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Salinity tolerance and site of K⁺ accumulation in four maize varieties grown in Khyber Pakhtoonkhwa region of Pakistan

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Crops face different types of biotic and abiotic stresses. Among abiotic stresses, salinity is very harmful and adversely affects the yield and production of crops. In Pakistan, maize is the third most important cereal crop after wheat and rice. In this research work, salt stress was applied to four maize varieties. The experiment was conducted in glass house. The effect of salinity on some physiological parameters that is, fresh and dry biomass of whole plant, above ground part and root of maize (*Zea mays* L.) was observed and the site of plant which accumulates K⁺ was determined. Five salinity levels of 30, 60, 110, 160 and 240 mmol each for NaCl and KCl were checked. The results showed that NaCl had significant effect on fresh and dry whole plant biomass, above ground biomass and root biomass. KCl had significant effect on whole plant fresh biomass and above ground dry biomass while its effect on above ground, root fresh biomass, whole plant and root dry biomass was insignificant. The combined effect of NaCl and KCl was also insignificant on fresh as well as dry biomass. With increasing salinity however, the cultivar Azam followed by Pop-2006 were the most tolerant to salinity. Contrarily, Pahari and Sarhad white were the least salinity tolerant varieties. Flam photometric analysis indicate that K⁺ accumulates in roots and above ground biomass (shoot), accumulation is more in the shoot than in the root. Azam and Pahari cultivars accumulated less K⁺ than Pop-2006 and Sarhad white.

Key words: Maize, salinity, stress, Pakistan, biomass.

INTRODUCTION

Maize (*Zea mays* L.) is one of the significant crops, which serves as food and oil for human consumption, as feed for livestock and as raw material for industry (Khatoon et al., 2010; Ullah et al., 2010). In Pakistan, maize is the third most important cereal crop after wheat and rice (Ministry of Food, Agriculture and Livestock, 2006; Khan et al., 2009). Salinity is probably the most pervasive

problem affecting crop production in irrigated lands. The degree and extent of salinity in cultivated lands is not certain due to lack of standardization of characterization criteria as no comprehensive salinity survey has ever been conducted. This is because the salinity status of agricultural land is continuously changing (Wyn-Jones, 1981). Estimates of the extent of world's saline land range up to 955 million ha (Szaboles, 1993). Salinity is a worldwide problem. Pakistan is situated in the arid and semi arid regions, where evaporation exceeds precipitation by a factor of more than 22 (Sandhu and Qureshi, 1986), thus leading to the development of salinity and sodicity. The economic importance of salinity is strongly realized from a 10% increase of saline areas all over the world on yearly basis (Shannon et al., 2000).

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Abbreviations: CCRI, Cereal Crops Research Institute; RCBD, randomized complete block design.

Table 1. Analysis of variance table on whole plant fresh biomass, dependent variables, whole plant fresh weight, test of between-subjects effects.

Source	Type III sum of square	Df	Mean square	F	Sig
Corrected model	24.820 ^a	27	0.919	6.487	0.000
Intercept	238.296	1	238.296	1681.52	0.000
Type	11.582	3	3.861	27.243	0.000
Na	4.131	4	1.033	7.287	0.000
K	3.096	4	0.774	5.461	0.001
Na * K	6.011	16	0.376	2.651	0.003
Error	10.203	72	0.142		
Total	273.319	100			
Corrected total	35.024	99			

a R squared = 0.709 (adjusted R squared = 0.599), sig. >0.05 = insignificant, sig. <0.05 = significant.

Salinity generally decrease growth at low concentration and is lethal at higher concentration. Salt affected plants appear dark green and are stunted (have shorter and fewer internodes), or may develop succulence or a rosette growth habit (Shannon and Nobel, 1995). Salinity affects plant growth at all stages of development and sensitivity to salinity varies from one growth stage to another. Salt tolerance varies considerably during the development of plants (Mass et al., 1983). This study was carried out to see the effect of various salt concentrations on maize cultivars to screen out salinity tolerant cultivars for saline areas of Khyber Pakhtoonkhwa and to evaluate the morphological and physiological traits.

MATERIALS AND METHODS

Seeds of four Pakistani maize (*Zea mays* L.) varieties grown in Khyber Pakhtoonkhwa were obtained from Cereal Crops Research Institute (CCRI) Pirsabak, Nowshera, Pakistan. The experiment was conducted in glass house. For experimental work, disposable glasses were used as pots for sowing of seeds. The area of each glass pot was 116.899 cm². Surface soil (0 to 15 cm) in bulk was collected from the normal field. The soil was air-dried and ground. Each glass pot was filled with 180 g of the dried soil. Three seeds of each that is, of the maize varieties Azam, Pahari, Sarhad white and Pop-2006 were sown in each glass pot. All the treatments were applied at the time of sowing of seeds. 20 ml each of NaCl and KCl solutions was added to each pot. Thirty glass pots were used for each variety in which twenty five received various treatments while five were kept as control. The glass pots were irrigated with water of different qualities: one series of 5 glass pots received the control treatment and were irrigated with tap water and the rest of 25 glass pots received different saline treatments including 30 mmol, 60, 110, 160 and 240 mmol of NaCl and KCl in combination according to randomized complete block design (RCBD).

The data collection was accomplished on daily bases immediately after the germination of seeds was observed. Data collection on Germination rate of seeds was started immediately after germination while the other aspects including whole plant height (shoot length), root length, shoot fresh and dry biomass, root fresh and dry biomass and determination of Na and K were studied after fifteen days of germination when the seedlings were uprooted. The effect of NaCl and KCl in combination on whole plant fresh Biomass, fresh above ground (shoot) biomass, whole plant dry Biomass, dry above ground (shoot) biomass, root fresh biomass

and root dry Biomass were determined according to the methods of Xiong et al. (2002).

Flame photometry

The shoots and roots previously dried at 60°C for 72 h were kept in China dishes in an oven at 160°C for 24 h. These completely dried shoots and roots were then ground with the help of mortar and pestle. The dried ground shoot and root material (0.20 g) was digested in 4 M HNO₃ (5 ml) and the volume of extracts was made 20 ml with distilled water, shaken, filtered and used for the determination of K by flame photometer.

Statistical analysis

Software SPSS and Graph pad were used for the statistical analyses of data. Results of analysis were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1980).

RESULTS

Effect of NaCl and KCl on whole plant fresh, above ground fresh and root fresh biomasses

Effects of different level of NaCl and KCl salinity on growth parameter of four maize varieties were studied. Statistical analysis was performed for the separate effect of NaCl and KCl, as shown in Tables 1, 3 and 5 and combined effect of NaCl and KCl on whole plant fresh biomass, ground fresh biomass and root fresh biomass as shown in Tables 2, 4 and 6, respectively. There is a significant difference between mean values of whole plant fresh biomass between different treatments of NaCl and KCl. The combined effect of NaCl and KCl on whole plant fresh biomass is also significant.

There is significant difference between mean values of above ground fresh biomass between different treatments of NaCl. KCl has no significant effect. The combined effect of NaCl and KCl on above ground fresh biomass is also insignificant. There is no significant difference between mean values of root fresh biomass between different treatments of NaCl and KCl. The

Table 2. Multiple comparison test table of four maize varieties. Pair wise comparisons dependent variable: Whole plant fresh weight.

(I) Type of plant	(J) Type of plant	Mean difference (I-J)	Std. error	Sig. ^a	95% Confidence interval for difference	
					Lower bound	Upper bound
Azam	Pahari	0.822*	0.106	0.000	0.610	1.034
	Sarhad White	0.053	0.106	0.619	-0.159	0.265
	Pop. 2006	0.060	0.106	0.573	-0.152	0.273
Pahari	Azam	-0.822*	0.106	0.000	-1.034	-0.610
	Pop-2006	-0.762*	0.106	0.000	-0.974	-0.549
	Sarhad white	-0.769*	0.106	0.000	-0.981	-0.557
Sarhad white	Azam	-0.053	0.106	0.619	-0.265	0.159
	Pop-2006	0.007	0.106	0.946	-0.205	0.219
	Pahari	0.769*	0.106	0.000	557	981
Pop-2006	Azam	-0.060	0.106	0.573	-0.273	0.152
	Sarhad white	-0.007	0.106	0.946	-0.219	0.205
	Pahari	0.762*	0.106	000	0.549	0.974

Based on estimated marginal means. *The mean difference is significant at the 0.05 level. a. Adjustment for multiple comparisons: least significant difference (equivalent to no adjustments).

Table 3. Analysis of variance table on above ground fresh biomass. Tests of between-subjects effects dependent variable: above ground fresh biomass.

Source	Type III sum of square	df	Mean square	F	Sig
Corrected model	3.138 ^a	27	0.116	2.892	0.000
Intercept	35.321	1	35.321	878.820	0.000
Type	1.369	3	0.456	11.355	0.000
Na	0.956	4	0.239	5.946	0.000
K	0.304	4	0.076	1.892	0.121
Na * K	0.509	16	0.032	0.792	0.690
Error	2.894	72	0.040		
Total	41.353	100			
Corrected total	6.032	99			

a. R squared = 0.520 (adjusted R squared = 0.340), sig. >0.05 = insignificant, sig. <0.05 = significant.

combined effect of NaCl and KCl on root fresh biomass is also insignificant at 95% confidence interval.

Effect of NaCl and KCl on whole plant dry, above ground dry and root dry biomasses

Similarly the separate effect of NaCl and KCl on whole plant dry, above ground dry and root dry biomasses are presented in Tables 7, 9 and 11, respectively. There is significant difference between mean values of whole plant dry biomass and root dry biomass between different treatments of NaCl. The KCl has no significant effect on both the parameters. There is significant difference between mean values of above ground dry biomass

between different treatments of NaCl and KCl. The combined effect of NaCl and KCl on whole plant dry biomass, above ground dry and root dry biomasses of maize are presented in the Tables 8, 10 and 12, respectively. The combined effect of NaCl and KCl were insignificant on the studied parameters.

Site of potassium accumulation

Flame photometry was accomplished in order to detect the part of the plant which accumulates K⁺. The concentration K⁺ was determined in the seedling's roots and shoot (above ground) of maize varieties. The results show that K⁺ accumulates more in the shoot than in the

Table 4. Multiple comparison test table of four maize varieties. Pair wise comparisons dependent variable: Above ground fresh biomass.

(I) Type of plant	(J) Type of plant	Mean difference (I-J)	Std. error	Sig. ^a	95% Confidence interval for difference	
					Lower bound	Upper bound
Azam	Pahari	0.273*	0.057	0.000	0.160	0.386
	Sarhad White	0.112	0.057	0.051	-0.001	0.225
	Pop. 2006	-0.023	0.057	0.691	-0.136	0.090
Pahari	Azam	0.273*	0.057	0.000	-0.386	-0.160
	Pop-2006	-0.161*	0.057	0.006	-0.274	-0.048
	Sarhad white	-0.296*	0.057	0.000	-0.409	-0.183
Sarhad white	Azam	-0.112	0.057	0.051	-0.225	0.001
	Pop-2006	0.161*	0.057	0.006	0.048	0.274
	Pahari	-0.135*	0.057	0.020	-0.248	-0.022
Pop-2006	Azam	0.023	0.057	0.691	-0.090	0.136
	Sarhad white	0.296*	0.057	0.000	0.183	0.409
	Pahari	0.135*	0.057	0.020	0.022	0.248

Based on estimated marginal means *. The mean difference is significant at the 0.05 level a. adjustments for multiple comparisons: least significant difference (equivalent to no adjustments).

Table 5. Analysis of variance table on root fresh biomass. Tests of between-subjects effects, dependent variable: Root fresh weight.

Source	Type III sum of square	df	Mean square	F	Sig.
Corrected model	1.946 ^a	27	0.072	3.549	0.000
Intercept	17.342	1	17.342	854.024	0.000
Type	1.386	3	0.462	22.759	0.000
Na	0.174	4	0.044	2.148	0.084
K	0.093	4	0.023	1.145	0.342
Na * K	0.292	16	0.018	0.898	0.574
Error	1.462	72	0.020		
Total	20.749	100			
Corrected total	3.408	99			

a. R squared = 0.571 (adjusted R squared = 0.410), sig. >0.05 = insignificant, sig. <0.05 = significant.

Table 6. Multiple comparison test table of four maize varieties. Pair wise comparisons dependent variable: Root fresh weight.

(I) Type of plant	(J) Type of plant	Mean difference (I-J)	Std. error	Sig. ^a	95% Confidence interval for difference	
					Lower bound	Upper bound
Azam	Pahari	0.327*	0.040	0.000	0.247	0.408
	Sarhad White	0.213*	0.040	0.000	0.132	0.293
	Pop. 2006	200*	0.040	0.000	0.120	0.281
Pahari white	Azam	-0.327*	0.040	0.000	-0.408	-0.247
	Pop-2006	-0.115*	0.040	0.006	-0.195	-0.034
	Sarhad	-0.127*	0.040	0.002	-0.207	-0.047
Sarhad white	Azam	-0.213*	0.040	0.000	-0.293	-0.132
	Pop-2006	0.115*	0.040	0.006	0.034	0.195
	Pahari	-0.012	0.040	0.763	-0.093	0.068
Pop-2006	Azam	-0.200*	0.040	0.000	-0.281	-0.120
	Sarhad white	0.127*	0.040	0.002	0.047	0.207
	Pahari	0.012	0.040	0.763	-0.068	0.093

Based on estimated marginal means, * the mean difference is significant at the 0.05 level. a adjustment for multiple comparisons: least significant difference (equivalent to no adjustments).

Table 7. Analysis of variance table for whole plant dry biomass. Tests of between-subjects effects dependent variable: whole plant dry weight.

Source	Type III sum of square	df	Mean square	F	Sig.
Corrected model	0.344 ^a	27	0.013	1.4	0.145
Intercept	10.365	1	10.365	***	0.000
Type	0.026	3	0.009	0.937	0.428
Na	0.186	4	0.046	5.0	0.001
K	0.025	5	0.006	0.675	0.611
Na * K	0.107	16	0.007	0.724	0.761
Error	0.669	72	0.009		
Total	11.378	100			
Corrected total	1.013	99			

a. R Squared = 0.340 (adjusted R squared = 0.092), sig. >0.05 = insignificant, sig. <0.05 = significant.

Table 8. Multiple comparison test table of four maize varieties. Pair wise comparisons dependent variable: Whole plant dry weight.

(I) Type of plant	(J) Type plant	Mean difference (I-J)	Std. error	Sig. ^a	95% confidence interval for difference	
					Lower bound	Upper bound
Azam	Pahari	0.040	0.027	0.142	-0.014	0.095
	Sarhad white	0.035	0.027	0.209	-0.020	0.089
	Pop. 2006	0.036	0.027	0.192	-0.018	0.090
Pahari	Azam	-0.040	0.027	0.142	-0.095	0.014
	Pop-2006	-0.005	0.027	0.866	-0.059	0.050
	Sarhad white	-0.006	0.027	0.828	-0.060	0.048
Sarhad white	Azam	-0.035	0.027	0.209	-0.089	0.020
	Pop-2006	0.001	0.027	0.961	-0.053	0.056
	Pahari	0.006	0.027	0.828	-0.048	0.060
Pop-2006	Azam	-0.036	0.027	0.192	-0.090	0.018
	Sarhad white	-0.001	0.027	0.961	-0.056	0.053
	Pahari	0.005	0.027	0.866	-0.050	0.059

Based on estimated marginal means. a. Adjustment for multiple comparisons: Least significant difference (equivalent to no adjustments).

Table 9. Analysis of variance table on above ground biomass. Tests of between-subjects effects dependent variable: above ground dry biomass.

Source	Type III sum of square	df	Mean square	F	Sig.
Corrected model	0.133 ^a	27	0.005	3.229	0.000
Intercept	1.611	1	1.611	1058.32	0.000
Type	0.015	3	0.005	3.369	0.023
Na	0.086	4	0.022	14.139	0.000
K	0.018	4	0.004	2.925	0.027
Na * K	0.013	16	0.001	0.522	0.909
Error	0.110	72	0.002		
Total	1.853	100			
Corrected total	0.242	99			

a. R Squared = 0.548 (Adjusted R Squared = 0.378), Sig. >0.05 = Insignificant, Sig. <0.05 = Significant.

Table 10. Multiple comparison test table of four maize varieties. Pair wise comparisons dependent variable: Above ground dry biomass.

(I) Type of plant	(J) Type of plant	Mean difference (I-J)	Std. error	Sig. ^a	95% Confidence interval for difference	
					Lower bound	Upper bound
Azam	Pahari	0.022*	0.011	0.049	0.000	0.044
	Pop-2006	-0.013	0.011	0.260	-0.035	0.009
	Sarhad white	0.005	0.011	0.674	-0.017	0.027
Pahari white	Azam	-0.022*	0.011	0.049	-0.044	0.000
	Pop-2006	-0.035*	0.011	0.002	-0.057	-0.013
	Sarhad	-0.017	0.011	0.119	-0.039	0.005
Sarhad white	Azam	-0.005	0.011	0.674	-0.027	0.017
	Pop-2006	-0.017	0.011	0.123	-0.039	0.005
	Pahari	0.017	0.011	0.119	-0.005	0.039
Pop-2006	Azam	0.013	0.011	0.260	-0.009	0.035
	Pahari	0.017	0.011	0.123	-0.005	0.039
	Sarhad white	0.035*	0.011	0.002	0.013	0.057

Based on estimated marginal means, *. The mean difference is significant at the .05 level a. adjustments for multiple comparisons: Least significant difference (equivalent to no adjustments).

Table 11. Analysis of variance table on above ground biomass. Tests of between-subjects effects dependent variable: Root dry biomass.

Source	Type III sum of square	df	Mean square	F	Sig.
Corrected model	0.121 ^a	27	0.004	1.423	0.120
Intercept	3.757	1	3.757	1194.80	0.000
Type	0.033	3	0.011	3.544	0.019
Na	0.034	4	0.008	2.679	0.038
K	0.018	4	0.005	1.469	0.221
Na * K	0.035	16	0.002	0.699	0.786
Error	0.226	72	0.003		
Total	4.104	100			
Corrected total	0.347	99			

a. R Squared = 0.348 (adjusted R squared = 0.103), sig. >0.05 = insignificant, sig. <0.05 = significant.

Table 12. Multiple comparison test table of four maize varieties. Pair wise comparisons dependent variable: Root dry biomass.

(I) Type of plant	(J) Type of plant	Mean difference (I-J)	Std. error	Sig. ^a	95% Confidence interval for difference	
					Lower bound	Upper bound
Azam white	Pahari	0.016	0.016	0.305	-0.015	0.048
	Pop-2006	0.048*	0.016	0.003	0.016	0.080
	Sarhad	0.035*	0.016	0.028	0.004	0.067
Pahari white	Azam	-0.016	0.016	0.305	-0.048	0.015
	Pop-2006	0.032	0.016	0.050	0.000	0.063
	Sarhad	0.019	0.016	0.233	-0.013	0.051
Sarhad white	Azam	-0.035*	0.016	0.028	-0.067	-0.004
	Pop-2006	0.013	0.016	0.432	-0.019	0.044
	Pahari	-0.019	0.016	0.233	-0.051	0.013
Pop-2006	Azam	-0.048*	0.016	0.003	-0.080	-0.016
	Pahari	-0.013	0.016	0.432	-0.044	0.019
	Sarhad white	-0.032	0.016	0.050	-0.063	0.000

Based on estimated marginal means, *. The mean difference is significant at the 0.05 level. a. Adjustment for multiple comparisons: Least significant difference (equivalent to no adjustments).

root.

DISCUSSION

In the present study whole plant fresh biomass was greatly affected by salinity treatments in all of the four varieties. There was an increase in whole plant fresh biomass at low concentration of NaCl and KCl while decrease at concentration above 110 mmol (Data not shown). NaCl and KCl in combination had significant effect on whole plant fresh biomass. The difference in whole plant fresh biomass in both NaCl and KCl treatments and cultivars was also significant (Table 1). Salinity had drastically affected the whole plant fresh biomass of Pahari cultivar. Cicek and Cakirlar (2002) worked on two maize cultivars and also reported the same results. Reduction in whole plant fresh biomass as a result of salt stress has also been reported in several other plant species (Brugnoli and Lauteri, 1991; Alberico and Cramer, 1993). Kayani and Rahman (1988) also found the same results. An increase in fresh above ground biomass with increasing NaCl concentration up to 110 mmol and then decrease at concentration above 110 mmol was observed. The highest fresh above ground biomass was observed at 110 mmol concentration of NaCl while the lowest fresh above ground biomass was observed at 240 mmol concentration (Data not shown).

The separate effect of KCl as well as in combination with NaCl had no significant effect on fresh above ground biomass. However, the difference in fresh above ground biomass in maize cultivars was significant (Table 3). The effect of salinity treatments on fresh above ground biomass was more pronounced in Pahari while Pop-2006 was least effected. Dionisio-Sese and Tobita (2000) also found decrease in fresh above ground biomass as a result of salt stress. The results are in close consistency with that of Richardson and McCree (1985) Cramer et al. (1985) Izzo et al. (1993) also found reduction in fresh above ground biomass in plants. Salinity has little or no effect on root fresh biomass. NaCl separately has slight effect on root fresh biomass. KCl separately as well as in combination with NaCl had no significant effect. Neumann (1995) also found decrease in root biomass with high NaCl concentration. Salinity can rapidly inhibit root biomass and hence capacity of water uptake and essential mineral nutrition from soil (Demir and Arif, 2003). The results of Jeannette et al. (2002) also strongly correlate with our work. KCl separately and in combination with NaCl has no significant effect on whole plant dry biomass. However, NaCl separately has significant effect on whole plant dry biomass. The difference in whole plant dry biomass in maize cultivars was also significant (Table 7). Maximum whole plant dry biomass was observed at 60 mmol concentration of NaCl while 240 mMol concentration of NaCl resulted in drastic reduction in whole plant dry biomass. Similar results have also been reported by Cicek and Cakirlar (2002).

Our results were also similar to the findings of Kurth et al. (1986) and Nasim et al. (1993). Saqib and Qureshi (1998) worked on the effect of salinity and hypoxia on wheat line and found similar results as well. An increase in above ground dry biomass at low concentration of NaCl and KCl while decrease at high concentration. The dry biomass obtained at 60 and 110 mmol concentration of NaCl was almost similar. The difference in above ground dry biomass in both treatments of NaCl and KCl and in maize cultivars was significant. However, the combined effect of NaCl and KCl on above ground dry biomass was insignificant (Table 9). NaCl had more pronounced effect on dry biomass than KCl. Shafqat (1994) studied the effect of salinity on maize cultivars and reported the same results. Yeo et al. (1985) results are also similar to our results. Saqib and Qureshi (1998) demonstrated similar results as well. The present results indicated that KCl separately and in combination with NaCl has no significant effect on root dry biomass. However, NaCl separately has significant effect on whole plant dry biomass. The difference in whole plant dry biomass in maize cultivars was also significant (Table 7). Maximum root dry biomass was observed at 60 mmol concentration of NaCl while 240 mmol concentration of NaCl resulted in drastic reduction in root dry biomass. There was no much difference between the root dry biomass obtained as a result of 60 and 240 mmol concentration of KCl. Turner (1986) also found decrease in root dry biomass after the maize plants are subjected to salinity. Peterson et al. (1988) studied the effect of salinity on maize crop and found similar results. Hajibagheri et al. (1989) and Zeynalabedin and Jafari, (2002) results are similar to our work as well. The present results demonstrate that K^+ is accumulated more in the shoot than in the root of maize plant. Furthermore, K^+ was accumulated more in Pop-2006 cultivar while least in Azam.

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