Response of broomcorn millet (*Panicum miliaceum* L.) genotypes from semiarid regions of China to salt stress

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**ARTICLE INFO**

**Article history:**
Received 19 March 2014
Received in revised form
27 August 2014
Accepted 28 September 2014
Available online 5 October 2014

**Keywords:**
Salinity tolerance
Genetic variation
Ion response
Broomcorn millet

**ABSTRACT**

Salt tolerance of crops is becoming more and more important, owing to the constant increase of salinity in arid and semi-arid regions. Broomcorn millet (*Panicum miliaceum* L.), generally considered tolerant to salinity, can be an alternative crop for salt affected areas. To assess genotypic variation for vegetative-stage salinity tolerance, 195 broomcorn millet accessions from a core collection were evaluated for germination percentage, shoot length, and root length during germination in 8 mL of deionized water (control) or 8 mL of a 120 mmol L\(^{-1}\) salt solution (treatment). Six genotypes with different levels of salt tolerance were selected based on the growth parameters and ion concentrations in plant at the seedling stage and used for confirmation of the initial salinity response. Substantial variation for salinity tolerance was found on the basis of salt damage index [(germination percentage under control – germination percentage under salinity) / germination percentage under control \&bull; 100, SDI] and 39 accessions exhibited strong salt tolerance with SDI lower than 20%. The salt tolerance performance of the genotypes was generally consistent across experiments. In the seedling growth study, seedling number, root length and belowground biomass were adversely affected (showing more than 70%, 50%, and 32% reduction, respectively) in sensitive genotypes compared to tolerant genotypes (35%, 31%, and 3% reduction, respectively) under 160 mmol L\(^{-1}\) NaCl treatment. In general, whole-plant salinity tolerance was associated with increased Na\(^+\) concentration and Na\(^+\)/K\(^+\) ratio, and salt-tolerant genotypes often had higher root and lower shoot Na\(^+\) concentration than sensitive ones. Na\(^+\) concentration in root was closely related to salt tolerance and may be considered as a selection criterion for screening salt tolerance of broomcorn millet at the seedling or vegetative stages.

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**Abbreviations:** GP, germination percentage; RGP, relative germination percentage; SDI, salt damage index; RRL, relative root length; RSL, relative shoot length; CV, coefficient of variation.

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Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.
1. Introduction

Salinity is a major environmental factor adversely affecting plant growth and development, and severely reduces agricultural productivity and yield [1]. More than 6% of the world’s total land area and 20% of the irrigated land are salt-affected [2]. The salinity problem is particularly severe for arid and semiarid areas [3,4]. In China, there are 33 million acres of salinized cultivated land, distributed mainly in the northern interior of the Yangtze River Basin [5]. Minimizing the effects of salt on crop yield is necessary to maintain global food production for an increasing world population, which will increase to nine billion by 2050 [6]. Although different remedial and management methods, including reclamation and improved irrigation techniques, have been recommended to render salt-affected soil fit for agriculture, these methods are costly in terms of finance, energy use, and labor [7,8]. In addition, there is a need to improve salinity tolerance of important crops, because salt-tolerant crops have lower requirements for leaching of salt from the soil than do sensitive crops [9]. The development of salt-tolerant cultivars of staple crops is an effective approach to obtaining acceptable yields under moderately saline conditions [10,11].

Despite numerous efforts, few salt-tolerant genotypes have been released [12], owing to insufficient genetic knowledge of the tolerance traits, lack of effective selection criteria and evaluation methods, and poor understanding of the interaction between salinity and environment [13,14]. Strategies for salt tolerance selection have been proposed for many crops, including soybean (Glycine max Merr.), wheat (Triticum aestivum L.) and rice (Oryza sativa L.) [15–17]. The evaluation of salt-tolerant phenotypes under field conditions is very difficult, owing to high spatial and temporal variation [18]. For this reason, most screening experiments are conducted under controlled environmental conditions, such as in the greenhouse [19–22], based on plant vigor (germination rate and plant growth during early growth) or visual damage to vegetative tissues [23]. Screening for genetic diversity in physiological characters has been proposed and could be effective in salt tolerance breeding [12,24–29]. Ion uptake is a character of particular interest, and Na⁺ exclusion and grain K⁺/Na⁺ ratio have been suggested as reliable traits for salt-tolerant crop selection [21,30–32]. The lack of a single reproducible screening scheme and differential salinity sensitivities during various growth stages greatly limit breeding for salt tolerant varieties [33]. Seed germination and early seedling growth are crucial periods for crop cycles under salt stress [34], and determine the survival of plants. Several studies have shown that the salt tolerance of crops varies with growth stage [19,33,35–37]; for example, wheat is more salt tolerant during germination, flowering and grain filling stages than in seedling and vegetative stages [38]. However, differences in salt tolerance among crop genotypes may also occur at different growth stages [19,36]. The salt tolerance of different crop genotypes should thus be evaluated at different growth stages.

Broomcorn millet (Panicum miliaceum L.) is a seed crop that has been cultivated in China for more than 10,000 years [39], and has also been planted in India, central Europe, the USSR, and the Middle East [40–42]. This species grows at a wide range of altitudes with a short growth cycle of 10–12 weeks and requires little water for growth and development [43,44]. Broomcorn millet is considered a health-food crop, owing to its unique nutritional value, including higher grain alkaline protein content than wheat, rice and oat (Avena sativa L.) [45,46]. Broomcorn millet is more tolerant to salt stress than maize (Zea mays L.), wheat, rice, or foxtail millet (Setaria italica (L.) P. Beauv.) [47]. There is large genotypic variation in salt tolerance in broomcorn millet [48], suggesting that it possesses rich genetic resources for improving productivity in saline soil. However, the evaluation and identification of salt-tolerant genotypes lag behind efforts in other crops, and very few genes associated with salt tolerance have been found in broomcorn millet [25,48].

Broomcorn millet is currently planted mainly in the northern part of China, including the northwest and northeastern regions. Over 8500 accessions (varieties and landraces) of broomcorn millet are conserved in the National Centre for Crop Germplasm Conservation, Beijing, China. A core collection including 780 accessions has been established. In the present study, 195 accessions from the core collection were screened for salt tolerance by measurement of the germination salt damage index under mixed salt conditions, and the seedling performance of salt-tolerant and salt-sensitive genotypes selected according to germination-stage tolerance was reassessed under different salt stresses to validate the results from the germination stage and to investigate physiological traits for possible use as the salt-tolerance screening criteria for broomcorn millet. The aims of this study were to optimize the evaluation and identification of broomcorn millet as well as to identify promising genetic accessions from the core collection of broomcorn millet for the improvement of salt tolerance.

2. Materials and methods

2.1. Plant materials

A total of 195 accessions were selected from the core collection of broomcorn millet, including 90 landraces, 45 breeding lines, 20 commercial cultivars, 20 wild accessions, and 20 entries from other countries/organizations including Poland (2), Russia (2), India (2), Australia (1), France (1), Canada (1), ICRISAT (2), Japan (1), Hungary (1), and the USA (7). Seeds of all accessions were provided by the National Center for Crop Germplasm Conservation, Beijing, China.

2.2. Salt tolerance evaluation at germination stage in 195 accessions

Fifty seeds of each accession were surface-sterilized with 5% sodium hypochlorite for 20 min and germinated on filter paper in closed Petri dishes for 7 days in 8 mL deionized water (control) or in 8 mL of a 120 mmol L⁻¹ mixed (on a 1:1 molar basis) salt solution of NaCl and Na₂SO₄ (treatment) using a randomized complete block design with three replications in a growth chamber at 25/20 °C day/night with 12 h light. Seeds were considered as germinated when the plumule length
accounted for half of the seed length and the radicle length was equal to the seed length.

Germinated seeds were counted daily for each replicate and the growth parameters were calculated on the seventh day. According to the method of Wang and Wang [49], salt damage index (SDI, %) was adopted to evaluate the salt tolerance of broomcorn millet.

\[
\text{Salt damage index (SDI, \%)} = \left( \frac{GP_{\text{CK}} - GP_{\text{T}}}{GP_{\text{CK}}} \right) \times 100,
\]

where \( GP_{\text{CK}} \) represents germination percentage under the control condition and \( GP_{\text{T}} \) represents germination percentage under salt stress.

Accessions with SDI lower than or equal to 20.00% were considered as strongly tolerant, those with SDI between 20.01% and 40.00% as salt tolerant, those with SDI between 40.01% and 60.00% as intermediately tolerant genotypes, and those with SDI ranges of 60.01–80.00% and 80.00–100.00% as intermediate sensitive and sensitive, respectively. Seven days after germination, the seeds in the treatment regime were subjected to post-NaCl recovery by washing three times with deionized water, and allowed to germinate for an additional 5 days. Relative germination percentage (RGP), relative root length (RRL), and relative shoot length (RSL) were calculated as the ratios of the values under saline conditions to those under control conditions. Relative germination percentage (RRGP), relative shoot length (RRSL) and relative root length (RRRL) after recovery were calculated as the ratios of the values under recovery conditions to the values under control condition.

2.3. Salt tolerance of typical genotypes at seedling stage under a series of salt concentrations

Six broomcorn millet genotypes with differing salt tolerance at the germination stage were selected for determining the most appropriate salt concentration for further identifying seedling salt tolerance among genotypes and to evaluate ionic variation and distribution in different tissues under salt stress. Among these genotypes, Zhongwei Dahuangmi and Ningmi 4 were tolerant, with SDI between 40.01% and 60.00% as intermediately tolerant genotypes, and those with SDI ranges of 60.01–80.00% and 80.00–100.00% as intermediately sensitive and sensitive, respectively. Seven days after germination, the seeds in the treatment regime were subjected to post-NaCl recovery by washing three times with deionized water, and allowed to germinate for an additional 5 days. Relative germination percentage (RGP), relative root length (RRL), and relative shoot length (RSL) were calculated as the ratios of the values under saline conditions to those under control conditions. Relative germination percentage (RRGP), relative shoot length (RRSL) and relative root length (RRRL) after recovery were calculated as the ratios of the values under recovery conditions to the values under control condition.

2.4. Statistical analysis

Analyses of variance and correlation were performed for each measured or scored character using the statistical program SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The differences between genotypes were compared by a post hoc least significant difference (LSD) test at \( P < 0.05 \).

3. Results

3.1. Evaluation of salt tolerance of 195 broomcorn millet accessions at the germination stage

Forty accessions, including all entries from other countries that exhibited germination percentages lower than 80% under the control condition, were omitted to avoid confusion between poor seed germination and the effect of salt on early vegetative growth. The effects of salt stress on the germination parameters of 155 broomcorn millet accessions are shown in Table 1. The means of relative germination percentage and of relative shoot and root length were 36.7%, 42.2%, and 12.9%, respectively. These results indicated that salt stress strongly inhibited seed germination and plant growth of broomcorn millet during early vegetative growth. After 5 days in the post-NaCl recovery period, RGP, RSL and RRL were significantly increased, with means of 65.3%, 58.4%, and 55.4%, respectively. Among the broomcorn millet accessions tested, the coefficient of variation (CV) was the largest for the reduction in RGP and the lowest for RRRL. There was highly significant genotypic variation in the response of germination to salinity as measured by RGP and RSL, as well as by RRGP and RRRL after recovery (Table 1).

Based on salt damage indices, the 155 accessions examined were divided into five groups (Fig. 1). The first group, strongly tolerant genotypes, contained 39 accessions with salt damage indices lower than or equal to 20.00% (Table 2). The second, tolerant, group contained 22 accessions with salt damage indices between 20.01% and 40.00%. The third, moderately tolerant, group contained 26 accessions with salt damage indices between 40.01% and 60.00%. The fourth and fifth moderately sensitive and sensitive groups included 47 genotypes.

3.2. Effect of salt stress on seedling growth of P. miliaceum

Large genotypic variation was found for seedling growth under different levels of salt stress (Table 3). The percentage of surviving seedlings, a statistic directly reflecting the salt...
tolerance of genotypes, was significantly affected by salt concentration. The relative surviving seedling numbers of Zhongwei Dahuangmi, Ningmi 4, Huishu, Zigan Mizi, and Wahui Ruanmi were greater than 90% under 80 mmol L$^{-1}$ salt stress but only 10% under 200 mmol L$^{-1}$ salt stress.

The RSL markedly decreased with increasing salt concentration, but the trend in variation was different among genotypes. At 80 mmol L$^{-1}$ salt stress, the RSL of Zigan Mizi was the highest and that of Ningmi 4 was the lowest, with the six genotypes showing a mean value of 48.95%. With the increase in salt concentration, the decreases of Zigan Mizi and Yimen Yidianzong were greater than those of Zhongwei Dahuangmi and Ningmi 4. The former were 19.5% and 18.9% under 160 mmol L$^{-1}$ and 6.9% and 12.3% under 200 mmol L$^{-1}$ salt stress, respectively; while the latter were 29.4% and 20.0% under 160 mmol L$^{-1}$ and 20.8% and 16.3% under 200 mmol L$^{-1}$ salt stress, respectively.

The RRL of genotypes was greater than 100% under 80 mmol L$^{-1}$ mixed salt stress, indicating that low salt stress can stimulate root growth. With increasing salt stress, root growth began to be inhibited, and the RRL of most genotypes was lower than that of the control under 160 mmol L$^{-1}$ salt stress. However, a significant reduction in root growth was observed under 200 mmol L$^{-1}$ stress ($P < 0.05$), and the mean value of RRL was 22.2%. The variation in RRL among genotypes under salt stress was not significant.

The aboveground biomass decreased markedly with the increase in salt stress. The genotypic variation was significant for salt stress ranging from 160 to 200 mmol L$^{-1}$. Under 80 mmol L$^{-1}$ salt stress, Zigan Mizi had the highest relative biomass, whereas Huishu had the lowest and the mean of the six genotypes was 54.5%. When the salt concentration was 160 mmol L$^{-1}$, the aboveground biomass of Zigan Mizi significantly decreased and was only half of its original value under 80 mmol L$^{-1}$ salt stress. The decrease in aboveground biomass in other genotypes, except for Zhongwei Dahuangmi, was 10% lower than that in the control. Under high salt stress (200 mmol L$^{-1}$), the aboveground biomass of the sensitive genotypes Yimen Yidianzong and Wahui Ruanmi were significantly lower than that under low salt stress. For the tolerant genotypes, the aboveground biomass under 200 mmol L$^{-1}$ salt stress decreased slightly compared to that under 160 mmol L$^{-1}$ salt stress.

Low levels of salt stress had very little effect on belowground biomass and even stimulated root growth, as observed for Huishu and Wahui Ruanmi. However, when salt stress was increased to 160 mmol L$^{-1}$, the belowground biomasses of all genotypes were lower than those of the controls. With increased salt stress, the difference in belowground biomass among genotypes was significant ($P < 0.01$). The decrease in the belowground biomass of the tolerant genotypes was less than that of the sensitive genotypes.

### Table 1 - Growth characteristics at germination stage in 155 accessions of *Panicum miliaceum* under 120 mmol L$^{-1}$ salt stress.

<table>
<thead>
<tr>
<th>Germination index</th>
<th>Mean</th>
<th>Range of variation</th>
<th>Standard deviation</th>
<th>Coefficient of variation</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGP</td>
<td>36.7</td>
<td>3.0–97.9</td>
<td>34.4</td>
<td>93.7</td>
<td>8.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RSL</td>
<td>42.2</td>
<td>4.6–92.3</td>
<td>20.2</td>
<td>47.9</td>
<td>10.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RRL</td>
<td>12.9</td>
<td>1.3–75.1</td>
<td>9.6</td>
<td>74.4</td>
<td>1.38</td>
<td>0.042</td>
</tr>
<tr>
<td>RRGP</td>
<td>65.3</td>
<td>24.5–94.1</td>
<td>21.9</td>
<td>33.5</td>
<td>12.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RRSL</td>
<td>58.4</td>
<td>9.1–92.3</td>
<td>19.6</td>
<td>33.6</td>
<td>7.56</td>
<td>0.002</td>
</tr>
<tr>
<td>RRRL</td>
<td>55.4</td>
<td>12.5–87.5</td>
<td>18.1</td>
<td>32.7</td>
<td>1.24</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Values are expressed as percentages of the control. RGP, relative germination percentage; RSL, relative shoot length; RRL, relative root length; RRGP, relative germination percentage after recovery; RRSL, relative shoot length after recovery; RRRL, relative root length after recovery.
3.3. Ion change under different levels of salt stress

The Na\(^+\) concentrations in both shoots and roots increased with salt concentration (Table 4). When the salt stress was 80 mmol L\(^{-1}\), the mean concentration of Na\(^+\) was 0.036 mmol g\(^{-1}\) in shoots and 0.062 mmol g\(^{-1}\) in roots, and the values were not significantly increased compared to those of the control (0.027 and 0.074 mmol g\(^{-1}\), respectively). With further increase in salt stress, Na\(^+\) concentrations in shoots and roots significantly increased. When the salt stress was increased to 120 mmol L\(^{-1}\), the shoot Na\(^+\) concentration was 0.118 mmol g\(^{-1}\), and root Na\(^+\) concentration was 0.37 mmol g\(^{-1}\), approximately four- and sixfold higher, respectively, than those under the control condition. The Na\(^+\) concentration in root was greater than that in shoot, confirming that root was the first organ exposed to salt stress. The concentration of Na\(^+\) varied significantly among genotypes under salt stress. The shoot Na\(^+\) concentration in Yimen Yidianzong was 0.870 mmol g\(^{-1}\) and was significantly higher than that of Zhongwei Dahuangmi (0.297 mmol g\(^{-1}\)) at 200 mmol L\(^{-1}\) salt stress. However, the root Na\(^+\) concentration in Zhongwei Dahuangmi (2.363 mmol g\(^{-1}\)) was higher than that in Yimen Yidianzong (1.058 mmol g\(^{-1}\)). These results indicated that the Na\(^+\) concentration in roots was different among genotypes. The salt-tolerant genotypes usually accumulated more Na\(^+\) in roots than in shoots to reduce the transport of Na\(^+\) into aboveground parts and to avoid damage from salt stress. In contrast, the sensitive genotypes lacked this ability and often accumulated more Na\(^+\) in shoots than in roots.

Salt stress can reduce the K\(^+\) accumulation of shoot in broomcorn millet (Table 4). The mean decrease in K\(^+\) concentration in shoot under 80 mmol L\(^{-1}\) salt stress was 0.048 mmol g\(^{-1}\). When the salt concentration was greater than 120 mmol L\(^{-1}\), the variation of K\(^+\) concentration in the shoot was small. The effect of salt stress on the K\(^+\) concentration in root was small and not consistent. The K\(^+\) concentration among genotypes varied significantly under salt stress, and the tolerant genotypes often had higher values in root and lower values in shoot compared to the sensitive genotypes.

The Na\(^+\)/K\(^+\) ratio is important for the balance of ion concentration in plant cells under salt stress. We found that the Na\(^+\)/K\(^+\) ratio was increased not only in shoot but also in root with increasing salt stress (Fig. 2). Under 80 mmol L\(^{-1}\) salt
stress, the variation in shoot Na⁺/K⁺ ratio among the genotypes was not significant. When the salt stress was increased to 200 mmol L⁻¹, the Na⁺/K⁺ ratios of the sensitive genotypes were much higher than those of the tolerant genotypes. The variation of Na⁺/K⁺ ratio in root differed from that in shoot, and the tolerant genotypes usually had higher Na⁺/K⁺ ratios than the sensitive genotypes. The results indicated that the tolerant genotypes had greater Na⁺ restriction than the sensitive genotypes and that this restriction increased with salt stress (Fig. 3).

**Table 3 – Effects of salt treatment on seedling traits of Panicum miliaceum L.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NaCl treatment</th>
<th>Percentage surviving seedlings</th>
<th>Relative root length</th>
<th>Relative shoot length</th>
<th>Relative biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 mmol L⁻¹</td>
<td>96.5</td>
<td>127.8</td>
<td>44.7</td>
<td>61.6</td>
</tr>
<tr>
<td>Zhongwei</td>
<td>120 mmol L⁻¹</td>
<td>79.9</td>
<td>95.4</td>
<td>25.4</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td>160 mmol L⁻¹</td>
<td>66.5</td>
<td>62.4</td>
<td>14.1</td>
<td>28.2</td>
</tr>
<tr>
<td></td>
<td>200 mmol L⁻¹</td>
<td>10.4</td>
<td>17.7</td>
<td>14.3</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>80 mmol L⁻¹</td>
<td>98.5</td>
<td>170.4</td>
<td>53.5</td>
<td>51.8</td>
</tr>
<tr>
<td>Ningmi 4</td>
<td>120 mmol L⁻¹</td>
<td>82.6</td>
<td>109.7</td>
<td>45.5</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
<td>160 mmol L⁻¹</td>
<td>61.0</td>
<td>75.1</td>
<td>20.6</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>200 mmol L⁻¹</td>
<td>7.0</td>
<td>29.2</td>
<td>18.5</td>
<td>34.9</td>
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<tr>
<td>Huishu</td>
<td>80 mmol L⁻¹</td>
<td>96.5</td>
<td>137.7</td>
<td>39.6</td>
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<td></td>
<td>120 mmol L⁻¹</td>
<td>74.4</td>
<td>97.4</td>
<td>33.5</td>
<td>36.1</td>
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<td>160 mmol L⁻¹</td>
<td>45.0</td>
<td>83.0</td>
<td>29.4</td>
<td>44.4</td>
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<td></td>
<td>200 mmol L⁻¹</td>
<td>1.5</td>
<td>25.5</td>
<td>20.8</td>
<td>37.5</td>
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<tr>
<td>Zigan Mizi</td>
<td>80 mmol L⁻¹</td>
<td>98.5</td>
<td>202.1</td>
<td>62.1</td>
<td>48.1</td>
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<tr>
<td></td>
<td>120 mmol L⁻¹</td>
<td>81.4</td>
<td>132.5</td>
<td>48.6</td>
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<td>56.5</td>
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<td>76.5</td>
<td>175.4</td>
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<td></td>
<td>120 mmol L⁻¹</td>
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<td></td>
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<td>1.2</td>
<td>21.1</td>
<td>6.9</td>
<td>20.2</td>
</tr>
<tr>
<td>Wahui Ruanmi</td>
<td>80 mmol L⁻¹</td>
<td>93.0</td>
<td>111.6</td>
<td>28.0</td>
<td>49.4</td>
</tr>
<tr>
<td></td>
<td>120 mmol L⁻¹</td>
<td>69.2</td>
<td>102.2</td>
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<td>35.5</td>
<td>101.9</td>
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<td></td>
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<td>16.3</td>
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<tr>
<td>LSD0.05</td>
<td></td>
<td>18.94</td>
<td>76.28</td>
<td>17.38</td>
<td>9.98</td>
</tr>
</tbody>
</table>

Relative value was calculated as the ratio of the value under saline conditions to that under control conditions.

3.4. Correlations among salt damage index, growth parameters, and ion concentration at the seedling stage

The relationships among salt damage index, growth parameters and ion concentration at the seedling stage were analyzed as pairwise correlation coefficients (Table 5). There were significant negative correlations between salt damage index and percentage survival seedlings (P = 0.001), root Na⁺ concentration (P = 0.029) and Na⁺ restriction (P = 0.008), as well as significant positive correlations with aboveground biomass (P = 0.001), shoot Na⁺ concentration (P = 0.007) and shoot Na⁺/K⁺ ratio (P = 0.012). Shoot Na⁺ concentration exhibited the highest levels of correlation with most parameters examined, including a significant positive correlation with salt damage index and aboveground biomass and a significant negative correlation with survival seedling number, root K⁺ concentration, shoot Na⁺/K⁺ ratio and Na⁺ restriction. Shoot Na⁺/K⁺ ratio exhibited close correlations with the parameters of seedlings and salt damage index during germination, including significant negative and positive correlations with survival seedling number and aboveground biomass, respectively, and a significant positive correlation with belowground biomass.

4. Discussion

The results of this study indicated that salt stress caused a substantial decrease in germination rate of all broomcorn millet accessions, but that the accessions differed significantly in their germination rates under saline conditions. Such a variable response of genotypes to salt stress has been previously described in different crops [25,50,51]. A total of 39 entries, accounting for 25.2% of all the tested entries, were classified as strongly salt-tolerant in this study. These entries will be useful for salt-tolerance improvement in broomcorn millet.

Many crops are sensitive to salt stress at the seedling stage [52]. Seedling number strongly affects yield by reducing plant density. For this reason, the inclusion of seedling growth parameters in evaluation of salt tolerance is necessary for improving crop production under salinity. There were significant differences among genotypes in two parameters (RAB and RBB) under 80 mmol L⁻¹, three (RSL, RAB, and RBB) under 120 mmol L⁻¹, and four (PSS, RSL, RAB, and RBB) under 160 mmol L⁻¹ (Table 3). So the most efficient parameters for evaluation of seedling salt tolerance in broomcorn millet are aboveground and belowground biomass. The seedling salt tolerance evaluation showed that salt-tolerant genotypes such as Ningmi 4...
and Zhongwei Dahuangmi often had higher survival rates than salt-sensitive genotypes, especially under high salt stress. Correlation analysis showed that survival rate was negatively correlated with Na+ concentration among genotypes \((R^2 = -0.80, P = 0.001)\) but aboveground biomass positively \((R^2 = 0.77, P = 0.029)\) correlated with salt damage index.

Under saline conditions, plants usually accumulate large amounts of Na+ in vacuoles for osmoregulation \([53]\). Salt tolerance is associated with the low accumulation of Na+ \([54]\) and the partial exclusion and compartmentalization of salt in cells \([3]\). Na+ is the main toxic ion under salinized conditions, and the partial exclusion and compartmentalization of salt in cells \([53]\). Salt stress not only imposes osmotic and ion toxicity on plants but also affects uptake and transport of essential nutrients, such as K+ \([56]\). A decrease in K+ with an increase in Na+ in plant shoots has been reported \([57-59]\). In addition, the capacity to concentrate K+ in response to salinity stress is accompanied by reduced growth and accordingly does not represent adaptation to salt stress \([60]\). Our results for the relationship between Na+ and K+ are consistent with those reported earlier. However, K+ concentrations in shoot and root showed no obvious variation under different salt conditions. There are two reasons for the decrease in K+ concentration. First, the competition between Na+ and K+ to enter the membrane channels reduces the absorption of K+ under salt stress. Second, a large amount of Na+ accumulates in the cell, destroying the membrane and resulting in a great loss of K+ in the membrane \([61]\). Identifying the reason for the decrease in

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NaCl treatment</th>
<th>Na+ concentration (mmol g(^{-1}) DW)</th>
<th>K+ concentration (mmol g(^{-1}) DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seedling shoot</td>
<td>Seedling root</td>
<td>Seedling shoot</td>
</tr>
<tr>
<td>Zhongwei Dahuangmi</td>
<td>Control</td>
<td>0.027</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>80 mmol L(^{-1})</td>
<td>0.036</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>120 mmol L(^{-1})</td>
<td>0.129</td>
<td>0.482</td>
</tr>
<tr>
<td></td>
<td>160 mmol L(^{-1})</td>
<td>0.204</td>
<td>1.547</td>
</tr>
<tr>
<td></td>
<td>200 mmol L(^{-1})</td>
<td>0.297</td>
<td>2.363</td>
</tr>
<tr>
<td>Ningmi 4</td>
<td>Control</td>
<td>0.040</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>80 mmol L(^{-1})</td>
<td>0.054</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>120 mmol L(^{-1})</td>
<td>0.108</td>
<td>0.593</td>
</tr>
<tr>
<td></td>
<td>160 mmol L(^{-1})</td>
<td>0.200</td>
<td>1.782</td>
</tr>
<tr>
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<td>0.280</td>
<td>2.194</td>
</tr>
<tr>
<td>Huishu</td>
<td>Control</td>
<td>0.019</td>
<td>0.044</td>
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<tr>
<td></td>
<td>80 mmol L(^{-1})</td>
<td>0.031</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>120 mmol L(^{-1})</td>
<td>0.098</td>
<td>0.102</td>
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<tr>
<td></td>
<td>160 mmol L(^{-1})</td>
<td>0.334</td>
<td>0.952</td>
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<tr>
<td></td>
<td>200 mmol L(^{-1})</td>
<td>0.479</td>
<td>1.123</td>
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<td>Zigan Mizi</td>
<td>Control</td>
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<tr>
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<td>0.049</td>
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<td>120 mmol L(^{-1})</td>
<td>0.105</td>
<td>0.223</td>
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<td>160 mmol L(^{-1})</td>
<td>0.347</td>
<td>0.883</td>
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<td></td>
<td>200 mmol L(^{-1})</td>
<td>0.412</td>
<td>2.361</td>
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<tr>
<td>Yimen Yidianzong</td>
<td>Control</td>
<td>0.022</td>
<td>0.028</td>
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<td></td>
<td>80 mmol L(^{-1})</td>
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<td>120 mmol L(^{-1})</td>
<td>0.114</td>
<td>0.516</td>
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<td>160 mmol L(^{-1})</td>
<td>0.299</td>
<td>1.712</td>
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<tr>
<td></td>
<td>200 mmol L(^{-1})</td>
<td>0.870</td>
<td>1.058</td>
</tr>
<tr>
<td>Wahui Ruanmi</td>
<td>Control</td>
<td>0.035</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>80 mmol L(^{-1})</td>
<td>0.041</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>120 mmol L(^{-1})</td>
<td>0.155</td>
<td>0.306</td>
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<tr>
<td></td>
<td>160 mmol L(^{-1})</td>
<td>0.241</td>
<td>1.079</td>
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<tr>
<td></td>
<td>200 mmol L(^{-1})</td>
<td>0.252</td>
<td>1.553</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>0.0508</td>
<td>0.2132</td>
<td>0.2307</td>
</tr>
</tbody>
</table>

Control: treated with deionized water.
K+ in broomcorn millet awaits further study by noninvasive micro-test techniques (NMT) for detecting K+ flux in roots and shoots.

A low Na+/K+ ratio in cytoplasm is essential for maintenance of several enzymatic processes [62]. El-Hendawy et al. [7] found that the order of wheat genotypes for Na+/K+ ratio in the upper and lower two leaves of the main stem under different conditions correlated well with their salt-tolerance ranking. This result suggests that the Na+/K+ ratio would be a valuable selection criterion for screening salt-tolerant genotypes under different conditions. In our study, the Na+/K+ ratios of salt-tolerant genotypes such as Zhongwei Dahuangmi and Ningmi 4 were significantly lower than those of sensitive genotypes such as Yimen Yidianzong and Wahui Ruanmi. Thus, the Na+/K+ ratio clearly increased with salt concentration.

The Na+/K+ ratio in roots was 2.5–6.5-fold higher than that in shoots under 80 mmol L−1 stress and 5.2–14.6-fold higher under 240 mmol L−1 (Table 4). The Na+/K+ ratio in shoot was significantly correlated with salt damage index (R² = 0.64, P = 0.020), belowground biomass (R² = 0.62, P = 0.026), percentage survival seedlings (R² = −0.749, P = 0.010) and aboveground biomass (R² = 0.865, P < 0.001). These results confirmed the importance of Na+/K+ selectivity in the salt tolerance of broomcorn millet.

5. Conclusion

Clear variation in salt tolerance was observed at the germination and seedling stages in 155 broomcorn millet accessions, including 39 entries that were salt-tolerant according to
a salt damage index. These salt-tolerant entries usually showed higher survival rates of seedlings and greater belowground biomass than sensitive entries when exposed to salt stress. Salt damage index and seedling survival rate were highly correlated with Na⁺ concentration and Na⁺/K⁺ ratio in shoot, indices that were proposed as potential criteria for identification of salinity tolerance in broomcorn millet.

**Acknowledgments**

This research was supported by the National Millet Crops Research and Development System (CARS-07-12[1].S-A1) and the National Key Technology R&D Program of China (2013BAD01B05-2).

**References**


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**Table 5 – Correlation coefficients among salt damage index, growth parameters, and ion accumulation during seedling stage.**

<table>
<thead>
<tr>
<th>SDI</th>
<th>PSS</th>
<th>RSL</th>
<th>RRL</th>
<th>RAB</th>
<th>RBB</th>
<th>SN</th>
<th>RN</th>
<th>SK</th>
<th>RK</th>
<th>SNK</th>
<th>RNK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−0.80**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSS</td>
<td>0.12</td>
<td>0.28</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RSL</td>
<td>0.29</td>
<td>−0.35</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RRL</td>
<td>0.77**</td>
<td>−0.79**</td>
<td>0.13</td>
<td>0.37</td>
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<tr>
<td>RAB</td>
<td>0.29</td>
<td>−0.43</td>
<td>−0.10</td>
<td>0.13</td>
<td>0.39</td>
<td></td>
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<tr>
<td>RBB</td>
<td>0.92</td>
<td>−0.80**</td>
<td>0.05</td>
<td>0.20</td>
<td>0.83**</td>
<td>0.27</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RN</td>
<td>−0.65*</td>
<td>0.22</td>
<td>−0.49</td>
<td>0.13</td>
<td>−0.34</td>
<td>−0.12</td>
<td>−0.54</td>
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<tr>
<td>LK</td>
<td>0.16</td>
<td>0.50</td>
<td>0.02</td>
<td>0.03</td>
<td>−0.57</td>
<td>−0.32</td>
<td>−0.35</td>
<td>−0.23</td>
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<tr>
<td>RK</td>
<td>−0.84**</td>
<td>0.88**</td>
<td>0.05</td>
<td>−0.17</td>
<td>−0.83**</td>
<td>−0.49</td>
<td>−0.76**</td>
<td>0.52</td>
<td>0.36</td>
<td></td>
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</tr>
<tr>
<td>LNK</td>
<td>0.64</td>
<td>−0.75**</td>
<td>0.09</td>
<td>0.03</td>
<td>0.87**</td>
<td>0.62</td>
<td>−0.78**</td>
<td>−0.34</td>
<td>−0.62**</td>
<td>−0.80**</td>
<td></td>
</tr>
<tr>
<td>RNK</td>
<td>0.10</td>
<td>−0.59*</td>
<td>−0.66*</td>
<td>0.31</td>
<td>0.37</td>
<td>0.27</td>
<td>0.13</td>
<td>0.61*</td>
<td>−0.52</td>
<td>0.36</td>
<td>0.34</td>
</tr>
<tr>
<td>NR</td>
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<td>−0.24</td>
<td>−0.70*</td>
<td>0.89**</td>
<td>0.23</td>
<td>0.65*</td>
<td>−0.53</td>
</tr>
</tbody>
</table>

SDI, salt damage index; PSS, percentage surviving seedlings; RSL, relative shoot length; RRL, relative root length; RAB, relative aboveground biomass; RBB, relative belowground biomass; SN, shoot Na⁺ accumulation; RN, root Na⁺ accumulation; SK, shoot K⁺ accumulation; RK, root K⁺ accumulation; SNK, Na⁺/K⁺ ratio in shoot; RNK, Na⁺/K⁺ ratio in root; NR, Na⁺ restriction.

* Significant at P < 0.05.
** Significant at P < 0.01.


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