Screening of salt-tolerance of maize varieties based on the value of the membership function and under GGE biplot analysis (#90694)

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Screening of salt-tolerance of maize varieties based on the value of the membership function and under GGE biplot analysis

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This study aimed to screen for highly salt-tolerant maize varieties using four NaCl treatments at concentrations of 0, 60, 120, and 180 mMol/L. Agronomic traits, physiological and biochemical indices related to salt tolerance were measured, and salt tolerance was evaluated using principal component analysis, membership function method, and GGE biplot analysis. A total of 41 local maize varieties were assessed based on their D values. Results show: (1) The results revealed significant differences in plant height, stem thickness, germ length, etc. among the different NaCl treatments. As the salt concentration increases, the lengths of embryo shoot and root, leaf area, etc. decreased, while electrical conductivity and salt damage index increased. (2) Correlations were found among most of the indicators, and principal component analysis identified eight main components. (3) In the GGE biplot analysis, Youqi 909, Xuanhe 8, and Qunze 888 exhibited superior salt tolerance and adaptability in salt stress environments. The optimal stress concentration was determined to be 120 mMol/L NaCl solution. (4) The top five varieties for salt tolerance and stability were Yougi 909, Xuanhe 8, Qunze 888, and Wugu 568, followed by New Jade 66. This study further validated the reliability of GGE biplot analysis in germplasm selection, expanded the genetic resources of salt-tolerant maize in Xinjiang, and provided theoretical references and germplasm utilization for introducing maize into saline-alkali areas in southern Xinjiang. These research findings contributed to improving our understanding of maize salt tolerance and promoting its cultivation in harsh environments.

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- 2 the Value of the Membership Function and under GGE
- 3 biplot analysis

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Abstract

This study aimed to screen for highly salt-tolerant maize varieties using four NaCl treatments at concentrations of 0, 60, 120, and 180 mMol/L. Agronomic traits, physiological and biochemical indices related to salt tolerance were measured, and salt tolerance was evaluated using principal component analysis, membership function method, and GGE biplot analysis. A total of 41 local maize varieties were assessed based on their D values. Results show: (1) The results revealed significant differences in plant height, stem thickness, germ length, etc. among the different NaCl treatments. As the salt concentration increases, the lengths of embryo shoot and root, leaf area, etc. decreased, while electrical conductivity and salt damage index increased. (2) Correlations were found among most of the indicators, and principal component analysis identified eight main components. (3) In the GGE biplot analysis, Youqi 909, Xuanhe 8, and Qunze 888 exhibited superior salt tolerance and adaptability in salt stress environments. The optimal stress concentration was determined to be 120 mMol/L NaCl solution. (4) The top five varieties for salt tolerance and stability were Youqi 909, Xuanhe 8, Qunze 888, and Wugu 568, followed by New Jade 66. This study further validated the reliability of GGE biplot analysis in germplasm selection, expanded the genetic resources of salt-tolerant maize in Xinjiang, and provided theoretical references and germplasm utilization for introducing maize into saline-alkali areas in southern Xinjiang. These research findings contributed to improving our understanding of maize salt tolerance and promoting its cultivation in harsh environments.

Introduction

The sustainability of global agriculture has been severely compromised by soil salinisation, resulting in significant reductions in crop yield and quality worldwide [1,2]. The area of saline land in the world is as much as 954 million hectares, and the area of saline land in China reaches 37 million hectares, accounting for 4.9% of arable land [3-4]. In the process of growth and development, salt stress causes ionic toxicity, osmotic stress, oxidation stress, etc., resulting in cell membrane damage, root drying, metabolic disorders, and reduced plant photosynthesis, which seriously affects plant growth. [5]. Maize (Zea mays L.), a widely cultivated crop globally, plays a central role in the economic development of China. It is a moderately saline crop with low salt tolerance and is severely affected by salt stress at the seedling stage. It is severely affected by salt stress at the seedling stage and is a moderately salt-sensitive crop with poor salt tolerance. It is of great significance to study the salt tolerance of maize varieties in China and its comprehensive evaluation methods and identification indexes for the breeding, production and application of salt-tolerant germplasm resources.

Previous research has shown that salt damage significantly impacts maize yield stability. It affects phenotypic traits, physiological and biochemical indices of maize, especially during reproductive stages like germination and seedling growth. Salt stress causes a significant reduction in seed germination and also leads to a reduction in plant growth rate and yield, while at the same time reducing the plant's water uptake capacity [6-8]. Increased salt concentration inhibited germination and early seedling growth in several rice varieties, as shown by Song J et al [9]. Salt-tolerant varieties tend to have higher peroxidase activity under salt stress conditions [10]. The conductance and malondialdehyde (MDA) levels of leaf in Maize increased significantly after salt stress, resulting in peroxidative damage to the cell plasma membrane, and plant proline (PRO) levels increased significantly after salt stress to improve plant growth [11-12]. The salt tolerance indicators selected for the different fertility periods and treatments were



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also distinct. In previous studies, germination rate, germination potential, radicle length and radicle length have been used as indicators to reflect the salt tolerance of seeds at the germination stage from various perspectives [13-14]. Seedling condition, plant height and rate of change in dry weight have also been used as grading indices to identify salt tolerance in maize seedlings by previous authors [15].

Many domestic and foreign scholars have proposed identification methods based on salinity resistance, including the membership function method as one of the important methods to identify the salinity resistance of maize, and the higher value of the membership function indicates more salinity resistance [16]. Based on the membership function values, Yu Ying et al. used an affiliation function method to classify maize inbred lines into four categories of salinity sensitivity [16]. Zhang Haiyan and colleagues, based on the salt tolerance coefficient of germination and seedling stage identification index as the evaluation basis, divided the weights of each index according to the coefficient of variation of the value of the salt tolerance coefficient of the index membership function, and classified the salt tolerance of test maize into four levels by using the weighted membership function method [17]. GGE biplot analyses have been used to classify environments, to assess the ranking of genotypes, and to determine the discriminative and representative properties of environments [18]. It can be analysed in terms of crop yield and stability [19-20], identification of the ideal environment for crop growth [21], and screening and evaluation of germplasm resources [22-23]. The "GGE biplot Chart" graphically illustrates the relationships among varieties, identification indicators or assessment methods using auxiliary lines. It provides accurate and intuitive insights into the performance of different varieties in various environments, as well as the appropriate indicators for identification or assessment methods [24].

Although there have been numerous studies on the salt tolerance mechanisms of maize, most of the previous research focused on individual stages without considering other reproductive phases. Few studies have utilized GGE biplot analysis to investigate maize germination and seedling stages under salt stress. In this study, we simulated several agronomic traits and physiological indicators related to salt tolerance in maize using NaCl solutions of 0, 60, 120, and 180 mMol/L under different salt stress conditions, building upon previous work. By incorporating fuzzy membership function and GGE biplot analysis, we comprehensively evaluated the salt tolerance of 41 maize varieties. We assessed the overall salt tolerance of these varieties, identified appropriate concentrations and key evaluation indicators, and screened out more salt-tolerant maize varieties, providing guidance for the selection and promotion of maize varieties in Xinjiang's saline-alkali areas.

Materials & Methods

100 Plant materials

- 101 For this trial, 41 maize varieties popularized in production were selected as test material, and the
- specific material information was listed in Table 1.
- 103 Experimental Design

104 Germination test for seeds indoors

- Germination experiments were conducted in an artificial climate chamber with a day/night
- temperature of 25°C/15°C (day/night) and 75% humidity at the The study took place at the Key
- 107 Laboratory of Genetic Improvement and Efficient Production for Specialty Crops in Arid
- Southern Xinjiang of Xinjiang Corps in April-May 2022. The experiment was set up with a total
- of four concentration gradients of 0 (CK), 60, 120 and 180 mmol/L NaCl solutions and each
- treatment was replicated three times. The Petri dish was first sterilised with 70% alcohol, two
- 111 layers of filter paper were placed in the dish and then the seeds were placed evenly in the dish.
- 112 Incubation was carried out with four concentrations of NaCl solution, 0, 60, 120 and 180



- 113 mmol/L, and the solution in each Petri dish soaked more than 1/3 of the seeds. Each day, the
- Petri dish was filled with the appropriate amount of NaCl solution at the appropriate 114
- 115 concentration. Seed germination was recorded at 16:00 h each day, seed germination potential
- was determined at 4 d of treatment and germination rate was determined after 7 d of treatment 116
- 117 and relative germination potential, relative germination rate, salt tolerance index and salt injury
- index were calculated. 118

119 **Tolerance test for potting salt outdoors**

- 120 The study took place at the Tarim University Agronomy experiment station from May-July 2022.
- 121 25×25 seedling pots were used. The growing medium was fine sandy soil with a soil weight of
- 122 4.5 kg. Maize seeds of each cultivar were sown at a depth of about 3 cm with 10 seeds per pot,
- 123 selected for uniform and consistent size of 120 grains each. When the seedlings grew to three
- 124 leaves and one heart, they were treated with nutrient solution containing 1/4 concentration of
- 125 Hoagland nutrient solution with different concentrations of salt solution, the treatment
- 126 concentrations were 0 (CK), 60, 120 and 180 mmol/L NaCl solution and each treatment was
- 127 repeated three times. After 7 days of treatment, the fresh and dry weight of each plant was
- determined, the root-crown ratio was calculated, conductivity was determined and physiological 128
- 129 indices including MDA content, PRO content, SOD activity and POD activity were measured
- 130 using the kit method.
- 131 Calculation formula
- 132 Based on the salt tolerance coefficient (salt tolerance coefficient = mean value of salt stress
- 133 index/mean value of control index) of each identification index of the test varieties, 41 maize
- 134 varieties were comprehensively evaluated for salt tolerance by using the subordinate function
- 135 method in fuzzy mathematics, and the calculation formula was as follows
- 136 Coefficient of variation (CV) = (standard deviation SD/mean) \times 100%.
- 137 The formula for calculating the value of the membership function is where Xi is the salt
- 138 tolerance coefficient of each test material based on the identification index i, Xi max and Xi min
- are the maximum and minimum values of Xi in the test materials respectively, and U(Xi) is the 139
- 140 value of the membership function of each test material Xi.
- Inverse membership function formula: μ (Xi)=1-(Xi-Xi min)(Xi max-Xi min). 141
- The weighting formula:Wi = CVi/ $\sum_{i=1}^{n}$ CVi (i = 1,2,3,...,n) .CVi is the coefficient of variation of each test material μ (Xi) and Wi is the ratio of CVi to the total variation. 142
- 143
- Weighted membership function value formula : $D = \sum_{i=1}^{n} [\mu(Xi) \cdot Wi)](i = 1,2,3,...,n)$. $\mu(Xi)$ is 144
- 145 the value of the membership function of each test material Xi and Wi is the weight of each
- 146 identification index.
- 147 **Data Analysis**
- 148 Microsoft Excel was used for data processing, SPSS software for ANOVA and factor analysis,
- 149 and Origin 2022 software for correlation analysis. The analysis of adaptability of cultivation
- 150 varieties, discrimination analysis of NaCl concentration, representativeness analysis of NaCl
- 151 concentration, and analysis of ideal salt-tolerant cultivated varieties were conducted using
- 152 GenStat software for GGE biplot analysis. The analytical method partially referred to previous
- 153 studies [25].
- Results 154
- 155 Analysis of difference among salt tolerance indexes



156 An analysis of variance (ANOVA) was conducted on the phenotypic, physiological, and germination indices of 41 maize varieties (refer to Figures 1-3). Plant height, stem thickness, 157 radicle length, leaf area, conductivity, chlorophyll SPAD, germination rate, water content, 158 germination index, salt tolerance index, salt injury index, SOD activity, MDA content, and POD 159 160 activity exhibited significant difference among the four NaCl concentration treatments. While the root crown ratio and PRO content did not show any significant difference. Germ length, radicle 161 162 length, leaf area, germination rate, germination index, salt tolerance index, and germination potential decreased with increasing salt concentration, while conductivity and salt injury index 163 164 increased. The increase in water content with salt concentration could be attributed to the 165 continuous uptake of external water by the root system to maintain physiological metabolism 166 balance under adverse conditions, facilitating the survival and growth of seedlings under salt stress. Plant POD activity was an important indicator of resistance, reflecting the ability of plants 167 168 to scavenge reactive oxygen radicals. [26-27]. In this study, the POD activity of maize leaves differed significantly from the control group under 60 mMol NaCl treatment, but the changes 169 170 were minimal as the NaCl concentration increased. This finding contradicted a previous study [28] and may be attributed to variations in POD activity levels among different varieties under 171 172 NaCl treatment. The SOD activity of antioxidative enzymes in the functional leaves of 41 salt-173 tolerant maize varieties decreased and the MDA content increased under 120 mM NaCl 174 treatment. However, with higher concentrations of NaCl treatment, the SOD activity increased 175 and the MDA content decreased. This indicates that the ability to remove reactive oxygen 176 species (ROS) generated during normal growth and development could be enhanced at specific 177 salt concentrations. Subsequently, by clearing ROS and reducing lipid peroxidation, it mitigates 178 plant damage and enhances their adaptation to high salt stress at higher concentrations. 179 Analysis of the correlation among the salt tolerance indicators 180 Correlation analyses were conducted among the 18 indicators, and the relationships among these 181 indicators were depicted in Figure 4. Within the phenotypic traits, plant height exhibited 182 significant positive correlations with stem thickness, leaf area, germ, length, radicle length, 183 chlorophyll SPAD, and salt tolerance index; while displaying negative correlations with water content, root crown ratio, and conductivity. Leaf area displayed a negative correlation with root 184 crown ratio. Water content showed a negative correlation with germ length, radicle length, and 185 186 SOD activity. The content of MDA was negatively correlated with both SOD activity and chlorophyll SPAD. Additionally, SOD activity exhibited a negative correlation with POD 187 188 activity. Notably, there were significant positive correlations observed among germination 189 potential, germination percentage, germination index, salt tolerance index, germ length, and 190 radicle length. 191 There was no significant correlation observed between conductivity and the activities of SOD and POD in the physiological indices. Under adverse stress conditions, plants commonly respond 192 193 by increasing POD activity in their tissues [29]. Conductivity served as an indicator of leaf 194 damage severity caused by salt stress, and it was expected that as leaf damage increased, POD 195 activity would also increase. However, contrary to expectations, this study found that there was 196 no significant correlation between POD activity and conductivity. This discrepancy could be attributed to the continuous adversity that the plants experienced during this period, leading to a 197 198 saturation point in POD activity, increased membrane lipid peroxidation, disruption of normal cell metabolism, leaf damage, and inhibition of plant growth. Therefore, our research findings 199 deviated from previous studies. 200 Principal component analysis and membership function analysis of salt tolerance index 201 Factorial analyses were conducted on the 18 indicators, as presented in Table 2. Based on a 202 203 cumulative contribution exceeding 80%, eight principal components were extracted. The 204 eigenvalue of the first principal component was 5.141. The germination index had the highest



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- 205 loading value and was therefore referred to as the salt tolerance index factor. Principal 206 component 2 had an eigenvalue of 2.825 and was called the germ length factor. The plant height 207 factor was assigned to principal component 3, which had an eigenvalue of 1.541. The SOD 208 activity factor was assigned to principal component 4, which had an eigenvalue of 1.472. 209 Principal component 5, named the chlorophyll SPAD factor, possessed an eigenvalue of 1.067. Similarly, the sixth principal component, with an eigenvalue of 1.006, was associated with the 210 211 highest loading value of MDA content, called the MDA content factor. Principal component 7 212 had an eigenvalue of 0.941 and was called the POD activity factor. Principal component 8, 213 known as the PRO content factor, had an eigenvalue of 0.781. 214 The eight factors obtained from the principal component analysis were comprehensively 215 evaluated by membership function, including the germination index, chlorophyll SPAD, germ length, SOD activity, plant hight, POD activity, PRO content and MDA content. The D values 216 217 for these factors were calculated using the membership function method and further computed for the four NaCl concentrations listed in Table 4. In the absence of treatment, cultivar no. 23 218 219 exhibited the highest D value, while under a 60 mM NaCl solution treatment, cultivar no. 16 had the highest D value. Conversely, cultivar no. 12 showed the highest D value in 120 mM NaCl 220 221 solution treatments. Meanwhile, cultivar no. 18 showed the highest D value in 180 mM NaCl 222 solution treatments. It is important to note that a higher value of the membership function 223 indicates greater salt tolerance. Therefore, among all cultivars, cultivar no. 23 displayed the 224 highest salt tolerance in the 60 mM NaCl solution treatment, cultivar no. 12 exhibited higher salt 225 tolerance in the 120 mM NaCl solution treatment, and cultivar no. 18 showed higher salt 226 tolerance compared to other cultivars in the 180 mM NaCl solution treatment.
- 227 Salt tolerance or adaptation of different maize varieties on the basis of the analysis of the 228 GGE biplot

The D values of the test varieties under four stress concentrations were analyzed using GGE biplot analysis. The first principal component accounted for 56.62% of the total variation, while the second principal component explained 20.12% of the variation. The GGE biplot was employed to visualize varietal adaptation functions, where sectors represented the growth environments under the stress of the four NaCl solutions. The maize varieties for trail number 8, 38, 18, 33, 23, and 35 located in the apex of the sector area. The polygon was divided into six sectors, with the four environmental NaCl stress concentrations distributed in two sectors. C2, C3, and C4 resided in the first sector, whereas C1 was situated in the second sector. Cultivar no23 exhibited closer proximity to the C1 region, suggesting its superior adaptation to the C1 environment compared to other varieties. On the other hand, cultivar no. 18, 16, and 12 demonstrated a higher adaptability to high salt stress conditions, as they were positioned closer to regions C2, C3, and C4 respectively. This observation implies that these three varieties possessed relatively higher salt tolerance under salt stress conditions when compared to the remaining 38 varieties.

Representativeness and discriminatory power of different concentrations of NaCl

244 An important factor in assessing the suitability of an environment under NaCl stress 245 concentration was its discriminatory power and representativeness. In the GGE biplot analysis, 246 circles connected the mean environment axis and mean environment value. The smaller the circle 247 associated with a stress concentration environment point, the higher the overall level of that 248 particular environment. It was evident that the comprehensive ranking of the four stress environments was C3 > C2 > C4 > C1 (Figure 6). This implied that C3 exhibited a stronger 249 ability to identify the salt tolerance of maize germplasm, while C1 had the weakest ability to do 250 251 so. It should be noted that C1 served as the control environment, which explained its limited 252 capacity to identify the salt tolerance of maize.



- 253 The environments under different stress concentrations were analyzed and depicted (Figure 7).
- 254 The angle among the environment under C1 stress concentration and the environments under C2.
- 255 C3, and C4 stress concentrations was found to be less than 90°, indicating a strong positive
- 256 correlation. This suggests that the ranking of varieties was similar across the four stress
- 257 concentrations. In terms of representativeness, the environment under C2 stress concentration
- showed the best representation, as it had the smallest angle to the mean axis. On the other hand,
- the C4 environment had the longest line segment, indicating its strong discriminatory power for varieties.
- Furthermore, the analysis of variance for the test maize's identification indexes (Figures 1-3)
- revealed a significant downward trend. There was also a significant distinction among multiple
- varieties and concentrations. These results suggest that the measured indexes at 160 mmol/L
- NaCl concentration can effectively identify the salt tolerance of different maize varieties. Thus,
- 265 this concentration can be considered an ideal environment for determining maize varieties' salt
- 266 tolerance, providing strong persuasiveness and credibility. Moreover, the C2 environment
- 267 indicated less discrimination among varieties, as it had the shortest line segment. Overall, the
- 268 environments at the four stress concentrations can be divided into two regions: one comprising
- 269 C2, C3, and C4, and another comprising C1.

270 Analysis of the salt tolerance of different maize varieties

- 271 The central point of the circle on the environmental axis represents the average stress
- 272 concentration. Combined with this experiment, the varieties closer to the center circle exhibit the
- best overall performance in terms of salt tolerance and stability. The top six varieties in terms of
- 274 combined salt tolerance and stability were ranked as follows:18>12>16>21>32>40 (Figure 8).
- 275 On the other hand, the bottom six varieties in terms of combined salt tolerance and stability were
- 276 ranked as follows: 8<35<38<13<23<34. When comparing the variety rankings obtained from the
- 277 GGE biplot analysis with the membership function values calculated using the affiliation
- 278 function method, it was found that after conducting the GGE dual standard analysis of the
- 279 membership function values, the stability of the varieties could be further evaluated based on the
- assessment of salt tolerance in different maize varieties. This facilitates the identification of salt-
- tolerant germplasm resources with high potential. stability.

Discussion

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Effect of salt stress on maize phenotypic and physiological indicators

- 284 Salinity stress is a significant abiotic factor that greatly affects maize yields in maize-producing
- countries. It is crucial to develop and utilize crop varieties adapted to saline soils in order to
- address this issue [30]. With the increase of soil salinity and the expansion of saline-alkali land,
- it is urgent to screen and cultivate maize varieties with enhanced salt-tolerance. [17]. Salt stress
- 288 has negative impacts on crucial plant processes, impedes growth and development, and disrupts
- 289 cellular structures [31-32].
- 290 Previous studies on maize salt tolerance mainly focused on individual growing seasons, without
- 291 considering multiple reproductive periods. Furthermore, results may be various when diverse
- 292 germplasm were employed. For instance, Fu et al. [33] found that a concentration of 100
- 293 mMol/L NaCl promoted maize seed germination, while treatments with 200 mMol/L NaCl and
- 294 higher concentrations inhibited it. In our experiment, various indicators related to seed
- 295 germination, such as germ length, germination rate, and germination index, decreased with
- 296 increasing salt concentration. The NaCl solution impeded maize seed germination. It is important
- 297 to note that the results of this study may be biased due to the use of different germplasm.
- 298 Nevertheless, it aligns with the finding that high concentrations of NaCl solution inhibit maize
- 299 germination. When we analyzed the combined germination and seedling stages, most of the
- 300 evaluation indices for all tested maize varieties exhibited a significant declining trend at NaCl
- 301 concentrations ≥120 mMol/L. In conclusion, the index measured at 120 mMol/L NaCl



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concentration could effectively identify the salt tolerance of different maize varieties. These findings contradict previous conclusions that reported inconsistent levels of salt tolerance among different maize varieties across different test materials and measurement periods. It was speculated that the different salt tolerance exhibited by the same plant in different growth stages arose from distinct underlying mechanisms [33].

Screening of maize salt-tolerant germplasm by means of the membership function method and the GGE biplot analysis

The GGE biplot analysis method encompassed the assessment of genotype and genotype-byenvironment interactions [35]. These scatterplots visually represented bidirectional data, with genotypes as inputs and environments as outputs. This method is frequently used to describe the super-environment, rank genotypes, and determine stable environments. [36]. Furthermore, we found that this method has been widely utilized in assessing the adaptability and productivity of agricultural crops across multiple experimental sites. Previous researchers utilized GGE biplot analysis to investigate heat tolerance in wheat by analyzing late yield and multi-point trials [37, 38]. Similarly, this analysis was commonly utilized in adaptation and stability analyses for crops such as sorghum, sugarcane, and soybean [36, 39, 40]. Most prior studies applied the affiliation function method to calculate affiliation function values, which evaluated the comprehensive performance of plants under abiotic stresses such as drought tolerance, salt tolerance, and cold tolerance. Furthermore, we discovered that numerous studies employed a combination of analytical approaches, including throughput and hierarchical analyses, along with GGE biplot analysis, to evaluate diverse varieties. However, no previous study had evaluated the resistance of varieties by combining the membership function values under the membership function method with GGE biplot analysis [41-42]. In our study, we employed GGE biplot analysis to analyze the membership function values at four concentrations of NaCl solutions. This approach further enhanced the accuracy and reliability of the analyses compared to the observed results. In the GGE biplot polygons (Figure 5), varieties were positioned within specific environments, with genotypes represented at the top. This positioning indicated the superior performance of these genotypes in those environments [43,44]. The angle among environment vectors in GGE biplot analysis reflected their correlation, with a smaller angle indicating a higher correlation [45]. The length of the environment vectors approximated the standard deviation within each environment and served as an indicator of environmental distinctiveness. Consequently, environments with longer vector lengths had larger standard deviations and were more distinguishable [44, 46]. In our study (Figure 7), maize membership function values under NaCl solution stress and their corresponding environments were analyzed. The environment with a concentration of 160 mMol/L NaCl exhibited the longest vector line segment, followed by the environment with a concentration of 180 mMol/L NaCl. The small angle among these two segments indicated a positive correlation with better salt tolerance. Both of these environments were ideal for screening maize varieties for salt tolerance [46]. In summary, the three varieties examined in this study, namely Youqi 909, Xuanhe 8, and Qunze 888, demonstrated excellent adaptability to saline-alkali soil.

These findings provided significant insights for further investigations into the adaptability of maize in saline-alkali soil. Moreover, our study underscored the efficacy of utilizing GGE biplot analysis to assess the potential salt tolerance of maize varieties. A comprehensive evaluation of maize performance under specific environmental conditions was conducted, taking into account factors such as inter-environmental correlation, standard deviations, and vector lengths. This bore considerable significance in the selection of high-quality maize varieties that were well-suited to saline-alkali soil, ultimately leading to improved productivity and quality within maize cultivation in these regions.



350 Nevertheless, it was imperative to conduct subsequent research to validate these findings and 351 expand the scope of the sample size. Such endeavors would facilitate a more profound understanding of the underlying mechanisms governing maize's salt tolerance and the genetic 352 traits that contributed to adaptation under diverse saline-alkali soil conditions. These efforts 353 354 would contribute to the establishment of a robust theoretical foundation for maize cultivating practices, thereby fostering sustainable agricultural development in saline-alkali regions. 355 356 Significance of screening salinity tolerant germplasm resources for future cultivating and development of salinity tolerant crops 357 358 Land salinization became a prominent issue that hindered ecological and environmental 359 development as well as economic progress. The development of Salt Lake agriculture carried substantial practical significance for Chinese food industry, guaranteeing efficient food supply 360 and maintaining the ecological equilibrium of the region [47]. Therefore, cultivating salt-alkali-361 362 tolerant plant varieties and enhancing the salt-alkali tolerance of plants proved to be an effective biological measure in alleviating the impact of saline-alkali land on plants. It also generated 363 364 favorable ecological and economic benefits, promoting the sustainable development of agriculture [48]. In many countries, plant salt tolerance tests and cultivation of salt-tolerant 365 366 plants are used to improve saline-alkali land and develop its potential. At that time, over 100 367 countries grappled with saline soils, including Chinese major grain-producing areas in the 368 northwest, north, and northeast, where grain production and quality were affected. Therefore, 369 apart from soil improvement measures, cultivating salt-tolerant crop varieties became imperative 370 due to their absence, which was the leading cause of low maize yields in saline soils across 371 China [49]. It was worth noting that there was a lack of systematic research on the response of 372 plants in Xinjiang to soil salinity and the stress resistance of unique plant species [50]. Therefore, 373 it has profound significance to evaluate the salt-tolerance of maize varieties comprehensively, 374 screen the salt-tolerant varieties, and cultivate the salt-tolerant germplasm suitable for saline-375 alkali soil.

376 377

Conclusions

378 Given the increasing soil salinity levels, identifying maize genotypes with high salt tolerance has become crucial for expanding maize cultivation on saline-alkali soil, while also enhancing both 379 380 its quality and yield potential. The objective of this study was to further validate the reliability of 381 GGE biplot analysis in selecting maize germplasm through the utilization of the Membership 382 Function method combined with GGE biplot analysis. Through our research, we identified five 383 suitable maize genotypes, namely Youqi 909, Xuanhe 8, Qunze 888, Wugu 568, and New Jade 384 66, which demonstrate promising performance for planting in saline-alkali soil in Xinjiang. These findings provide a theoretical foundation for the cultivation and promotion of maize 385 386 genotypes in other regions of Xinjiang. 387

This study contributes to the field by confirming the effectiveness of the combined approach of GGE biplot analysis and the Membership Function method in the selection of salt-tolerant maize germplasm. Future studies could focus on exploring the salt tolerance of other maize germplasm resources, improving the GGE biplot analysis method, and investigating alternative cultivation techniques applicable to saline-alkali soil conditions. Such research endeavors will further advance our understanding of maize cultivation in challenging environments and pave the way for sustainable agriculture practices.

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Analysis of difference in various morphological indicators under salinity stress.



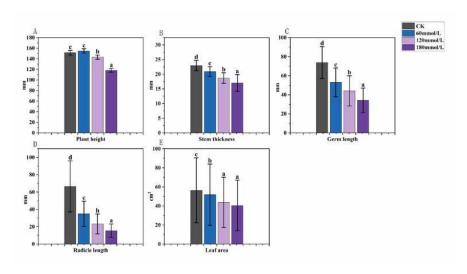


Figure 1. Analysis of difference in various morphological indicators under salinity stress. A: Analysis of the difference in plant height among four NaCl solutions; B: Analysis of the difference in stem thickness among four NaCl solutions; C: Analysis of the difference in germ length among four NaCl solutions; D: Analysis of the difference in radicle length among four NaCl solutions; E: Analysis of the difference in leaf area among four NaCl solutions; a, b, c and d are markers of significance of differences at 0.05 level.



Analysis of difference in various physiological indicators under salinity stress.



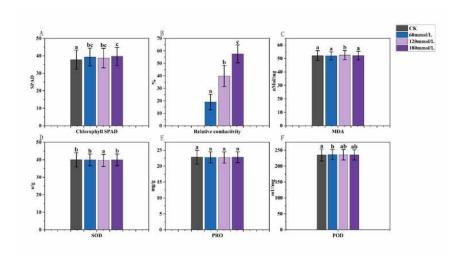


Figure 2. Analysis of difference in various physiological indicators under salinity stress. A: Analysis of the difference in chlorophyll content among four NaCl solutions; Analysis of the difference in conductivity among four NaCl solutions; Analysis of the difference in MDA content among four NaCl solutions; Analysis of the difference in PRO content among four NaCl solutions; Analysis of the difference in PRO content among four NaCl solutions; Analysis of the difference in POD activity among four NaCl solutions; a, b, c and d are significant difference markers at 0.05 level.



Analysis of difference in various germination indicators under salt stress.



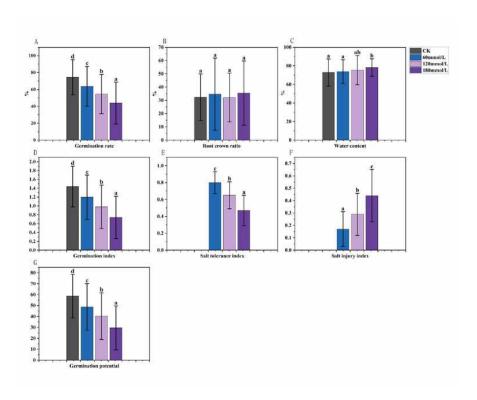


Figure 3. Analysis of difference in various germination indicators under salt stress. Analysis of the difference in germination percentage among four NaCl solutions; Analysis of the difference in root-crown ratio among four NaCl solutions; Analysis of the difference in water content ratio among four NaCl solutions; Analysis of the difference in germination index among four NaCl solutions; Analysis of the difference in salt tolerance index among four NaCl solutions; Analysis of the difference in germination potential among four NaCl solutions; a, b, c and d are significant markers of difference at the 0.05 level.



Correlation of agronomic traits with physiological indicators.



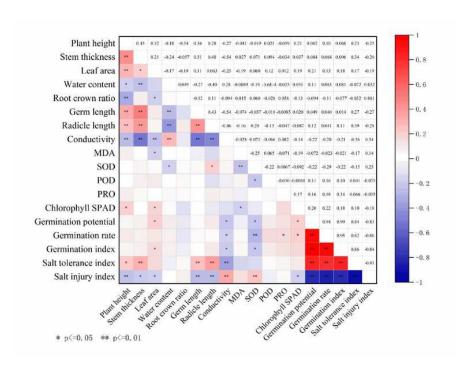


Figure 4. Correlation of agronomic traits with physiological indicators.



Adaptation analysis of maize varieties based on GGE biplot analysis.



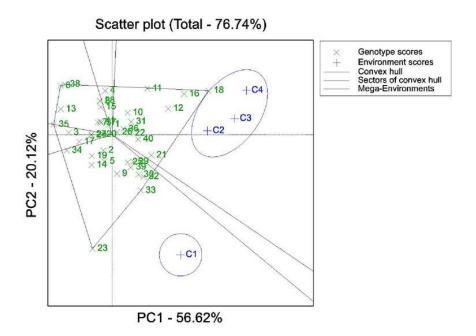


Figure 5. Adaptation analysis of maize varieties based on GGE biplot analysis. 1, 2, 3, etc. are the cultivar numbers of 41 maize varieties; C1, C2, C3 and C4 are the environments under stress of 0 mMol/L NaCl solution, 60 mMol/L NaCl solution, 120 mMol/L NaCl solution and 180 mMol/L NaCl solution, respectively.



Analysis of optimal NaCl stress solutions based on GGE biplots.



Comparison biplot (Total - 76.74%)

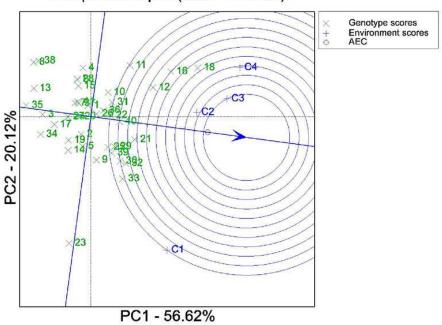


Figure 6. Analysis of optimal NaCl stress solutions based on GGE biplots. 1, 2, 3, etc. are the cultivar numbers of 41 maize varieties; C1, C2, C3 and C4 are the environments under stress of 0 mMol/L NaCl solution, 60 mMol/L NaCl solution, 120 mMol/L NaCl solution and 180 mMol/L NaCl solution, respectively.



Representative analysis of NaCl stressed solutions based on GGE biplot.



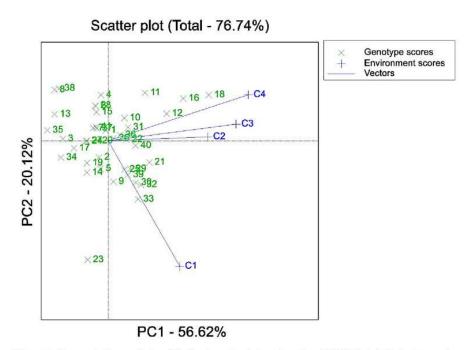


Figure 7. Representative analysis of NaCl stressed solutions based on GGE biplot. 1, 2, 3, etc. are the cultivar numbers of 41 maize varieties; C1, C2, C3 and C4 are the environments under stress of 0 mMol/L NaCl solution, 60 mMol/L NaCl solution, 120 mMol/L NaCl solution and 180 mMol/L NaCl solution, respectively.



Varietal analysis of salt tolerance stability based on GGE biplot.



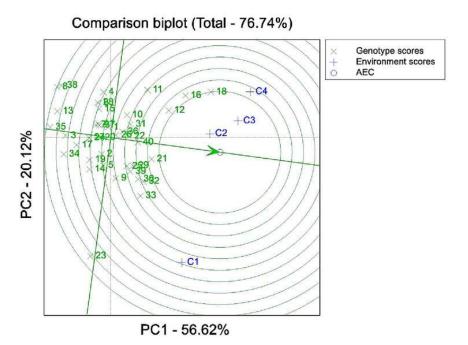


Figure 8. Varietal analysis of salt tolerance stability based on GGE biplot. 1, 2, 3, etc. are the cultivar numbers of 41 maize cultivars; C1, C2, C3 and C4 are the environments under stress of 0 mMol/L NaCl solution, 60 mMol/L NaCl solution, 120 mMol/L NaCl solution and 180 mMol/L NaCl solution, respectively.



Table 1(on next page)

Maize materials tested.



Table 1. Maize materials tested.

	Table 1. Waize materials tested.						
Nu		Nu			_		
mb	Name	Source	mb	Name	Source		
er			er				
	Hong	Jilin Hongxing Seed		Zheng	Henan Academy of		
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	Ping'a	Jilin Ping'an Seed		HengY	Jilin Hengyu Seed Industry		
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	712	Co		e 9918	Agricultural Co.		
	Seedst	Inner Mongolia Seedstar		Xingn	Zhongyan Seed Industry		
4	ar 618	Co	25	ong	Co.		
				No.1			
	Simon	Inner Mongolia Simon		Bixian	Beijing Huanong Weiye		
5	6	Co	26	g 809	Seed Industry Co.		
	Hong	Jilin Hongxing Seed		Jinfeng	Zhengzhou Fengjie Seed		
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	528	•		· ·			
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0	ng 188		29	01	Seed Co.		
	Yuhe	Hanan Vuyay Saad		Vinym	Vinijana Vinski Saad Ca		
9		Henan Yuyu Seed	30	Xinyu	Xinjiang Xinshi Seed Co.		
	536	Industry Co		24			
	*** 0	Shanxi Vodafone Co.		Dengh	Shandong Denghai Seed		
10	Wofe		31	ai 3672	Industry Co.		
10	ng		51				
	No.9						
11	Nongf	Inner Mongolia	32	Xinyu	Urumqi Shengyang		
11	u 99	Zhongnong Co	32	66	Agricultural Company		
	Xuanh	Xuanhui Agriculture Co		Xinno	Inner Mongolia Lanhai		
12	e 8	C	33	ng 008	Xinnong Agricultural		
				8	Company		
	Simon	Inner Mongolia Simon		Xianyu	Tieling Pioneer Seed Co.		
13	668	Co	34	335	Trening Francer Seed Co.		
		Yunnan Linpeng			Chifeng Fengtian Science		
1.4	Linyu		25	Toyota 14			
14	1339	Agriculture Co	35	14	and Technology Seed		
		W7-:-1 T		Г.,	Industry Co.		
1	3.7	Weishan Jiyuan	2.0	Fuyu	Zhongyan Seed Industry		
15	Yuany	Agricultural Co.	36	109	Co.		
	uan 1						



16	Qunze	Sichuan Qunze Seed	37	Simon	Inner Mongolia Simon Co.
	888	Industry Co		3358	
17	Shann	Ningxia Jinyu Seed Co	38	Jixing2	Jilin Xingnong Seed
1 /	ing 23		30	18	Industry Co.
	Youqi	Jilin Hongxiang Seed		Sanme	Changtu Zewei
18	909	Industry Co	39	ng	Agricultural Science
				9599	Research Institute
19	Jin'ai	Inner Mongolia Jin'ai	40	Simon	Inner Mongolia Simon Co.
19	588	Ailite Co	40	208	
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20	n	Agricultural Research	41	338	
	2818	Institute			
21	Wugu	Gansu Wugu Seed			
21	568	Industry Co			



Table 2(on next page)

Maize trait contribution eigenvalues. Extraction method: Principal component analysis.



Table 2. Maize trait contribution eigenvalues. Extraction method: Principal component analysis.

7		

		Initial Eigenv	alues	Sum of squared rotating loads			
Fact or	Tot	Percentage	Cumulativ	Tot	Percentage	Cumulativ	
———	al	variance	e %	al	variance	e %	
1	5.14 1	28.561	28.561	5.14 1	28.561	28.561	
2	2.82 4	15.688	44.250	2.82 4	15.688	44.250	
3	1.54 1	8.560	52.810	1.54 1	8.560	52.810	
4	1.47 2	8.181	60.990	1.47 2	8.181	60.990	
5	1.06 7	5.929	66.919	1.06 7	5.929	66.919	
6	1.00 6	5.589	72.508	1.00 6	5.589	72.508	
7	0.94 1	5.227	77.735	0.94 1	5.227	77.735	
8	0.78 1	4.337	82.072	0.78 1	4.337	82.072	
9	0.71 5	3.971	86.043				
10	0.63 7	3.541	89.584				
11	0.56 2	3.122	92.706				
12	0.39 9	2.214	94.920				
13	0.37 4	2.080	97.000				
14	0.29 8	1.656	98.656				
15	0.15 0	0.831	99.487				
16	0.06 6	0.365	99.852				
17	0.01 9	0.104	99.956				
18	0.00	0.044	100.000				



Table 3(on next page)

Matrix of factor loadings after rotation.

Table 3. Matrix of factor loadings after rotation.

Characters				Ingre	dients			
Characters	1	2	3	4	5	6	7	8
Plant height	0.064	0.503	0.568	0.094	0.266	0.008	0.021	0.091
Stem thickness	0.074	0.759	0.098	0.139	0.057	0.030	0.113	0.034

0.906

0.254

0.061

0.062

0.004

0.052



Table 4(on next page)

D-values of eight indices such as germination index and chlorophyll SPAD under four NaCl concentration stresses.



Table 4. D-values of eight indices such as germination index and chlorophyll SPAD under four NaCl concentration stresses.

Cultivar	CKD	60mMol/LD	120mMol/LD	180mMol/LD
number	value	value	value	value
1	1.82	2.03	1.43	1.86
2	2.11	1.65	1.57	1.63
3	1.54	2.02	0.99	0.94
4	1.41	1.17	2.14	1.87
5	2.23	1.30	2.12	1.36
6	1.51	1.97	1.07	2.05
7	1.65	1.73	1.84	1.36
8	0.90	1.44	1.57	0.86
9	2.40	2.35	1.61	1.44
10	1.76	2.03	2.13	1.90
11	1.49	2.52	2.74	1.84
12	1.91	2.73	2.78	2.18
13	1.30	1.24	1.12	1.22
14	2.21	1.21	1.75	1.31
15	1.51	1.63	1.90	1.56
16	1.90	2.92	2.64	2.75
17	1.71	1.78	1.59	0.86
18	2.25	2.40	2.53	3.92
19	2.07	1.79	1.28	1.40
20	1.85	1.59	1.92	1.41
21	2.42	2.22	2.58	1.87
22	2.00	2.36	1.93	1.80
23	3.21	1.83	1.12	0.96
24	1.89	1.51	1.16	1.79
25	2.39	1.70	2.27	1.68
26	1.86	2.31	1.90	1.48
27	1.78	1.75	1.48	1.37
28	1.41	1.83	1.74	1.57
29	2.46	1.87	2.01	1.97
30	2.57	2.22	2.22	1.66
31	1.88	2.09	2.20	1.85
32	2.69	2.42	1.77	2.05
33	2.86	1.98	2.05	1.91
34	1.78	1.69	1.10	0.89
35	1.46	1.10	0.96	1.13
36	1.88	2.50	1.76	1.69
37	1.75	1.84	1.37	1.78
38	0.97	1.83	0.91	1.31
39	2.43	1.93	2.35	1.54
40	2.16	2.48	1.80	2.05



3 41 1.74 1.17 2.04 1.62

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