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Alleviation of Salinity Hazards in Different Maize Genotypes Using Inorganic Ions (Ca^{++} and K^{+})

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Abstract. A hydroponic experiment was conducted to evaluate and compare the efficiency of Ca and K ions and different maize genotypes with imposed salinity. Two levels of K^{+} and Ca^{++} (5 and 10 mM) each were tested on two maize genotypes (Pioneer-3335 and Syngenta-8441) under 100 mM NaCl stress. Saline treatment adversely affected the plant physiological parameters and disturbed the ionic balance and resulted in poor plant growth. However, Syngenta-8441 was more prone to salt stress as compared to Pioneer-3335. Both of the inorganic ions showed significant effects on physiological and ionic components of both genotypes. Among the inorganic ions K was found to be more efficient than Ca in improving the plant growth. Improved physiological and ionic traits were observed more significant with 10 mM K ion treatment followed by 5 mM K. Among Ca treatments higher level of Ca (10 mM) showed more significant results as compared to 5mM Ca^{++}. This study revealed that Ca^{++} and K^{+} both are compulsory for maintaining the crop growth because of their mutual effects under salt stress. However, the alleviative efficiency of K is far better than Calcium but its (Ca^{++}) role could not be neglected, while regarding genotypic differential response imposes the fact that Pioneer-3335 is more tolerant to salinity menace as compared to Syngenta-8441 but both of the genotypes were significant in their recovery from stress to applied Ca^{++} and K^{+}.

Keywords: maize, genotypic variation, inorganic ions, salt stress

Introduction

Salinity menace is ever expanding concern in agricultural productivity in Pakistan with a salt affected area of 6.30 Mha distributed in all four provinces. An annual conversion of 40,000 ha of productive soils into salt affected area is a clear depiction of problem extent (Alam *et al*., 2000). This increasing trend is an alarming situation for sustainable agriculture and ultimately the food security in Pakistan. Salts build up in soils is responsible for the deterioration of soil structure and ultimately disturbing the media for optimum crop growth.

There are certain mechanisms that are salinity mediated, forcing the plants to uptake more Na ions likewise Na ion permeable transporters along with nutrient cation channels and transporters. Na ion channels seems to be the fundamental reason in elevated cytoplasmic concentration of Na ions, uptake by plants through root cells, up to toxic levels. Na ion uptake is also enhanced by certain nutrient cation channels and transporters (Ca^{++} and K^{+}) and further aggravated by Na ion intrusion through Ca^{++} sensitive and insensitive pathways (Essah *et al*. 2003, Rubio *et al*. 2003). K ion channels are selective to K^{+}:Na^{+} and under saline conditions K^{+} channels might be involved in sodium uptake by the cells (Amtmann and Sanders, 1999) as K ion channels and transporters were involved in Na ion uptake in the roots of *S. maritima* under salt stress (Wang *et al*., 2007). The Na^{+} flow in the cells could be controlled by using certain channel blockers (Essah *et al*., 2003).

Ca has a diverse role in plant growth as it is required in smaller amounts for regulating certain processes in cell metabolism, while in higher amounts for development of cell wall (Ashraf *et al*., 2008; Rubio and Botella , 2004) and maintenance of cell membrane integrity when the plant is facing salt stress (Mahmood, 2009; Ashraf, 2004). Among all other macro nutrients potassium has the significant role in plant survival under salt stress (Mengel and Kirkby, 2001). K helps the crop plants to maintain turgor pressure, membrane stability and antioxidant enzymes activation to survive the hazard of saline environment (Cherel, 2004) and besides potassium is also crucial for plant metabolism (synthesis of proteins and osmotic adjustment). Keeping in view these factors an experiment was designed to compare the efficiency of Ca^{++} and K^{+} in reducing the salinity

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hazards in maize which is an important cereal crop grown in Pakistan to meet the food requirements with yield averaging 3264 kg/ha and covering an area of more than 3 million hectares (Mehdi and Ahsan, 2000). The study was planned with following objectives;

- The response of two imported genotypes of maize to Ca^{++} and K^{+} (Inorganic ions) under salt stress
- Efficiency evaluation of Ca^{++} and K^{++} in alleviating salt stress in maize.

Materials and Methods

A hydroponic experiment was established in the glass house of University of agriculture Faisalabad. The seeds of two maize genotypes (Pioneer-3335 and Syngenta-8441) were collected from the respective companies Pioneer and Syngenta. A nursery was raised in trays containing acid washed sand. At two leaves stage the plants were transplanted in thermo pore sheets suspended over water tub containing ½ strength modified Hoagland's solution (Hoagland and Arnon, 1950) with pH maintained at 6.5 (on daily basis). Salt stress of 100 mM NaCl was applied in three increments. The treatments were control (½ strength Hoagland solution), saline (100 mM NaCl), 5 mM Ca^{++}, 10 mM Ca^{++}, 5 mM K^{+} and 10 mM K^{+} under salt stress (100 mM NaCl) with three replicates.

After 4 weeks chlorophyll contents were noted after that plants were harvested and growth parameters (plant height, fresh biomass and dry biomass of root and shoot) were recorded. Membrane stability index of plants was recorded using the method of Sairam *et al.*, (2000). Di-acid digested samples of shoot (Skoog *et al.*, 2000) were then analyzed using Flame photometer (Jenway PFP-7) to determine Na^{+}/K^{+}. The experiment was laid out using appropriate statistical design with three replications and data was subjected to Genstat® Discovery edition statistical package (Payne *et al.*, 1987).

Results and Discussion

Growth attributes. Results of this hydroponic short durational study show that the genotype Syngenta-8441 performed better under normal conditions as compared to genotype Pioneer-3335. But under stress conditions Syngenta-8441 was found to be more sensitive with a marked decrease of 46.4% and 40.2% in shoot and root length respectively, 88.29% and 59.8% in fresh biomass (shoot and root respectively) and 68.3% and 35.8% in dry biomass (shoot and root respectively) while that for Pioneer-3335 was 41.8% and 41.1% (shoot and root lengths respectively), 73.3% and 68.7% (shoot and root respectively) and 58.9% and 18.9% (shoot and root respectively) as compared to control. The increasing cytoplasmic concentration of salts reduces the elasticity of cell wall, metabolic malfunctioning and ultimately reduces the plant growth. When supplemented with inorganic ions under stress conditions Ca^{++} and K^{+} significantly reduced the harmful effects of salinity at all applied levels. Similar results were conferred by Jafari *et al.* (2009) that combine application of calcium and potassium boosts the plant growth under saline environment. However 10 mM KCl treatment under salt stress was found more effective in alleviating salt stress than any other treatment followed by 10 mM $CaCl_2$. An increase of 19% and 29% (shoot and root length), 5.4% and 61% (shoot and root fresh biomass) and 2.3% and 18% (shoot and root dry biomass) for Syngenta-8441 and 7.60% and 26% (shoot and root length), 12% and 54% (shoot and root fresh biomass) and 1.1% and 35% (shoot and root dry biomass) for Pioneer-3335 was observed for 5 mM $CaCl_2$ treatment under salt stress. While the increase in growth parameters was more promising with 10 mM $CaCl_2$ treatment under salt stress as Syngenta-8441 growth was improved by 11% and 35% (shoot and root lengths), 19% and 95% (shoot and root fresh weight) and 24% and 124% (shoo and root dry weight) and that for Pioneer-3335 was 24% and 46% (shoot and root lengths), 24% and 74% (shoot and root fresh weight) and 33% and 110% (shoot and root dry weight). The beneficial role of calcium in plant growth improvement under stress condition is in accordance with Arshi *et al*., (2006) that calcium plays a crucial role in the developmental and growth phases of plant because calcium couples a broad range of external stimuli to internal response as a messenger (Sneeden and Formm, 2001). Although effect of Ca^{++} was significant on growth parameters of both maize genotypes but KCl treatments showed more prominent results as under the treatment of 10 mM KCl Syngenta-8441 responded with an increase of 15% and 54% (shoot and root length), 19% and 100% (shoot and root fresh weight) and 38% and 133% (shoot and root dry biomass) while Pioneer-3335 responded via an increase of 32% and 475 (shoot and root length), 35% and 78% (shoot and root fresh biomass) and 40% and 128% (shoot and root dry biomass) growth attributes followed by 5 mM KCl treatment. These results are in alliance with Kaya *et al.,* (2001; 2003) who reported that potassium has a significant role in alleviating the salinity

Table 1. Effect of inorganic ions (Ca and K) on plant growth parameters of two maize genotypes grown under salt stress for 30 days

	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Root dry weight (g)		Shoot length (cm)		Root length (cm)	
Treatments	Pioneer (8441)	Syngenta (3335)	Pioneer (8441)	Syngenta (3335)	Pioneer (8441)	Syngenta (3335)	Pioneer (8441)	Syngenta (3335)	Pioneer (8441)	Syngenta (3335)	Pioneer (8441)	Syngenta (3335)
Control	7.49± 0.29	22.02± 0.98	0.72± 0.03	1.23±0.06	3.75± 0.08	4± 0.09	0.28± 0.01	0.45±0.08	53.33± 1.15	65.33± 1.15	41.33± 0.88	44± 0.88
100 mM NaCl	2.00± 0.29 (26.7)	2.58± 0.29 (11.71)	0.30± 0.02 (41.1)	0.39±0.04 (31.7)	1.17± 0.05 (31.3)	1.60± 0.05 (40.2)	0.23± 0.02 (82.1)	0.29±0.01 (64.2)	31± 0.80 (58.2)	35± 1.52 (53.6)	24.33± 1.20 (58.9)	26.33± 1.20 (59.8)
5 mM $CaCl_2$ +100 mM NaCl	2.88± 0.10 (38.5)	3.60± 0.18 (16.4)	0.35± 0.01 (42.2)	0.41±0.02 (33.1)	3.2± 0.12 (85.3)	4.05± 0.05 (101.2)	0.33± 0.02 (117.8)	0.37±0.12 (82.2)	35± 1.15 (65.62)	47.66± 0.88 (72.6)	35± 1.00 (84.6)	39±0.76 (88.63)
10 mM $CaCl_2$ +100 mM NaCl	3.75± 0.19 (50.2)	6.66± 0.26 (30.2)	0.54± 0.02 (74.3)	0.68±0.03 (55.6)	4.03± 0.07 (107.5)	5.50± 0.07 (135.5)	0.54±0.04 (192.8)	0.85±0.08 (188.8)	44± 0.90 (82.6)	42.33± 1.23 (64.7)	43.33± 0.88 (104.8)	46± 1.00 (104.6)
5 mM KCl +100 mM NaCl	3.54± 0.13 (47.2)	6.22± 0.26 (28.3)	0.43± 0.02 (59.2)	0.66±0.02 (53.9)	3.95± 0.05 (105.3)	4.45± 0.12 (111.3)	0.43±0.06 (153.6)	0.66±0.07 (146.4)	40± 1.10 (70.2)	44.66± 1.22 (67.5)	39± 1.50 (94.3)	43±1.15 (97.9)
10 mM KCl +100 mM NaCl	4.62± 0.23 (61.7)	6.72± 0.28 (30.5)	0.59± 0.03 (81.2)	0.85±0.04 (69.1)	4.1± 0.06 (109.3)	5.60± 0.17 (140)	0.59±0.03 (210.7)	0.89±0.09 (197.7)	48.33± 1.20 (90)	45.33± 1.43 (68.2)	43.66± 1.45 (105.6)	50±0.88 (113.6)

Each value is an average of three replications ± standard error; ( ) = percent of respective control

hazards and improving the growth of cucumber, pepper and strawberry. Similar results were documented by Akram *et al*. (2007) that a sufficient K amount is required for optimum plant growth and potassium application had significant effects on sunflower growth under salt stress.

Physiological and ionic attributes. Results in the Table. 2 clearly depict the hazards of salinity on chlorophyll contents, membrane stability and Na^+: K^+ in two maize genotypes grown under salt stress. A significant decrease of 42% and 81% (chlorophyll contents and membrane stability index) was observed for Syngenta-8441 when subjected to salt stress. A same decreasing trend was found in Pioneer-3335 with 54% decrease in chlorophyll contents and 87% membrane instability as compared to control under saline treatment. The reduction in chlorophyll contents is probably the result of disturbed metabolic activities due to higher salt accumulation in the cell wall that ultimately results in poor growth of the affected crop plants. These findings are parallel to that of Chookhampaeng, (2011), who stated that salinity adversely affects the plant at all physiological levels including chlorophyll contents. The effect of K was more promising as compared to Ca regarding physiological and ionic development in both genotypes under salt stress. It was clear that with the increasing K and Ca levels the salinity hazard was significantly minimized. because Ca and K are thought to be the regulator for certain physiological and biochemical processes in plants and keep the Na away to be adsorbed resulting in low Na uptake and accumulation. These findings are in alliance with Akram and Ashraf, (2011), who reported the chlorophyll contents recovery with the application of potassium under salt stress. A significant chlorophyll contents recovery was achieved with an increase of 23% in Syngenta-8441 and 36% in Pioneer-3335 when supplied with 10 mM $CaCl_2$ followed by 5 mM $CaCl_2$ treatment. While K affected more significantly on chlorophyll contents with an increase of 28% in Syngenta-8441 and 41% increase in Pioneer-3335 at 10 mM KCl level followed by 5 mM KCl level. In case of membrane stability index 10 mM KCl treatment was found to give the most prominent results with 70% increased membrane stability in Syngenta-8441 and 84% increase in Pioneer-3335 as compared to saline treatment with membrane stability index of 19% and 13% for Syngenta-8441 and Pioneer-3335 respectively. 10 mM KCl was followed by 10 mM $CaCl_2$ treatment with an increase

Table 2. Effect of inorganic ions (Ca and K) on chlorophyll content, membrane stability index and Na^+: K^+ of two maize genotypes grown under salt stress for 30 days.

	Chlorophyll contents		Membrane stability index(%)		Na+: K+	
Treatments	Pioneer (3335)	Syngenta (8441)	Pioneer (3335)	Syngenta (8441)	Pioneer (3335)	Syngenta (8441)
Control	49.77±0.63	53±0.88	63.69±0.77	67±1.04	0.26± 0.85	0.15± 0.83
100 mM NaCl	23±0.25 (46.3)	28±1.15 (58.1)	8.47±0.50 (13.3)	12.96± 0.50 (19.3)	2.59± 1.80	2.63± 1.79
5 mM CaCl2 +100 mM NaCl	39.83±1.25 (80.1)	42±1.32 (79.2)	52.5±1.44 (82.5)	50± 1.41 (74.6)	1.04± 1.00	0.83± 1.03
10 mM CaCl2 +100 mM NaCl	40.76±0.98 (82.1)	43.07±0.86 (81.3)	60±1.52 (95.2)	59± 1.25 (88.0)	0.75± 1.15	0.79± 1.07
5 mM KCl +100 mM NaCl	42.17±1.71 (84.2)	44±1.00 (83.1)	27.62±1.32 (43.4)	30± 1.03 (44.8)	0.83± 0.92	0.81± 1.02
10 mM KCl +100 mM NaCl	44.1±0.55 (88.7)	46±1.31 (86.8)	61.52±1.76 (97.6)	60.0±1.63 (89.5)	0.73± 1.06	0.76± 0.83

Each value is an average of three replications ± standard error; ( ) = percent of respective control

of 69% and 82% in membrane stabilities for Syngenta-8441 and Pioneer-3335 respectively.

Under salinity stress the uptake of nutrient is imbalance due to which plant growth reduces by affecting the accessibility, transport and distribution of nutrients. The reduction in growth and yield is basically due to reduction in quantum yield, chlorophyll pigments and K^+/Na^+ ratio.

As far as Na^+: K^+ is concerned, salt stress drastically increased the Na^+: K^+ in both genotypes but the extent of hazard was more in Syngenta-8441. Increased Na^+: K^+ in plants in turn hindered the metabolic activities resulting in stunted growth of both genotypes. The results depict that the application of Ca^{++} and K^+ significantly alleviated the salinity hazard by narrowing the Na^+: K^+ but among these inorganic ions K was more efficient in decreasing the Na^+: K^+. Ca^{++} K^+ have an interactive effect because under saline environment, Ca^{++} helps the plant to uptake more K^+ as compared to sodium, hence keeping the Na^+/K^+ in limit (Dabuxilatu and Ikeda, 2005). K treatment with a level of 10 mM KCl brought the Na^+: K^+ from 2.63 to 0.76 in Syngenta-8441, while in Pioneer-3335 the Na^+: K^+ was decreased from 2.59 to 0.73 followed by 10 mM $CaCl_2$ treatment with a Na^+: K^+ of 0.79 and 0.75 in Syngenta-8441 and Pioneer-3335 respectively. While all other levels of K and Ca was also significant in reducing the Na^+: K^+ of both genotypes.

Conclusion

The results of this study concluded that Ca^{++} and K^+ both are compulsory for maintaining the crop growth because of their mutual effects under salt stress. However the alleviative efficiency of K is far better than Calcium but its (Ca^{++}) role could not be neglected. While regarding genotypic differential response imposes the fact that Pioneer-3335 is more tolerant to salinity menace as compared to Syngenta-8441 but both of the genotypes were significant in their recovery from stress to applied Ca^{++} and K^+.

Conflict of Interest. The authors declare no conflict of interest

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