Effect of saline stress on the physiology and growth of maize hybrids and their related inbred lines

Meijie Luo[1,†], Yanxin Zhao[1,†], Weisong[1], Ruyang Zhang[1], Aiguo Su[1], Chunhui Li[1], Xiangpeng Wang[1], Jinfeng Xing[1], Zi Shi[1,‡*], Jiuran Zhao[1,‡*]

[1]Beijing Key Laboratory of Maize DNA Fingerprinting and Molecular Breeding, Maize Research Center, Beijing Academy of Agricultural and Forest Sciences, Shuguang Garden Middle Road N° 9, Haidian District, Beijing 100097, People's Republic of China

†These authors contributed equally to this work. ‡These authors also contributed equally to this work.
*Corresponding author: E-mail: shizi_baafs@126.com; maizezhao@126.com

Abstract

Salinity is one major abiotic stress that restrict plant growth and crop productivity. In maize (*Zea mays* L), salt stress causes significant yield loss each year. However, indices of maize response to salt stress are not completely explored and a desired method for maize salt tolerance evaluation is still not established. A Chinese leading maize variety Jingke968 showed various resistance to environmental factors, including salt stress. To compare its salt tolerance to other superior maize varieties, we examined the physiological and growth responses of three important maize hybrids and their related inbred lines under the control and salt stress conditions. By comparing the physiological parameters under control and salt treatment, we demonstrated that different salt tolerance mechanisms may be involved in different genotypes, such as the elevation of superoxide dismutase activity and/or proline content. With Principal Component Analysis of all the growth indicators in both germination and seedling stages, along with the germination rate, superoxide dismutase activity, proline content, malondialdehyde content, relative electrolyte leakage, we were able to show that salt resistance levels of hybrids and their related inbred lines were Jingke968 > Zhengdan958 > X1132 and X1132M > Jing724 > Chang7-2 > Zheng58 > X1132F, respectively, which was consistent with the saline field observation. Our results not only contribute to a better understanding of salt stress response in three important hybrids and their related inbred lines, but also this evaluation system might be applied for an accurate assessment of salt resistance in other germplasms and breeding materials

Keywords: maize, Jingke968, salt stress evaluation, Principal Component Analysis

Abbreviations: GR - Germination rate, SOD - Superoxide dismutase; Pro - Proline, MDA - Malondialdehyde, REL - Relative electrolyte leakage, SFW - Shoot fresh weight, SDW - Shoot dry weight, SL - Shoot length, RFW - Root fresh weight, RDW - Root dry weight, RL - Root length, ROS - reactive oxygen species, NBT - Nitro blue tetrazolium, TBA - Thiobarbituric acid, STI - Salinity tolerance index, PCA - Principal component analysis, CSTI - Comprehensive salt tolerance index, PC - Principal component Analysis

Introduction

Salinity is one major abiotic stress that seriously limits plant growth and productivity throughout the world (Chinnusamy et al, 2005; Deinlein et al, 2014; Parida et al, 2009; Shabala et al, 2014). Till now, over 800 million hectares of land have been affected by salinity, accounting for more than 6% of the world's total land area (Munns and Tester, 2008). Moreover, the amount of salt-affected land continues to increase due to the persistent non-sustainable farming practices. Hence, developing and breeding salt tolerant crops are essential for sustaining crop production.

Plants respond to salt stress through a series regulation of physiological and morphological adaptations (Bhaskar Gupta, 2014; Deinlein et al, 2014; Shabala et al, 2014). As a result of osmotic and ionic effects, plants accumulate various antioxidant enzymes and solute induced by salt stress to improve salt resistance. Pro is an effective osmotic adjustment agent, which accumulates in plant to maintain water uptake and regulate physiological metabolism, and to protect cells from damage (Gangopadhyay et al, 2000; Gao et al, 2014). Salt stress results in overproduction of ROS, leading to oxidative damage to plant cells (Yu et al, 2015), which triggers antioxidant defense system and results in the a rise of SOD (EC 1.15.1.1) enzyme activity, and these antioxidant enzymes can effectively remove ROS and alleviate oxidative damage (Bhatia et al, 2010). In addition, many physiological factors are used to assess the severity of salt stress, including MDA and REL. MDA is the product of membrane lipid peroxidation, which will seriously damage the biological membrane (Miao et al, 2010; Yazici et al, 2007). REL is an important indicator to measure the permeability of cell membrane, and the greater the value, the more the leakage of the

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electrolyte is together with the more the cell membrane is affected (Cui et al, 2015). Salinity also impairs seed germination, retards plant development and reduces grain yield (Gao et al, 2014). However, these responses vary in different genotypes.

Maize is an important food, feed and economic crop in most part of the world. Unfortunately, maize is a salt-sensitive crop with the salt tolerance limit of 1.02 ‰. Maize grain yield would reduce by 12% with every 0.6‰ increase in salt concentration (Zheng et al, 2010). Maize shows the most sensitivity to salt at germination and seedling stages. Salt tolerance evaluation of important maize hybrids in agricultural production and their related inbred line from their response to salt stress is a critical step for maize breeding. Growth and physiological parameters in seedling stage are frequently used for salt tolerance evaluation in Maize (Giaveno et al, 2007).

In the present study, we selected three major leading maize varieties Jingke968 (Wang et al, 2016), Zhengdan958 (Du et al, 2006) and X1132, all of which possess many desired traits such as high quality, high productivity, and wide resistance, as well as their related inbred lines. However, information regarding the effects of salt stress on those hybrids and their related inbred lines have not been specifically studied. Here, we analyzed the effect of salinity stress on the physiology and growth of the three important maize hybrids and their related inbred lines at different growth stages, and their salt tolerance levels were comprehensively evaluated by the principal component analysis. These results provided a better understanding and a more integrated picture of phenotypic response to salt stress and shed light on a useful salt tolerance evaluation approach in maize.

Materials and Methods

Plant materials and treatments

Local maize (*Zea mays* L) cultivar Jingke968 and its female parent inbred line Jing724, local maize cultivar Zhengdan958 and its parental inbred lines Zheng58 (female parent) and Chang7-2 (male parent), foreign maize cultivar X1132 and its parental inbred lines X1132M and X1132F, and inbred line B73 were collected from germplasm bank of Maize Research Center, Beijing Academy of Agriculture and Forestry Sciences, China. These three cultivars used in this study have been largely grown in China because of its broad stress resistance. Seeds were sown and hydroponically cultured in maize seedling identifying instrument (Chinese patent, patent number: ZL200920177285.0) in Hoagland's nutrient solution (Phyto Technology Laboratories Co Ltd, USA) under long day condition (12 light/12 dark, 150-180 μmol m^{-2} s^{-1}, 26 ± 1°C, 70% humility). For salt treatment in germination stage, seeds were sown in Hoagland medium with no (control) and 100 mM NaCl (Coolaber Co Ltd, Beijing, China) (stress), and cultured for seven days. For salt treatment in seedling stage, three-leaf maize seedlings were cultured in Hoagland medium containing no (control) and 100 mM NaCl (Stress), and shoot and root samples were collected for analysis after seven days.

Physiological assays

Activities of SOD were measured by nitro blue tetrazolium (NBT) photo reduction method (Beauchamp and Fridovich, 1971). Free Pro content of seedling shoot was extracted and quantified following the ninhydrin-based colorimetric assays (Hu et al, 1992). MDA content was determined by the TBA assay (Schmedes and Hølmer, 1989). REL assay was conducted according to the method described previously (Liu et al, 2012). All the physiological assays were performed with six biological replicates for each treatment.

Growth parameter measurements

In order to determine GR, thirty maize seeds were cultured in control and in saline condition for seven days with two biological replicates for each treatment. Seed was recorded as germinated when both radicle and plumule had emerged, and the plumule length reached more than 1/2 of seed length. Seed germination number was counted seven days after culture and GR was calculated using the following formula: GR = (seed germination number / total seed number) × 100%. After seeds were cultured in control and in salt treatment for seven days after culturing, SFW, SL, RFW, and RL were measured with six biological replicates each with five seedlings for both control and salt treatment. After dried at 80°C for three days, the SDW and RDW of seedlings in germination stage were measured. For growth parameters analysis in seedling stage, three-leaf seedlings were cultured in control and in saline condition for seven days. Then SL, RFW, and RL were determined using four biological replicates each with five seedlings for each treatment. SDW and RDW of seedlings in seedling stage were determined after dried at 80°C for three days.

Data analysis

STI of GR, SOD, Pro, MDA, REL, SFW, SDW, SL, RFW, RDW, and RL were calculated using the following formulas (Yang et al, 2011):

Formula 1: GR_{STI}, SFW_{STI}, SDW_{STI}, SL_{STI}, RFW_{STI}, RDW_{STI}, and RL_{STI} = value measured in salt stress / value measured in control

Formula 2: SOD_{STI} = (SOD activity in salt stress - SOD activity in control) / SOD activity in control

Formula 3: Pro_{STI} = (Pro content in salt stress - Pro content in control) / Pro content in control

Formula 4: MDA_{STI} = 1 - (MDA content in salt stress - MDA content in control) / MDA content in control

Formula 5: REL_{STI} = 1 - (REL in salt stress - REL in control) / REL in control

For comprehensive evaluation of salt tolerance level, PCA was performed based on the STI of all physiological and growth traits using SPSS software.

Differences in the average of physiological and growth parameters were compared by two-way ANOVA and Bonferroni post-tests using GraphPad Prism software. Values are shown as means ± SEM. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$.

The principal components (PC) of the PCA analysis were calculated using the following equation:

$$PC_i = \sum_{j=1}^{m} (\textit{loading}\ \text{matrix})_{ij} * STI_{ij}$$

where i = 1, 2, 3… and j = GR, SOD, Pro, MDA, REL, $SFW_{\text{(seedling stage)}}$, $SDW_{\text{(seedling stage)}}$, $SL_{\text{(seedling stage)}}$, $RFW_{\text{(seedling stage)}}$, $RDW_{\text{(seedling stage)}}$, $RL_{\text{(seedling stage)}}$, $SFW_{\text{(germination stage)}}$, $SDW_{\text{(germination stage)}}$, $SL_{\text{(germination stage)}}$, $RFW_{\text{(germination stage)}}$, $RDW_{\text{(germination stage)}}$, $RL_{\text{(germination stage)}}$.

To determine the comprehensive STI (CSTI) for each genotype using the principal components, the following formula was employed:

$$CSTI = \sum_{k=1}^{n} (\textit{contribution}\ \text{rate})_k * PC_i$$

where k = PC1, PC2, PC3.

Results

GR and physiological responses of maize hybrids to saline stress

To determine the effect of salt stress on the physiology of maize hybrids, we investigated GR, SOD activity, Pro content, MDA content, and REL in control and salt treatment (Figure 1A-E). Generally, GR and REL did not show significant difference among three maize hybrids (Figure 1A, E). In X1132, salt stress led to a significant increase in Pro concentration ($p < 0.001$) and no significant difference was observed in SOD activity and MDA content (Figure 1B-D). In contrast, for Jingke968, salt stress significantly increased SOD activity ($p < 0.001$) and significantly decreased Pro ($p < 0.05$) and MDA content ($p < 0.001$) (Figure 1B-D). Similarly, for Zhengdan958, SOD activity was substantially increased ($p < 0.05$), and MDA content was significantly decreased ($p < 0.001$) by salt treatment, while Pro content didn't significantly change compared to control (Figure 1B-D).

GR and physiological responses of inbred lines to saline stress

The changes of GR, SOD activity, REL and Pro and MDA concentrations were also evaluated in control and salt stressed six maize inbred lines (Figure 1F-J). Maize inbred lines X1132M, Jing724, and Zheng58 are female parent of X1132, Jingke968, and Zhengdan958, respectively, and maize inbred lines X1132F and Chang7-2 are the male parents. In all of these inbred lines, GR did not significantly change compared to the control (Figure 1F). In X1132M and Chang7-2, salt stress led to a substantial increase in Pro content ($p < 0.05$ and $p < 0.001$, respectively) and no significant difference was observed in SOD activity, MDA content and REL (Figure 1F-J). In X1132F, except for GR, salt stress resulted in a significant elevation in all of these parameters ($p < 0.001$ for SOD, Pro and MDA; $p < 0.01$ for REL) (Figure 1F-J). In B73 and Jing724, SOD activity was significantly increased with the salt treatment ($p < 0.001$) but the Pro content, MDA content and REL stayed the same (Figure 1G-J). In Zheng58, REL was significantly increased ($p < 0.001$) while SOD activity, MDA content and Pro content did not vary compared to control (Figure 1G-J).

Effect of saline stress on growth parameters of maize hybrids in the germination stage

To study the effects of salt stress on growth parameters of three maize hybrids in germination stage, SFW, SDW, SL, RFW, RDW and RL of three maize hybrids were measured, and all of them exhibited a significant decrease upon saline solution treatment ($p < 0.001$) (Figure 2A-F). In order to evaluate the inhibition degree of growth in maize hybrids, we compared the growth parameters between salt treatments and their control counterparts and calculated their percentage values. The higher the percentage value was, the represents less degree of growth inhibition was.

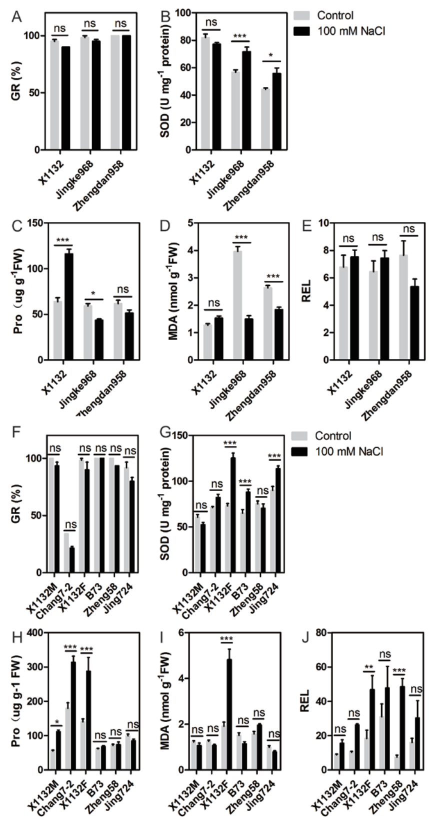

Figure 1 - GR and physiological analysis of maize hybrids and their related inbred lines under control and saline conditions. (A) GR, (B) SOD activity, (C) Pro content, (D) MDA content, and (E) REL. For germination rate, the bar charts represent Mean± SE of two biological replicates each with 30 seeds. For other parameters, the data represent Mean ± SE of six biological replicates. * represents the level of significance of 0.05, ** 0.01, and *** 0.001, respectively.

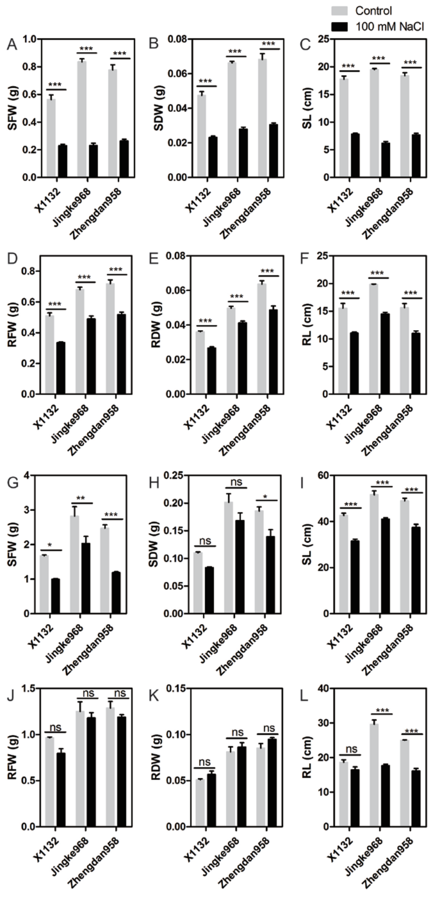

Figure 2 - Growth response of maize hybrids to salt stress in the germination and seedling stages. (A) SFW, (B) SDW, (C) SL, (D) RFW, (E) RDW, and (F) RL. The bar charts represent mean ± SE of six biological replicates each with five seedlings for all parameters for the germination stage or four biological replicates for the seedling stage. * represents the level of significance of 0.05, ** 0.01, and *** 0.001, respectively.

As shown in Table 1, the inhibition order of SFW, SDW and SL in the three hybrids during the germination stage was X1132 < Zhengdan958 < Jingke968, while it was Jingke968 < Zhengdan958 < X1132 for RFW, RDW and RL. In addition, results revealed that growth parameters of shoot were more severely affected than those of root upon salt stress.

Effect of saline stress on growth parameters of maize hybrids in seedling stage

Effects of salt stress on growth parameters of three maize hybrids in seedling stage were also investigated. In general, salt stress significantly decreased SFW and SL but no significant change in the RFW and RDW of three maize hybrids was observed (Figure 2G, I, J, K). In X1132, salt stress did not affect SDW and RL (Figure 2H, L), but they were substantially reduced in Zhengdan958 (Figure 2H, L). In addition, RL was significantly decreased upon salt stress in Jingke968, but SDW wasn't affected (Figure 2H, L).

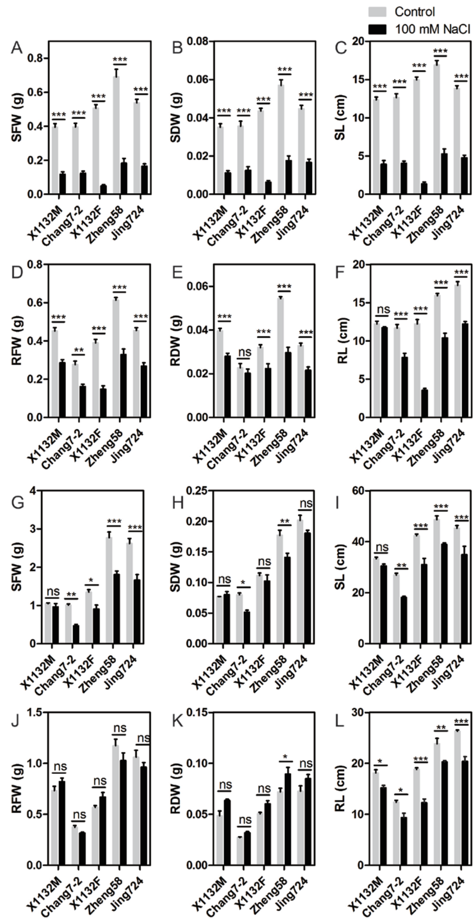

Figure 3 - Growth response of maize inbred lines to salt stress in the germination and the seedling stage. (A) SFW, (B) SDW, (C) SL, (D) RFW, (E) RDW, and (F) RL. Data represent mean ± SE of six biological replicates each with five seedlings for all parameters for the germination stage or four biological replicates for the seedling stage. * represents the level of significance of 0.05, ** 0.01, and *** 0.001, respectively.

Growth parameters between salt applications and their control counterparts were compared and their percentage values were calculated to illustrate the growth inhibition degree. As shown in Table 1, the inhibition order of SFW, SDW, and SL among the three hybrids in seedling stage was Jingke968 < X1132 < Zhengdan958, however, the inhibition degree of RFW, RDW and RL among three hybrids was not consistent. For RDW and RL, the inhibition order was X1132 < Zhengdan958 < Jingke968, while it was Jingke968 < Zhengdan958 < X1132 for RFW. Similar to the germination stage, the shoot was more sensitive to salt stress than the root during the seedling stage, but the susceptibility was reduced compared with the germination stage.

Effect of saline stress on growth parameters of maize inbred lines in germination and seedling stage

Table 1 - Effect of salt stress on the growth of maize hybrids and their related inbred lines.

Maize lines		SFW*(%)	SDW*(%)	SL*(%)	RFW*(%)	RDW*(%)	RL*(%)
Germination stage							
Hybrids	X1132	40.9	48.8	44.2	66.0	74.0	70.9
	Jingke968	27.6	42.1	31.6	71.9	83.1	73.1
	Zhengdan958	34.0	44.7	41.6	72.1	76.3	70.3
Inbred lines	X1132M	29.5	31.7	31.8	63.2	70	95.8
	Chang7-2	31.1	35.1	31.9	58.4	89.0	67.1
	X1132F	9.6	14.3	9.0	37.8	68.8	29.3
	Zheng58	26.3	30.8	31.3	53.6	55.1	65.3
	Jing724	30.8	37.1	34.7	59.3	65.9	70.5
Seedling stage							
Hybrids	X1132	59.4	75.8	74.0	82.5	111.1	88.1
	Jingke968	72.0	83.6	79.6	94.4	106.4	59.7
	Zhengdan958	48.1	75.1	76.5	92.4	111.1	64.6
Inbred lines	X1132M	91.8	104.8	91.5	112.1	130.8	83.9
	Chang7-2	46.3	64.2	67.5	85.0	117.1	75.7
	X1132F	67.4	91.7	73.0	117.7	119.3	65.3
	Zheng58	65.4	79.7	80.1	87.5	124.3	85.1
	Jing724	63.5	89.7	77.2	90.8	117	77.7

*displayed value = (observed value in salt treatment / observed value in control) ×100%

To demonstrate the salt sensitivity of the parent inbred lines of three hybrids, the effect of salt stress on growth parameters of five inbred lines in germination stage and in seedling stage were also studied. In germination stage, except for RDW of Chang7-2 and RL of X1132M, salt stress led to a significant decrease in all growth parameters (Supplementary Figure 1 and Figure 3A-F). As shown in Table 1, the inhibition order of SFW, SDW and SL among the five inbred lines was Jing724 < Chang7-2 < X1132M < Zheng58 < X1132F, it was X1132M < Jing724 < Chang7-2 < Zheng58 < X1132F for RFW and RL, and Chang7-2 < X1132M < X1132F < Jing724 < Zheng58 for RDW. In addition, as in the hybrid, root was much more resistant than the shoot in the inbred lines.

In seedling stage, RFW was not substantially altered in all the tested inbred lines by salt stress (Figure 3J). In X1132M, only RL was significantly reduced by salt stress (Figure 3L), but all growth parameters, except for RFW, were significantly affected by salt stress in Zheng58 (Figure 3G-L). In Chang7-2, SFW, SDW, SL and RL were significantly reduced by salt stress (Figure 3G-I, L). Additionally, in X1132F and Jing724, SFW, SL, and RL were significantly decreased by salt stress (Figure 3G, I, L). As shown in Table 1, for SFW, SDW, and SL, the inhibition order of five inbred lines was X1132M < X1132F / Zheng58 / Jing724 < Chang7-2, and it was X1132M / X1132F < Zheng58 / Jing724 / Chang7-2 for RFW, RDW, and RL. Furthermore, similar to the hybrid, shoot was more susceptible to salt stress than root in both germination and seedling stages, and the germination stage was more sensitive to the stress compared to its older stage.

Comprehensive evaluation of salt tolerance in maize hybrids and inbred lines

The salt tolerance levels of three hybrids and their related inbred lines varied according to different evaluation indices. To comprehensively evaluate salt tolerance levels of maize hybrids and their related inbred lines, PCA was performed based on STI of SFW, SDW, SL, RFW, RDW, and RL in both germination stage and in seedling stage, along with the STI of GR, SOD, Pro, MDA with REL. For maize hybrids, two PCs were obtained with the eigen value of 12.326 and 4.674, respectively, explained 100% of observed variations (Table 2). According to the loading values of different traits (Table 2), the value of each PC was obtained, and the CSTI was further determined according to the contribution rate of each PC (Tables 2 and 3).

Based on the formula, CSTI of X1132, Jingke968,

Table 2 - Loading matrix of each trait and contribution rate of principal components.

Maize lines PC	Eigen value	Contribution rate (%)	Cumulative contribution rate (%)	Loading matrix: Seedling stage GR	SOD	Pro	MDA	REL	SFW	SDW	SL	RFW	RDW	RL	Germination stage SFW	SDW	SL	RFW	RDW	RL
Hybrids																				
1	12.326	72.505	72.505	0.351	0.873	-0.904	0.992	-0.085	0.543	0.871	0.998	0.934	-0.866	-0.935	-1.000	-0.992	-0.947	0.851	0.962	0.746
2	4.674	27.495	100.00	0.936	0.488	-0.428	0.127	0.996	-0.840	-0.491	-0.062	0.358	0.500	-0.354	-0.022	-0.128	0.321	0.525	-0.275	-0.666
Inbred lines																				
1	8.031	47.240	47.240	-0.146	-0.912	-0.474	0.964	-0.101	0.123	-0.072	0.431	-0.601	0.341	0.900	0.964	0.934	0.982	0.961	-0.012	0.913
2	5.408	31.810	79.050	0.867	-0.238	0.257	-0.175	0.024	0.977	0.916	0.901	0.705	0.849	0.294	-0.222	-0.302	-0.158	0.008	-0.584	0.292
3	2.526	14.859	93.908	-0.422	0.060	0.697	0.167	0.873	0.171	0.182	0.039	0.376	0.077	-0.255	0.131	0.051	-0.027	0.268	0.786	0.285
4	1.036	6.092	100	0.219	0.329	-0.473	0.113	0.476	0.043	0.35	0.014	0.022	-0.396	-0.196	0.072	0.185	0.103	0.07	-0.201	-0.03

Table 3 - Salt tolerance levels of maize hybrids and inbred lines.

Maize lines	STI																	PC1	PC2	PC3	PC4	CSTI	Salt tolerance level
	Seedling stage											Germination stage											
	GR	SOD	Pro	MDA	REL	SFW	SDW	SL	RFW	RDW	RL	SFW	SDW	SL	RFW	RDW	RL						
Hybrids																							
X1132	0.95	-0.06	0.82	0.80	0.89	0.59	0.75	0.74	0.83	1.11	0.88	0.41	0.49	0.44	0.66	0.74	0.71	1.441	0.866			1.283	3
Jingke968	0.97	0.27	-0.26	1.62	0.85	0.72	0.84	0.80	0.94	1.06	0.60	0.28	0.42	0.32	0.72	0.83	0.73	4.628	1.496			3.767	1
Zhengdan958	1.00	0.26	-0.17	1.30	1.30	0.48	0.75	0.77	0.92	1.11	0.65	0.34	0.45	0.42	0.72	0.76	0.70	3.580	2.203			3.201	2
Inbred lines																							
X1132M	0.93	-0.14	1.00	1.13	0.23	0.92	1.05	0.92	1.12	1.31	0.84	0.30	0.32	0.32	0.63	0.70	0.96	3.903	5.401	2.417	-0.328	3.901	1
Chang7-2	0.63	0.16	0.74	1.15	-0.55	0.46	0.64	0.68	0.85	1.17	0.76	0.31	0.35	0.32	0.58	0.89	0.67	3.547	3.409	1.508	-0.673	2.943	3
X1132F	0.92	0.72	1.06	-0.52	-0.55	0.67	0.92	0.73	1.18	1.19	0.65	0.10	0.14	0.09	0.38	0.69	0.29	-0.184	4.771	1.250	-0.674	1.575	5
Zheng58	0.93	-0.06	0.03	0.75	-4.51	0.65	0.80	0.80	0.88	1.24	0.85	0.26	0.31	0.31	0.54	0.55	0.65	4.070	4.277	-2.888	-2.200	2.721	4
Jing724	0.87	0.26	-0.14	1.21	0.11	0.64	0.9	0.77	0.91	1.17	0.78	0.31	0.37	0.35	0.59	0.66	0.71	3.942	4.038	1.306	0.303	3.359	2

and Zhengdan958 was 1.283, 3.767, and 3.201 (Table 3), respectively, indicating that the order for salt tolerance levels of the three hybrids was Jingke968 > Zhengdan958 > X1132.

For inbred lines, four PCs were obtained by PCA and their eigen values were 8.031, 5.408, 2.526, and 1.036, respectively, explained 100% of observed variations. According to loading values and contribution rate (Table 2), four PCs and CSTIs were calculated, which revealed the CSTI of 3.900, 2.943, 1.575, 2.721, and 3.359 for X1132M, Chang7-2, X1132F, Zheng58, and Jing724, respectively (Table 3), indicating that the order for salt tolerance levels of was X1132M > Jing724 > Chang7-2 > Zheng58 > X1132F using our evaluation system.

Discussion

Salt stress is a complicated phenomenon, involving a series of growth and physiological responses (Gao et al, 2014; Wu, 2007; Ye et al, 2005). Tremendous efforts have been made to establish the system for screening and evaluation of salt tolerance based on multiple traits and indices. Pires comprehensive analyzed salt stress response of 56 rice phenotype seedlings with 15 phenotypic traits (Pires et al, 2015). Based on growth and physiological parameters, the salinity levels of Avicennia marina seedlings were evaluated (Yan et al, 2007). Zhang and Yang used five physiological and biochemical characters, including GR, SOD activity, Pro content, MDA content, and REL, to evaluate seedling tolerance levels of 26 maize hybrids and 67 maize inbred lines (Yang et al, 2011; Zhang et al, 2010). However, the evaluation of the salt tolerance only using the data from the seedling stage had its limitation, because the effect of salt may be considerable in the germination stage for some genotypes but only marginal during seedling stages. Therefore, in the present study, we investigated multiple growth parameters in both stages. To take different parameters into the assessment of a certain trait, PCA is widely used for plant tolerance comprehensive evaluation (Gandour et al, 2013). In this study, salt tolerance indices including SFW, SDW, SL, RFW, RDW, and RL in both germination and in seedling stages, along with GR, SOD, Pro, MDA, and REL were comprehensively evaluated by PCA for the salt tolerance evaluation of three important maize hybrids and their related inbred lines, and we found that the order of the salt tolerance levels for hybrids and inbred lines was Jingke968 > Zhengdan958 > X1132 and X1132M > Jing724 > Chang7-2 > Zheng58 > X1132F, respectively, which was consistent with the field observation under salt stress, indicating that our method is useful for evaluating maize salt tolerance.

Salt stress mainly affects plant via ionic stress, osmotic stress and oxidative stress (Zhang and Shi, 2013). The accumulation of Pro is important for osmotic stress adaptation (Gangopadhyay et al, 2000). A substantial increase of Pro content was found in stressed Salicornia europaes by Aghaleh (Aghaleh et al, 2011), and they also demonstrated a significant correlation between salt tolerance and the increase in Pro content (Aghaleh et al, 2011). SOD can alleviate oxidative damage (Sun and Tao, 2011). It was reported that salt tolerant cultivars of alfalfa had a higher SOD activity (Wang and Han, 2009). In our study, physiological responses varied among different genotypes. Explicitly, SOD activity was significantly increased by salt treatment in Jingke968 and Zhengdan958, while only Pro content was substantially elevated in the hybrid of X1132, suggesting that different salt tolerance mechanisms play roles in different maize varieties (Zhu, 2001). Similarly, in the inbred lines, SOD activity was increased in B73 and Jing724, whereas Pro content was dramatically improved in X1132M and Chang7-2. In addition, both SOD activity and Pro content were significantly increased in the line of X1132F, indicating that probably multiple mechanisms were involved in its salt tolerance (Pires et al, 2015). MDA content and REL represent the degree of cell membrane damage, and they are negatively correlated with plant salt tolerance (Cui et al, 2015; Miao et al, 2010; Yazici et al, 2007). In our study, no increase in MDA and REL was observed upon salt stress in all three maize hybrids indicating that probably no cell membrane damage in these hybrids, which supports the concept that these three hybrids are resistant to salt stress (Du et al, 2006; Wang et al, 2016).

Maize is a salt sensitive crop and their plant growth and development are greatly affected by salinity. In the present study, different growth and physiological parameters upon salt treatment at different developmental stages did not share the same variation pattern among genotypes. The SFW, SDW, SL, RFW, RDW, and RL were significantly reduced in all three hybrids and nearly all their related inbred lines in the germination stage, suggesting that maize is sensitive to salt stress at 100 mM in the germination stage. However, except for RDW of Zheng58, the RFW and RDW of all maize genotypes did not respond to salt stress in seedling stage, which suggests that maize is more susceptible to salt in the germination stage compared to the seedling stage, similar to the results found in salt treated wheat (Al-Naggar, 2015). Moreover, similar to the result in avocado (Bernstein et al, 2001), we observed that the shoot growth was more sensitive to salinity than root growth in all tested genotypes during both germination and seedling stages. Interestingly, we found that the RDW of Zheng58 inbred lines was significantly increased by salt treatment, and this phenomenon was also reported in sorghum (Chaugool et al, 2013).

Conclusion

Salt tolerance levels of three important hybrids and their related inbred lines were comprehensively evaluated based on their growth and physiological parameters, and the results were consistent with the observation of these lines growing in the saline soil, and thus this evaluation system might be applied for an accurate assessment of salt resistance in other germplasms and breeding materials. In addition, we showed that maize was more susceptible to salt stress at germination stage than at seedling stage, and that the shoot growth was more sensitive to salinity than root growth during both germination and seedling stages. Our data revealed different maize genotypes might utilize different salt tolerance mechanisms in maize seedling, but all of them involved the response of Pro and SOD activity.

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