.57–5.13 and 5.45–3.61 cm for control and 40 mM NaCl groups, respectively. It gradually decreased to 0.97–0.62 and 0.61–0.29 cm for 160 mM and 200 mM NaCl treated groups, respectively. The mean RSR decreased with increasing salt concentrations. The mean 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 had the highest RSRs under 160 and 200 mM NaCl treatments, whereas C16 had the lowest ratios. Both C6 and Shida No. 1 were the best genotypes as per their mean SFW under 0, 40 and 80 mM NaCl treatments, whereas both Gannong No. 4 and C6 were the best genotypes under 120, 160, 200 mM NaCl treatments as per their mean SFW. C6 and Gannong No. 4 were the best genotypes as per mean RFW under 0-120 mM NaCl treatments, whereas C6 and Gannong No. 2 were the best under 160 and 200 mM NaCl treatments. Furthermore, the highest mean SDW was observed for Shida No. 1 and C6 under 0-80 mM NaCl treatments, for C6 under 120 mM and 160 mM NaCl treatments and for Gannong No. 4 under 200 mM NaCl treatment. In contrast, the lowest mean SDW under high salt concentrations was exhibited by Zhongsi 1084 and C36. Gannong No. 4 and C6 were the most desirable genotypes under 0-120 mM salt treatments for RDW. It was also reported that different salinity concentrations caused considerable effects on GP%, GR, 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 (**Table 6)**. The highest positive correlation (r = 1.00) was observed between GP% and MDG. High significant positive correlations were observed among GR, GVI, GP%, MDG, SVI, and RL. Significant positive correlations were also observed among RL, SFW, RFW and RDW. SVI was significantly positively correlated with RL. GVI was significantly positively correlated with GE and SL. Significant positive correlations were observed between GE, SVI, and SL, and between SL and RL. Positive but non-significant correlations were observed between germination traits GR, GVI, GP%, MGT, GE and SVI and seedling traits RSR, SFW, RFW, SDW and

RDW. In contrast, highly significant negative correlations were observed between RSI and GR, GVI, GP%, and MDG. Significant negative correlations were also observed between MGT and RDW and between RSI and both SVI and RL.

PCA

In the current study, PCA classified the nine genotypes into four clusters based on their mean performance under different NaCl treatments (**Figure 3**). The first cluster was found in the 1st quadrant, which included triticale genotypes C6 and Gannong No. 4. Both genotypes scored the highest values for the seedling traits SFW, RFW, SDW, RDW and high values for RL, SVI, MDG and GVI. The second cluster was found in the 2nd quadrant and included the genotypes Zhongsi 1084, C23, and C25. These genotypes had high mean GR, GVI, GP%, MDG, SVI, SL and RL and low RSI. The third cluster was found in the 3rd quadrant and included Gannong No. 2 and C16 genotypes, whereas 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 GR, GVI, GP%, MDG, SVI, and RL. These results suggested considerable variability for salt tolerance in the studied triticale genotypes.

Tables 7 and 8 reveal that Gannong No. 4 was the most tolerant genotype with an average rank (AR) equal to 2.12 (**Figure 4**). However, Zhongsi 1084 was the least tolerant genotype (AR = 8.04). Both Gannong No. 2 and C25 were moderately tolerant as their ARs were 4.29 and 4.62, respectively. Higher AR suggested the lower tolerance of the genotype (**Table 8).**

Cluster analysis

SFW and RFW were used to construct a distance matrix and to generate a tanglegram exhibiting dissimilarity among all genotypes under control and the treatment with the highest salt concentration (200 mM) (**Figure 5**). The fuzzy C-means method elucidated that low overlap existed between clusters, thus 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, which were average, generalized average, single, and weighted.

The valued of agglomerative coefficients were 0.76, 0.81, 0.53, 0.77, 0.85, and 0.88 respectively, under control treatment, whereas under 200 mM NaCl treatment, they were 0.68, 0.72, 0.55, 0.73, 0.77, and 0.81 respectively. These results reveal that Joe's method had the highest coefficient compared to those of the other five methods under control and 200 mM NaCl treatments. Therefore, Joe's method was chosen to conduct cluster analysis. To identify the optimum number of clusters in the data, 30 internal validation indices were selected and screened (Charrad et al., 2014). As shown in **Figure 5**, all genotypes were separated into two clusters under control and 200 mM NaCl treatment groups (**Table 9**). The structure of the clusters changed markedly when the genotypes were subjected to 200 mM NaCl treatment except for genotypes Gannong No. 4 and C6, which migrated from cluster 1 under control to cluster 2 under the saline treatment because they were more tolerant than the other members of their cluster.

Heatmaps elucidate the relationship between the genotypes and the studied traits based on standardized (scaled) data using a color scale under control and 200 mM NaCl treatments (**Figures 6 and 7**). Before drawing the heatmap, the data were standardized by subtracting the mean from each value and dividing the obtained value by the standard deviation. Genotype C6 had the highest mean SFW and SDW in the control group, whereas genotype Gannong No. 4 had the highest mean SFW and SDW under the highest salinity treatment (200 mM). These results demonstrated that Gannong No. 4 was the most tolerant genotype. The lowest mean SFW and SDW under control treatment were observed in C16, whereas Zhongsi 1084 exhibited the lowest mean SFW and C26 had the lowest mean SDW under 200 mM NaCl treatment. Moreover, GP% of genotypes Gannong No. 4 and Gannong No. 2 was the highest and the lowest, respectively, under control treatment.

In contrast, 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 mean SFW, RFW, SL, and RSI. Gannong No. 4 had higher values of germination traits under control treatment. The heatmap does not reveal any association between germination traits and the tolerance indices of the genotypes, except for MGT, which was negatively associated with the tolerance of the genotypes.

359

360 **Discussion**

361 Soil salinity is a major environmental factor limiting crop growth and yield performance. Therefore,
362 understanding these intricate responses is crucial for identifying specific salt-tolerant genotypes and
363 optimizing agricultural practices in saline-prone regions. Therefore, the primary objective of this study was
364 to identify salt-tolerant and sensitive triticale genotypes during the early seedling stage to assess their
365 potential for salt tolerance. As previously noted, the impact of salinity on plant growth can differ
366 significantly between plant species and even among different genotypes of the same species. Therefore, it
367 is essential to monitor the genetic variability among genotypes (Van Dijk et al., 2021). This knowledge is
368 crucial for enhancing salt tolerance in crops and improving their resilience to saline conditions. In
369 consistent, our results indicated the differential responses of targeted genotypes to salinity stress. Based on
370 germination traits, genotypes Zhongsi 1084, C6, C23, and C25 showed the highest salinity stress tolerance.
371 Meanwhile, C6 and Gannong No. 4 were the most tolerant genotypes based on their seedling traits. In
372 contrast, the germination traits of Gannong No. 2 and Shida No. 1 genotypes were the most sensitive
373 genotypes. Genotype specific responses were also reported in the study of Kandil et al., (2012) who found
374 that different wheat genotypes significantly varied in their response to salinity stress at GP%, GR, SVI,
375 SL, RL, SFW, RFW, SDW, and RDW levels.

376 Different genotypes may demonstrate varying degrees of salinity stress tolerance at specific growth
377 stages, necessitating careful selection and monitoring to ensure optimal performance under saline
378 conditions. In this context, the effect of salinity stress is also associated with their growth stage (Shannon,
379 1997). Seed germination and seedling establishment are the most salt-sensitive stages of plants (Ashraf and
380 Foolad, 2005). Salinity-induced ion toxicity, particularly elevated levels of Na+ and Cl- ions, disrupts
381 cellular processes, causing damage to plant tissues and hindering the normal growth and development of
382 seedlings. Atak et al. (2006) found that high Na^+ accumulation induced germination inhibition. High salt
383 levels in the soil can disrupt nutrient and water uptake, leading to stunted growth, leaf wilting, and other
384 physiological stress symptoms (Zhao et al., 2020). In this regrad, the study of Akgun et al. (2011) found

that GR, SL, RL and dry weights of the green parts and roots considerably decreased with increasing salt concentrations. Kandil et al. (2012) and Atri et al., (2018) reported that with increasing salt concentrations, the average values of germination and seedling growth traits gradually reduced. Francois et al. (1988) also reported that at soil salinity of up 6.0 dSm^{-1} and 20.5 dSm^{-1} delayed the seed germination and reduced the final germination rate by 17%, respectively.

The salt tolerance index is commonly used to evaluate and rank the relative salt tolerance of different plant genotypes or varieties. Thus, correlating the salt tolerance index with these specific indices of germination and seedling growth shed the light on which traits contribute most to overall salt tolerance. This information can guide the selection and breeding of salt-tolerant plant varieties, ultimately leading to improved crop performance in saline-affected environments. In accordance with these results, Alom et al. (2016) reported that the salt tolerance index for seedling dry weight of wheat genotypes irrigated with saline water (15 dSm^{-1}) was positively correlated with salt tolerance indices GR, GVI, SL, and RL suggesting their role as selection criteria. Aflaki et al. (2017) investigated the effect of salinity on germination of different genotypes of wheat and found that MDG exhibited the highest correlation with GP%. In a previous study, PCA classified different genotypes of wheat and soybeans 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; Shelke et al., 2017). Overall, identifying which traits contribute most to overall salt tolerance, can guide the selection and breeding of salt-tolerant plant varieties, ultimately leading to improved crop performance in saline-affected environments.

Conclusions

In the current study, the researchers observed that as the salt concentration increased, the average performance of most traits showed a gradual decrease. This indicates that higher salt levels negatively affected the performance of the plant traits under investigation. Correlating the salt tolerance index with these specific indices of germination and seedling growth and plant breeders can gain valuable insights into which traits contribute most to overall salt tolerance. By correlating the salt tolerance index with these

specific indices such as GR, GVI, SL, and RL, we gain valuable insights into which traits contribute most to overall salt tolerance. Genotype Zhongsi 1084 exhibited the best germination performance. Line C6 and genotype Gannong No.4 resulted in best performance for shoot and root length and fresh and root dry weight. PCA analysis grouped the most desirable genotypes (Gannong No.4 and C6) in clusters 1 and 2), whereas other genotypes were grouped into clusters 3 and 4. Overall, the identification of salt-tolerant traits in genotypes is crucial for addressing the challenges of salinity stress in agriculture and ensuring food security in the face of changing environmental conditions. The findings of our study will establish a basis for future research and offer valuable insights into the selection and development of salt-tolerant genotypes at early seedling stage.

Author 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.

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 authors confirm that the data supporting the findings of this study are available within the supplementary materials.

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Table 1(on next page)

List of genotypes and names, of triticale investigated in this study

Number	Genotype 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 GASU)
6	C16 (Triticale line bred by GASU)
7	C23 (Triticale line bred by GASU)
8	C25 (Triticale line bred by GASU)
9	C36 (Triticale line bred by GASU)

GASU: Gansu Agricultural University of P.R. China

Table 2(on next page)

Abiotic stress screening indices

1

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

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Table 3(on next page)

Mean square estimates for the parameters of triticale genotypes under different salt treatments

Source of Variance	Treatment	Genotype	Treatment × Genotype	Error
degree of fredom	5	8	40	108
Germination rate	47.51**	8.59**	0.365**	0.06
Germination vigor index	2891.46**	470.32**	18.675**	4.16
Germination percentage (%)	18758.28**	3197.63**	170.011**	29.56
Mean daily germination	382.77**	65.25**	3.475**	0.60
Mean germination time (d)	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

**: highly significant differences at 0.01 level; *: significant differences at the 0.05 level; and ns: no significant differences

Table 4(on next page)

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

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Genotypes / Traits		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 g	0.50 c	0.51 c	0.64 a	0.31 f	0.47 cd	0.38 e	0.43 d	0.58 b	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
	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

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

The overall mean performance of the six salt treatments

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Treatments Traits		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
	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

2 Values followed by the different letter(s) are significantly different from
3 each other by Duncan's multiple range test at 5% level of probability
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Table 6(on next page)

Phenotypic correlation coefficients among the studied traits

Traits	GR	GVI	GP	MDG	MGT	GE	RSI	SVI	SL	RL	RSR	SFW	RFW	SDW
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	-0.219			
RFW	0.251	0.308	0.209	0.209	-0.654	0.445	-0.218	0.388	0.694*	0.533	-0.104	0.883**		
SDW	0.156	0.214	0.109	0.109	-0.572	0.492	0.065	0.409	0.835**	0.445	-0.199	0.963**	0.798**	
RDW	0.303	0.354	0.264	0.265	-0.672*	0.488	-0.212	0.467	0.782*	0.535	-0.191	0.913**	0.962**	0.837**

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 7 (on next page)

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

	Zhongsi 1084	Gannong No.2	Gannong No.4	Shida No.1	C6	C16	C23	C25	C36
Yield under normal condition (Yns)	457.67	342.25	433.00	480.33	488.33	324.67	385.00	373.33	422.33
Yield under stress condition (Ys)	70.00	101.87	144.00	88.33	121.17	117.80	93.34	97.97	76.33
Mean productivity (MP)	263.83	222.06	288.50	284.33	304.75	221.23	239.17	235.65	249.33
Geometric mean productivity (GMP)	1497.52	1884.53	2996.45	1935.96	2677.57	2122.58	1831.40	1892.90	1568.71
Harmonic mean (HM)	121.43	157.00	216.12	149.22	194.16	172.88	150.25	155.21	129.30
Stress Tolerance Index (STI)	0.19	0.21	0.37	0.25	0.35	0.23	0.21	0.22	0.19
Yield index (YI)	0.69	1.01	1.42	0.87	1.20	1.16	0.92	0.97	0.75
Modified stress tolerance index-I (MSTI1)	0.23	0.14	0.41	0.34	0.49	0.14	0.19	0.18	0.20
Modified stress tolerance index- II (MSTI2)	0.09	0.21	0.74	0.19	0.50	0.31	0.18	0.20	0.11
Yield stability index (YSI)	0.15	0.30	0.33	0.18	0.25	0.36	0.24	0.26	0.18
Relative stress index (RSI)	0.62	1.21	1.35	0.75	1.01	1.48	0.99	1.07	0.74
Drought index (DI)	0.11	0.30	0.47	0.16	0.30	0.42	0.22	0.25	0.14

Cont.

	Zhongsi 1084	Gannong No.2	Gannong No.4	Shida No.1	C6	C16	C23	C25	C36
stress/non-stress productivity index (SNPI)	145.06	187.21	261.72	175.84	228.31	212.80	176.52	183.04	152.50
relative efficiency index (REI)	0.77	0.84	1.50	1.02	1.42	0.92	0.86	0.88	0.77
mean relative performance (MRP)	1.80	1.84	2.47	2.04	2.38	1.95	1.86	1.87	1.78
golden mean (GM)	1.36	1.85	2.00	1.45	1.66	2.14	1.64	1.71	1.44
tolerance index (TOL)	387.67	240.38	289.00	392.00	367.17	206.87	291.66	275.37	346.00
stress susceptibility Index (SSI)	1.12	0.93	0.88	1.08	1.00	0.84	1.00	0.98	1.09
stress susceptibility percentage index (SSPI)	0.47	0.29	0.35	0.48	0.45	0.25	0.35	0.33	0.42
yield reduction (YR)	0.85	0.70	0.67	0.82	0.75	0.64	0.76	0.74	0.82
abiotic stress tolerance index (ATI)	17048.80	11028.18	17731.07	19839.54	21944.46	9940.17	13584.70	12939.30	15264.15
mean productivity index (MPI)	193.83	120.19	144.50	196.00	183.58	103.43	145.83	137.68	173.00
Schnieder's stress susceptibility index (SSSI)	0.09	-0.05	-0.09	0.06	0.00	-0.12	0.00	-0.02	0.06
sensitivity drought index (SDI)	0.85	0.70	0.67	0.82	0.75	0 .64	0.76	0.74	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) treatments as well as their average rank (AR).

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	Zhongsi 1084	Gannong No.2	Gannong No.4	Shida No.1	C6	C16	C23	C25	C36
Yield under normal condition (Yns)	3	8	4	2	1	9	6	7	5
Yield under stress condition (Ys)	9	4	1	7	2	3	6	5	8
Mean productivity (MP)	4	8	2	3	1	9	6	7	5
Geometric mean productivity (GMP)	9	6	1	4	2	3	7	5	8
Harmonic mean (HM)	9	4	1	7	2	3	6	5	8
Stress Tolerance Index (STI)	9	7	1	3	2	4	6	5	8
Yield index (YI)	9	4	1	7	2	3	6	5	8
Modified stress tolerance index-I (MSTI1)	4	8	2	3	1	9	6	7	5
Modified stress tolerance index- II (MSTI2)	9	4	1	6	2	3	7	5	8
Yield stability index (YSI)	9	3	2	7	5	1	6	4	8
Relative stress index (RSI)	9	3	2	7	5	1	6	4	8
Drought index (DI)	9	3	1	7	4	2	6	5	8

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3 Cont.

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	Zhongsi 1084	Gannong No.2	Gannong No.4	Shida No.1	C6	C16	C23	C25	C36
stress/non-stress productivity index (SNPI)	9	4	1	7	2	3	6	5	8
relative efficiency index (REI)	9	7	1	3	2	4	6	5	8
mean relative performance (MRP)	8	7	1	3	2	4	6	5	9
golden mean (GM)	9	3	2	7	5	1	6	4	8
tolerance index (TOL)	8	2	4	9	7	1	5	3	6
stress susceptibility Index (SSI)	9	3	2	7	5	1	6	4	8
stress susceptibility percentage index (SSPI)	8	2	4	9	7	1	5	3	6
yield reduction (YR)	9	3	2	7	5	1	6	4	8
abiotic stress tolerance index (ATI)	6	2	7	8	9	1	4	3	5
mean productivity index (MPI)	8	2	4	9	7	1	5	3	6
Schnieder's stress susceptibility index (SSSI)	9	3	2	7	5	1	6	4	8
sensitivity drought index (SDI)	9	3	2	7	5	1	6	4	8
AR	8.04	4.29	2.12	6.08	3.75	2.92	5.88	4.63	7.25

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Table 9(on next page)

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

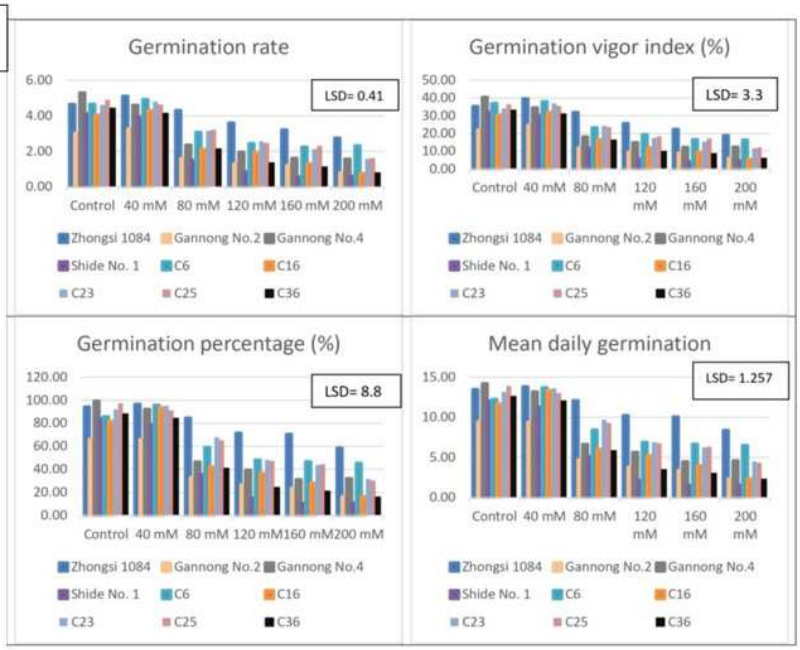
Table 9. Average of the studied traits for the two clusters under normal and water stress conditions

Treatment	Control		200 mM	
Group	1	2	1	2
Germination rate (GR)	4.28	4.65	1.35	1.54
Germination vigor index (GVI)	32.15	35.86	9.79	11.26
Germination percentage (GP)	86.89	88.71	27.17	31.13
Mean daily germination (MDG)	12.41	12.67	3.88	4.45
Mean germination time (MGT)	2.62	2.19	2.96	2.95
Germination energy (GE)	47.42	51.46	52.17	49.70
Relative salt injury (RSI)	0.00	0.00	69.46	65.02
Seedling vigor index (SVI)	14.32	14.73	0.55	0.81
Shoot length (SL)	9.84	9.80	1.56	2.19
Root length (RL)	6.49	6.73	0.48	0.48
Root/shoot ratio (RSR)	0.66	0.69	0.33	0.22
Shoot fresh weight (SFW)	410.15	415.33	87.97	127.66
Shoot fresh weight (RFW)	188.29	248.11	63.06	67.56
Shoot dry weight (SDW)	51.37	50.53	14.31	20.34
Root dry weight (RDW)	27.59	32.63	12.24	14.54

Figure 1

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

1 A


Germination rate
LSD= 0.41
Germination vigor index (%)
LSD= 3.3
Germination percentage (%)
LSD= 8.8
Mean daily germination
LSD= 1.257
Control 40 mM 80 mM 120 mM 160 mM 200 mM
Zhongsi 1084 Gannong No.2 Gannong No.4
Shide No. 1 C6 C16
C23 C25 C36

1 B

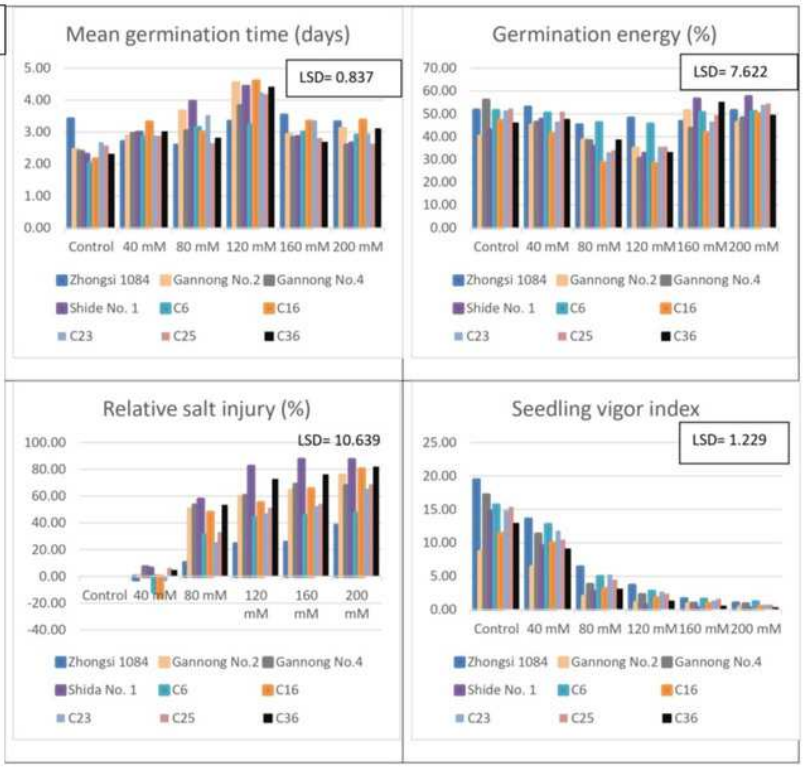
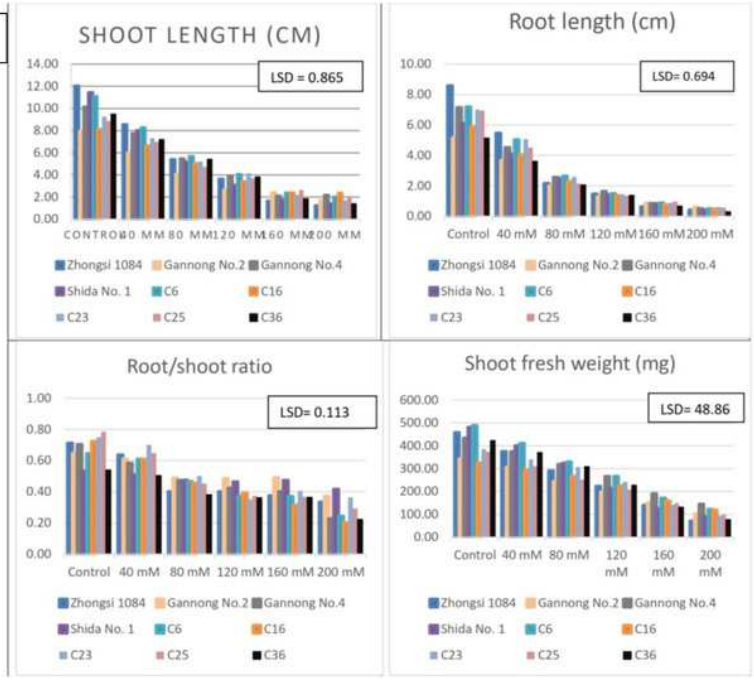

Mean germination time (days)
LSD= 0.837
Germination energy (%)
LSD= 7.622
Relative salt injury (%)
LSD= 10.639
Seedling vigor index
LSD= 1.229
Control 40 mM 80 mM 120 mM 160 mM 200 mM
Zhongsi 1084 Gannong No.2 Gannong No.4
Shide No. 1 C6 C16
C23 C25 C36

Figure 2

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

2 A


SHOOT LENGTH (CM)
LSD = 0.865
Root length (cm)
LSD= 0.694
Root/shoot ratio
LSD= 0.113
Shoot fresh weight (mg)
LSD= 48.86
Control 40 mM 80 mM 120 mM 160 mM 200 mM
Zhongsi 1084 Gannong No.2 Gannong No.4
Shida No. 1 C6 C16
C23 C25 C36

2 B

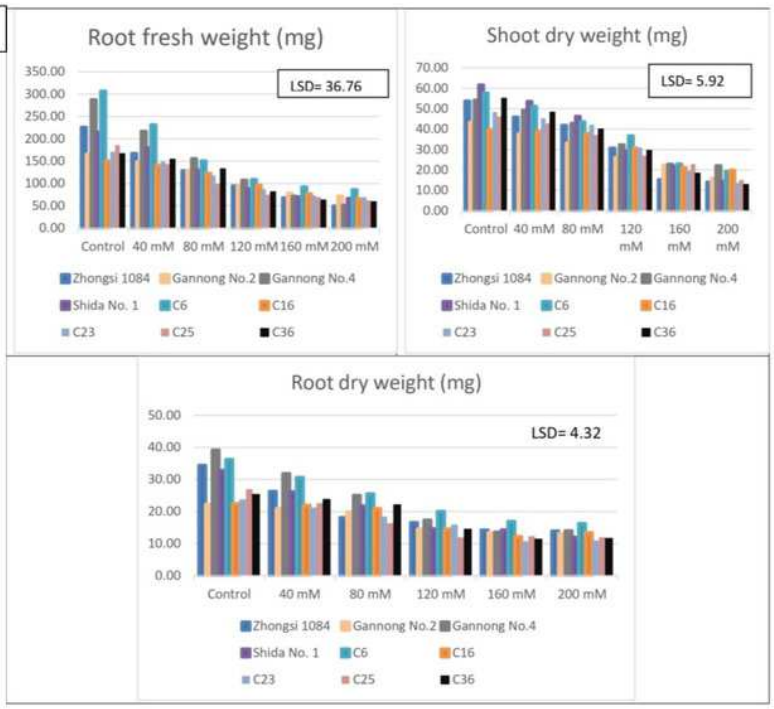

Root fresh weight (mg)
LSD= 36.76
Shoot dry weight (mg)
LSD= 5.92
Root dry weight (mg)
LSD= 4.32
Control 40 mM 80 mM 120 mM 160 mM 200 mM
Zhongsi 1084 Gannong No.2 Gannong No.4
Shida No. 1 C6 C16
C23 C25 C36

Figure 3

Two-dimensional ordination of the nine Triticale genotypes investigated in this study based on their overall mean performance under salt treatments

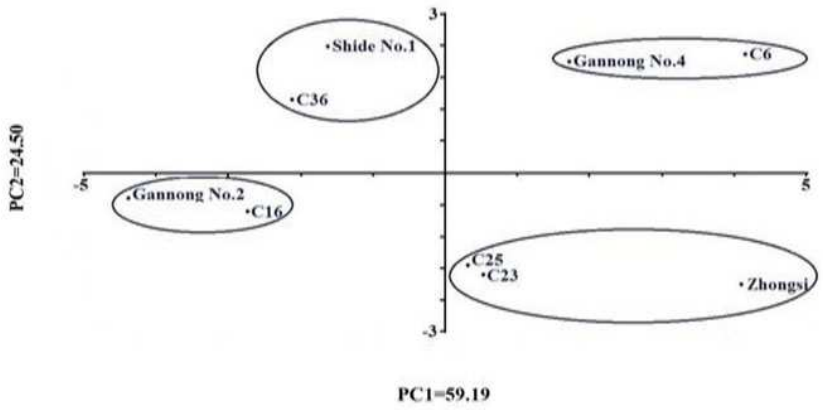

3
-3
-5
5
PC2=24.50
PC1=59.19
Shide No.1
C36
Gannong No.4
C6
Gannong No.2
C16
C25
C23
Zhongsi

Figure 4

Tolerance of genotypes according to the average rank of 22 abiotic stress indices (Lower average rank indicates higher tolerance

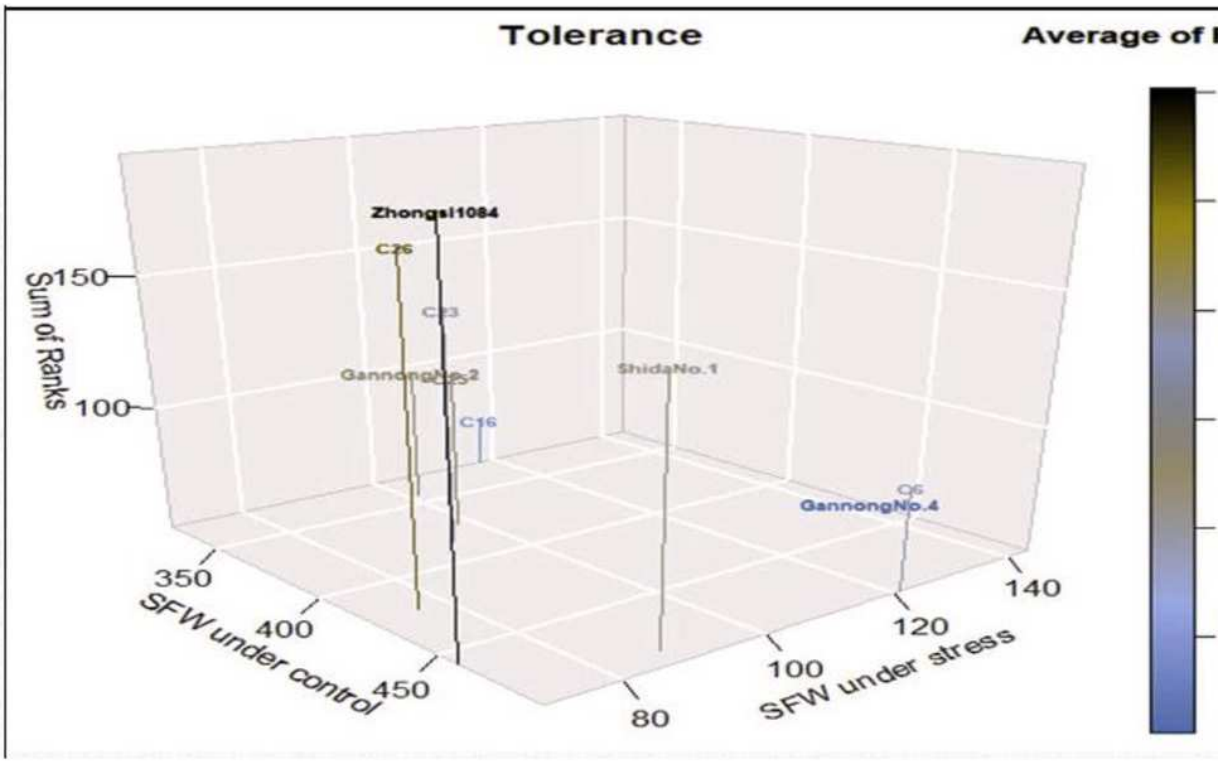

Tolerance
Average of
Zhongsl1084
C26
C23
GannongNo.2
C16
ShidaNo.1
Q6
GannongNo.4
Sum of Ranks
150
100
350
400
450
SFW under control
80
100
120
140
SFW under stress

Figure 5

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

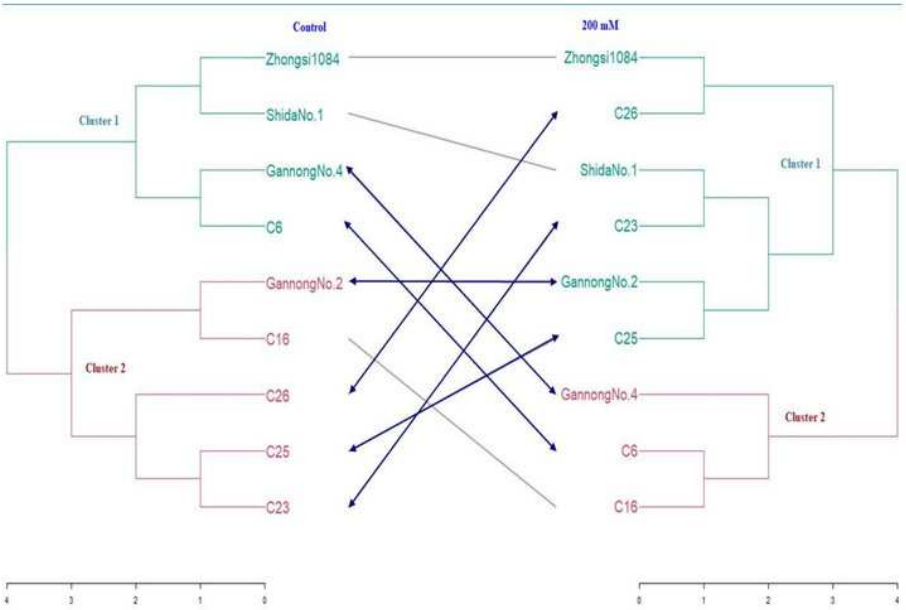

Control
200 mM
Zhongsi1084
ShidaNo.1
GannongNo.4
C6
GannongNo.2
C16
C26
C25
C23
Cluster 1
Cluster 2
Zhongsi1084
C26
ShidaNo.1
C23
GannongNo.2
C25
GannongNo.4
C6
C16
Cluster 1
Cluster 2

Figure 6

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

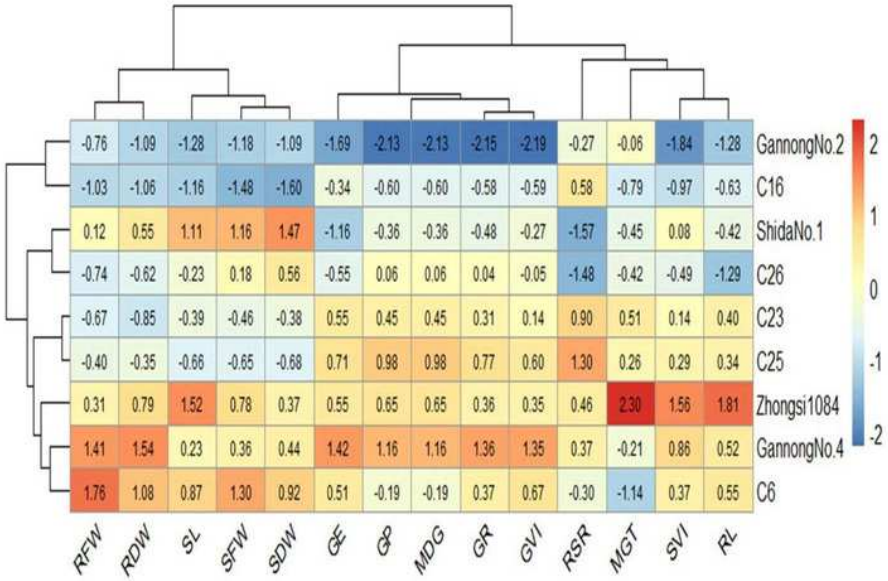
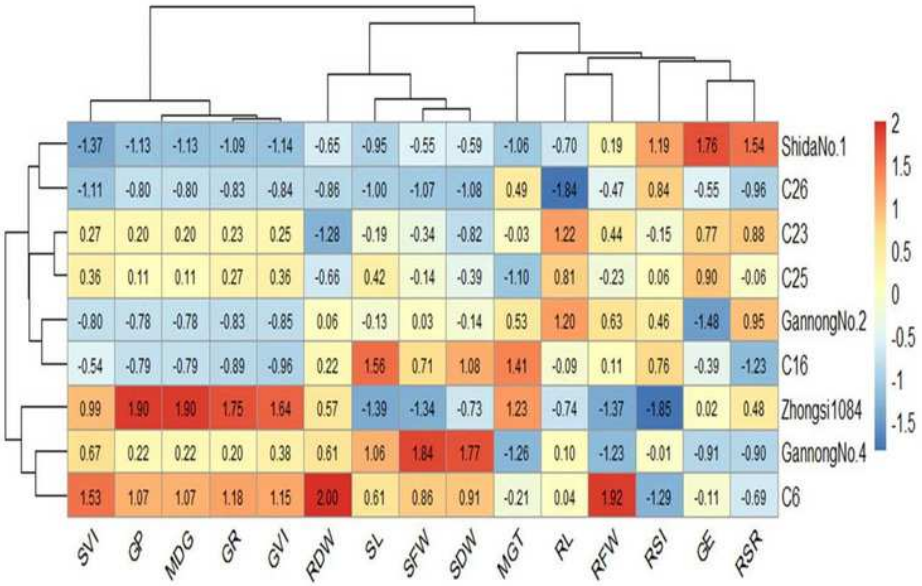

-0.76 -1.09 -1.28 -1.18 -1.09 -1.69 -2.13 -2.13 -2.15 -2.19 -0.27 -0.06 -1.84 -1.28 GannongNo.2
-1.03 -1.06 -1.16 -1.48 -1.60 -0.34 -0.60 -0.60 -0.58 -0.59 0.58 -0.79 -0.97 -0.63 C16
0.12 0.55 1.11 1.16 1.47 -1.16 -0.36 -0.36 -0.48 -0.27 -1.57 -0.45 0.08 -0.42 ShidaNo.1
-0.74 -0.62 -0.23 0.18 0.56 -0.55 0.06 0.06 0.04 -0.05 -1.48 -0.42 -0.49 -1.29 C26
-0.67 -0.85 -0.39 -0.46 -0.38 0.55 0.45 0.45 0.31 0.14 0.90 0.51 0.14 0.40 C23
-0.40 -0.35 -0.66 -0.65 -0.68 0.71 0.98 0.98 0.77 0.60 1.30 0.26 0.29 0.34 C25
0.31 0.79 1.52 0.78 0.37 0.55 0.65 0.65 0.36 0.35 0.46 2.30 1.56 1.81 Zhongsi1084
1.41 1.54 0.23 0.36 0.44 1.42 1.16 1.16 1.36 1.35 0.37 -0.21 0.86 0.52 GannongNo.4
1.76 1.08 0.87 1.30 0.92 0.51 -0.19 -0.19 0.37 0.67 -0.30 -1.14 0.37 0.55 C6
RFW RDW SL SFW SDW GE GP MDG GR GVI RSR MGT SVI RL
2
1
0
-1
-2

Figure 7

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


-1.37 -1.13 -1.13 -1.09 -1.14 -0.65 -0.95 -0.55 -0.59 -1.06 -0.70 0.19 1.19 1.76 1.54 ShidaNo.1
-1.11 -0.80 -0.80 -0.83 -0.84 -0.86 -1.00 -1.07 -1.08 0.49 -1.84 -0.47 0.84 -0.55 -0.96 C26
0.27 0.20 0.20 0.23 0.25 -1.28 -0.19 -0.34 -0.82 -0.03 1.22 0.44 -0.15 0.77 0.88 C23
0.36 0.11 0.11 0.27 0.36 -0.66 0.42 -0.14 -0.39 -1.10 0.81 -0.23 0.06 0.90 -0.06 C25
-0.80 -0.78 -0.78 -0.83 -0.85 0.06 -0.13 0.03 -0.14 0.53 1.20 0.63 0.46 -1.48 0.95 GannongNo.2
-0.54 -0.79 -0.79 -0.89 -0.96 0.22 1.56 0.71 1.08 1.41 -0.09 0.11 0.76 -0.39 -1.23 C16
0.99 1.90 1.90 1.75 1.64 0.57 -1.39 -1.34 -0.73 1.23 -0.74 -1.37 -1.85 0.02 0.48 Zhongsi1084
0.67 0.22 0.22 0.20 0.38 0.61 1.06 1.84 1.77 -1.26 0.10 -1.23 -0.01 -0.91 -0.90 GannongNo.4
1.53 1.07 1.07 1.18 1.15 2.00 0.61 0.86 0.91 -0.21 0.04 1.92 -1.29 -0.11 -0.69 C6
SVI GP MDG GR GVI RDW SL SFW SDW MGT RL RFW RSI GE RSR
2
1.5
1
0.5
0
-0.5
-1
-1.5