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EFFECT OF POTASSIUM APPLICATION ON AMMONIUM NUTRITION IN MAIZE (*Zea mays* L.) UNDER SALT STRESS

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Application of potassium has been found to minimize the toxic effect of NH_4^+ under salt stress. To study the interactive effect of K^+ and NH_4^+ under saline condition, maize (*Zea mays* L., cv. Pioneer-3335) was grown in a hydroponic culture with ammonium (5.0 and 10 mM) as $(NH_4)_2SO_4$ at two different levels (3.0 and 9.0 mM) of K^+ under control and 100 mM NaCl. Under saline condition, 5 mM NH_4^+ application along with 3.0 mM K^+ decreased the dry mass by 24% in maize while its addition at the rate of 10 mM showed a percent decline upto 70% than the control. A decrease in shoot dry mass induced by the combine application of 5.0 mM NH_4^+ and 9.0 mM K^+ was 19% relative to control whilst a decrease i.e. 52% was observed at 10 mM NH_4^+ level. The increasing concentration of potassium was found to alleviate the NH_4^+ toxicity and salinity stress partly by inhibiting the uptake of NH_4^+ and Na^+ and by stimulating the N assimilation in plant body. Growth improvement at combination of 5.0 mM NH_4^+ and 9.0 mM K^+ was reinforced by higher K^+ influx into root cells and its translocation to the growing tissues. Elevating the K^+ supply also resulted in the enhanced plant growth several times and reduction in NH_4^+ toxicity and salinity stress.

Key words: NH_4^+, K^+, NaCl, salinity stress, maize, crops

INTRODUCTION

Salinity is a major limiting agricultural factor in arid and semi-arid regions. Salt accumulation in soils may induce osmotic changes, interfere with nutrient uptake, inactivate the enzymes and disturb osmotic adjustment at the cytosol and vacuoles' level (Apse *et al*., 1999). Crops differ significantly in their resistance to high salt contents and maize is found to be a moderately salt sensitive crop (Maas and Hoffman, 1977). Salt tolerance differences exist among different plant species as well as among different cultivars or varieties of same species (Azevedo Neto *et al*., 2006). Mineral nutrient supply to plants also plays a critical role in improving tolerance potential of plant against various environmental stresses including, drought, salinity, disease and temperature (Marschner, 1995). Similarly, salt tolerance could also be associated with plant's capacity to regulate the rate of ion transport or accumulation in leaf tissues (Saqib et al., 2012). Ammonium accumulation has been reported in many species due to certain stresses (Husted *et al*., 2000). The low (micro molar) concentration of NH_4^+ is considered optimal in many plants (Kronzucker *et al*., 1999) but most of the plants cannot withstand at low concentrations (Britto and Kronzucker, 2002). The passive, high capacity low affinity transport system is involved in NH_4^+ transport in this toxic range where the low-affinity NH_4^+ influx and efflux are carried out alongside and cause futile cycling of NH_4^+ across plasma membrane (Britto and Kronzucker, 2006). The plant's mineral composition is affected by NH_4^+ nutrition significantly mainly because of decline in cation concentration (Szczerba *et al*., 2006).

It has been postulated that there may be some direct antagonism among NH_4^+ and other cations for the common channels and K^+ channels are considered the main candidates for low affinity NH_4^+ transport as these both cations are monovalent and having the same hydrated atomic radii (Wang *et al*., 1996). Ammonium (NH_4^+) assimilation into plant metabolism is disturbed under saline condition. It is not stored in cells and found in only small amounts in plant tissues. Ammonium toxicity is generally observed when a large amount of free ammonium and/or ammonia is present in plant tissues (Gill and Reisenauer, 1993). Potassium ion plays a substantial role in stimulating various enzymes essential for growth that otherwise be inhibited by NH_4^+ by activating protein synthesis to lower down the intracellular ammonium concentration (MacKown *et al*., 1982), resulting enhanced ammonium assimilation in the presence of potassium (Xu *et al*., 1992). Thus, the role of K^+ under salt stress is also very important with respect to stress physiology, biochemical and metabolic processes. The good insight and knowing the particular mechanisms and interaction will be very helpful in improving plant growth under salt stress conditions. Keeping in view the above observations, this study was aimed to figure out the effect of different combinations of K^+ and NH_4^+ on plant growth under salt stress.

MATERIALS AND METHODS

Plant growth conditions: Seeds of maize (*Zea mays* L., cv. Pioneer-3335) were surface-sterilized for 10 min in 1% sodium hypochlorite (Ashraf and McNeilly, 1990) and were washed with distilled water. The seeds were sown in trays containing acid-washed two inch layer of sand. The proper moisture content was maintained with distilled water. Ten-days-old seedlings were transplanted in polystyrene sheet with foam plugged holes suspended over nutrient solution contained in polythene lined iron tubs. The experiment was carried out in a controlled conditions chamber with day and night temperatures of 25 and 18 °C respectively; while relative humidity was 60-65% (for detail see Saqib et al., 2011). The solution was exchanged weekly to ensure that plants remained at a nutritional steady state. The pH was maintained at 6.0±0.5. The aeration was provided for about 8 hours daily with the help of aeration pump. The different concentrations of potassium and ammonium were supplied by using KCl and $(NH_4)_2SO_4$, respectively. The potassium was applied at the rate of 3 and 9 mM K^+ using KCl salt in combination with two levels of ammonium i.e. 5 and 10 mM as $(NH_4)_2SO_4$ using the Completely Randomized Design layout in factorial arrangement along with one control treatment having only nutrient solution (½ strength Hoagland's solution; Hoagland and Arnon, 1950). Each treatment was replicated four times. The NaCl salinity stress was imposed after three days of transplantation by adding NaCl salt in the solution to achieve the desired salinity levels. The plants were slowly acclimated to saline environment by applying NaCl salt in three installments until a final concentration of 100 mM NaCl was attained.

Measurements: The 30 days old plants after treatment application were harvested for growth data and other measurements For chemical analysis of Na^+ and K^+, plant shoot of maize was air dried and ground plant material was wet digested using di-acid (HNO_3+$HClO_3$) and heated at 250±50 °C (Skoog *et al*., 2000) and filtered aliquots were used for ionic analysis. Sodium and potassium contents of plant samples were determined with Sherwood 410 flame-photometer. Flag leaves were desorbed for 5 min in a medium having 10 mM $CaSO_4$ to remove extracellular ammonium contents. Plant samples were transferred after weighing to vials having liquid N_2 and stored at -80 °C till before starting analysis. For analysis, 0.5 g of shoot samples was homogenized in 6 mL of 10 mM formic acid using a mortar and pestle (Husted *et al*. 2000). After centrifugation at 2.53 *g* at 2°C for 10 min, I ml of the supernatant was filtered using 0.45 mm nylon and transferred to 2 mL tubes to centrifuge again at 53 000 *g* (2 °C) for 5 min. The supernatant was used for determining total tissue NH_4 content following the *O*-phthalaldehyde (OPA) method (Goyal *et al*. 1988).

Statistical Analysis: The data were analyzed statistically using appropriate analysis of variance techniques. The differences among the treatment means were compared using DMR test at 5% probability level (Steel *et al*. 1997).

RESULTS AND DISCUSSION

The combine application of NH_4^+ with increasing level of K^+ under saline condition improved significantly ($p< 0.05$) physiological attributes of maize. Increase in NH_4^+ from 5 to 10 mM resulted in a decrease in dry matter production while the high potassium (9 mM) supply caused a significant ($P<0.05$) reduction in total ammonium content (10 mM) and improve growth relative to plants supplied with 3 mM potassium. A significant interaction between ammonium application, salinity and K^+ treatment on the shoot fresh weight was observed (Table 1). Under the saline condition, the supplementation of 5 mM ammonium with 9 mM K^+ resulted in the reduction of shoot fresh weight by 32% and at 10 mM NH_4^+ concentration, the reduction was about 43% relative to control. Similarly, the NH_4^+ application at the rate of 5 mM together with 3 mM K^+ decreased the shoot fresh weight by 34% while the addition of NH_4^+ at the rate of 10 mM showed a percent decrease upto 36% relative to control. The data of shoot dry weight under control and 100 mM NaCl showed a significant difference but the increasing NH_4^+ concentration (5-10 mM) significantly reduced the shoot dry weight under salinity stress (Table 2). The increasing trend of potassium 3 and 9 mM significantly improved the shoot dry weight. The reduction in relative growth rate is correlated with the accumulation of free NH_4^+ in shoot (Schortemeyer *et al*. 1997), but the application of K^+ was found to increase the shoot dry weight and fruit weight of melons under salinity stress (Kaya *et al*., 2007). Root fresh and dry weight of maize as affected by salt stress and supplementation of potassium and ammonium showed a substantial variation (Table 3 and 4). A major reduction in root fresh and dry weight was observed at 10 mM NH_4^+ level and 100 mM salinity level. While the potassium supply at 9 mM significantly improved these growth parameters in Pioneer-3335. Under stressed condition, the percent decrease in root dry weight induced by the combine application of NH_4^+ (5 mM) and K^+ (9 mM) was about 24% in Pioneer-3335 whilst at 10 mM NH_4^+ concentration, the percent decrease was upto 33% relative to control.

Table 1. Effect of K^+ on shoot fresh weight (g $plant^{-1}$) of maize having different levels of NH_4^+ under saline conditions

NH_4^+ (mM) as $(NH_4)_2SO_4$	Control		100 mM NaCl		Means
	3 mM K^+	9 mM K^+	3 mM K^+		3 mM K^+
5 mM	38.27d	59.36a	25.36f	40.42c	40.85A
10 mM	28.16e	42.27b	18.22h	24.17g	28.21B
Means	33.22B	50.82A	21.79D	32.30C	

Means sharing same letter do not differ significantly at $p < 0.05$

Table 2. Effect of K^+ on maize dry weight (g $plant^{-1}$) having different levels of NH_4^+ under saline conditions

NH_4^+ (mM) as $(NH_4)_2SO_4$	Control		100 mM NaCl		Means
	3 mM K^+	9 mM K^+	3 mM K^+	9 mM K^+	
5 mM	4.11c	6.23a	3.11d	4.78b	4.56A
10 mM	3.32d	4.87b	0.99f	2.33e	2.88B
Means	3.72B	5.55A	2.05C	3.56B	

Means sharing same letter do not differ significantly at $p < 0.05$

Table 3: Effect of K^+ on root fresh weight (g $plant^{-1}$) of maize having different levels of NH_4^+ under saline conditions

NH_4^+ (mM) as $(NH_4)_2SO_4$	Control		100 mM NaCl		Means
	3 mM K^+	9 mM K^+	3 mM K^+	9 mM K^+	
5 mM	21.24b	28.19a	12.22g	20.25c	20.48A
10 mM	13.25f	18.43d	9.30h	13.50e	13.62B
Means	17.25B	23.31A	10.76D	16.88C	

Means sharing same letter do not differ significantly at $p < 0.05$

Table 4. Effect of K^+ on root dry weight (g $plant^{-1}$) of maize having different levels of NH_4^+ under saline conditions

NH_4^+ (mM) as $(NH_4)_2SO_4$	Control		100 mM NaCl		Means
	3 mM K^+	9 mM K^+	3 mM K^+	9 mM K^+	
5 mM	2.7d	4.3a	2.2f	3.4c	3.2A
10 mM	2.5e	4.0b	1.9g	2.7d	2.8B
Means	2.6C	4.2A	2.1D	3.1B	

Means sharing similar letter do not differ significantly at $p < 0.05$

Table. 5: K^+/Na^+ ratio of maize having different levels of NH_4^+ under saline conditions

NH_4^+ (mM) as $(NH_4)_2SO_4$	Control		100 mM NaCl		Means
	3 mM K^+	9 mM K^+	3 mM K^+	9 mM K^+	
5 mM	1.68d	2.07a	0.54g	0.76e	1.26A
10 mM	1.85b	1.77c	0.47h	0.63f	1.18B
Means	1.77B	1.92A	0.51D	0.70C	

Means sharing similar letter do not differ significantly at $p < 0.05$

The ammonium adversely affect the root growth in Arabidopsis in absence of K^+ (Cao *et al*., 1993) and this harmful effect can be alleviated by potassium supply which activates the enzymes involved in NH_4^+ assimilation (Hagin *et al*., 1990). The potassium was applied at the rate of 3 and 9 mM to ameliorate the adverse effects of salinity and NH_4^+ toxicity to improve its dry matter. The root dry weight of perennial ryegrass is decreased with increasing salinity levels (Alshammary *et al*., 2004) so the better way to increase salt defense is to enhance the dose of potassium salts (Zheng *et al*., 2008). Potassium in the solution culture is readily absorbed by roots and transported to plant body (Akram *et al*., 2007).

The K^+ ameliorating effect was found significant on the ionic parameters of maize regarding NH_4^+ nutrition and salinity. A high K^+: Na^+ ratio was observed in the shoot under high potassium concentration (9 mM). While the reduction in K^+/Na^+ ratio was found under salinity stress (100 mM NaCl) and at 10 mM NH_4 level (Table 5). The NH_4^+ (10 mM) application in combination with 9 mM K^+

supply significantly decreased the sodium content in maize. The treatment means were found significantly ($P \leq 0.05$) different from each other. When plants are grown under saline condition, their potassium content decreased in rice genotypes (Castillo *et al*., 2007). This ion toxicity as Na^+ displaces K^+ in the presence of high concentration of NaCl salt in root-zone, leads to metabolic imbalances which cause reduction in growth and dry matter production (Zhu, 2001).

The higher K^+ contents in plant tissue under salt stress could be due to ability of plant to do selective K^+ uptake and selective cellular K^+ and Na^+ compartmentation and their distribution in the shoots (Carden *et al*., 2003).

The genetic variations among genotypes was observed for their ability to restrict the entry of Na^+ and selectivity for K^+ at root level and maintaining high K^+: Na^+ ratio in leaf and shoot by controlling their influx into the root xylem from root cells (Hu and Schmidhalter, 1997). Dogan *et al*. (2010) found that the metabolic processes carried out in plants are badly affected by low K^+/Na^+ ratio. A considerable decline in $K^+:Na^+$ ratio and consequently reduction in plant height, fresh and dry biomass as also noted in soybeans cultivars (Cicek and Cakirlar, 2008).

The K^+/Na^+ ratio and dry matter under the K^+ application at different low and high rates showed the maximum K^+/Na^+ ratio and dry matter at 9 mM potassium supply (Fig.1). The ability to maintain high K^+: Na^+ ratio by roots shows the selectivity for plant for K^+ over Na^+ through preferential uptake of K^+ ion into xylem rather than Na^+ (Carden *et al*., 2003; Maqsood et al., 2008). This strategy of the plant was recognized as an important tool to minimize growth reduction in saline soils (Santa-Maria and Epstein, 2001). The effect of NH_4^+ application on K^+/Na^+ ratio and dry matter resulted in a decrease of K^+/Na^+ ratio and dry matter at 10 mM NH_4^+ as compared to 5 mM NH_4^+ concentration (Fig.2.). The ammonium application from 5 to 10 mM resulted an increase in NH_4^+ contents in maize under saline condition, but a significant decrease when additional external K was noted in this study. The treatment means were found significantly ($P \leq 0.05$) different from each other (Table 6). K^+ prevented plants from the deleterious effect of high ammonium deposition by reducing its uptake as these both cations are monovalent and having the same hydrated atomic radii (Wang *et al*., 1996; White, 1996). Results of ionic parameters showed that the effect of potassium was significant regarding NH_4^+ nutrition. The increased potassium level (9.0 mM) significantly reduced high ammonium deposition and salinity stress in maize.

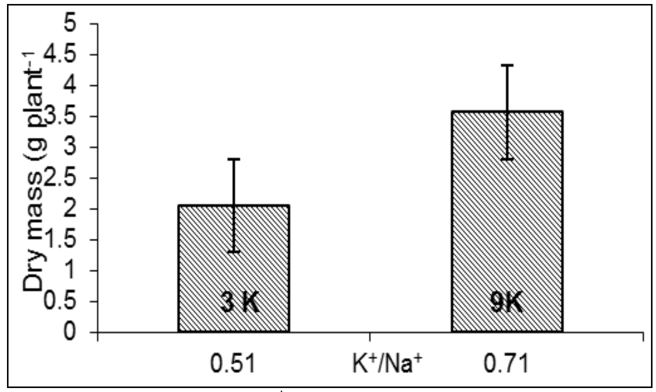

Figure 1. Impact of K^+ application (mM) as KCl in relation of K^+/Na^+ ratio and dry mass of shoot of maize under salt stress.

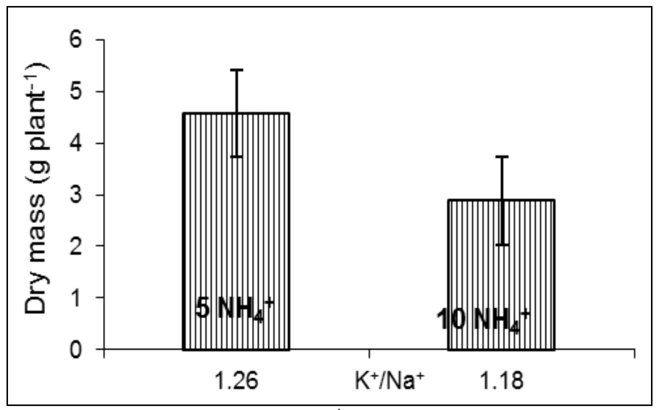

Figure 2. Impact of NH_4^+ application (mM) as $(NH_4)_2SO_4$ in relation of K^+/Na^+ ratio and dry mass of shoot of maize under salt stress

Potassium regulate NH_4^+ assimilation by activating many essential enzymes for growth that otherwise be suppressed by NH_4^+ (Xu *et al*., 1992). For instance, glutamine synthetase (GS) activity, which is basic NH_4^+ assimilation enzyme, in NH_4^+ sensitive species like barley and maize is increased with potassium application. Although, potassium is not a co-factor of GS, yet, it improves it indirectly by giving relief to plants and improving its overall growth by

Table 6. Effect of K^+ on ammonium (μmol g^{-1}) concentration in maize shoot having different levels of NH_4^+ under saline conditions

NH_4^+ (mM)	Control		100 mM NaCl		Means
as $(NH_4)_2SO_4$	3 mM K^+	9 mM K^+	3 mM K^+	9 mM K^+	
5 mM	30b	22d	26c	19d	24.25A
10 mM	38a	29b	29b	21e	29.25B
Means	34A	25.5C	27.5B	20D	

Means sharing same letter(s) do not differ significantly at $p < 0.05$

different phenomena which include (1) reduce NH_4^+ influx (2) reduce Na^+ influx (3) act as osmolyte (4) maintain cellular pH as NH_4^+ decrease cellular pH (5) act as enhancing GS activity in NH_4^+ sensitive species like barley and maize (Balkos *et al.* 2010). If plants have inadequate potassium level, ammonium is taken both by K^+ channels and non-selective cation channels (NSCCs). So increasing K^+ in NH_4^+ tolerant species e.g. rice diminishes excessive uni-directional NH_4^+ inflow that will help in less NH_4^+ uptake as needed in saline soil due to reduced growth.

Conclusion: The external application of K^+ play a positive role in the regulation of NH_4^+ toxicity and consequently, will help the salt stressed pant in reducing energy burden of toxic NH_4^+ efflux to uphold plant growth under high-NH_4^+ nutritional and salinity stress environment.

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