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Physiological Approach for Different Chloride Stress Tolerance and Ion Accumulation Through Cell Sap in Little Millet (*Panicum colonum* L.)

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Abstract. Salinity stress is one of the most serious environmental factors adversely affects plant growth and yield in Pakistan and around the world. Little millet (*Panicum colonum* L.) is an important cereal crop which is principal source of food and is extensively grown in Pakistan and other areas of the world. The pot experiment was carried out in CRD design with six treatments including control contained three replications in the Department of Food Science and Technology, Hamdard University Karachi. Plants of Little millet (*Panicum colonum* L.) watered as 00 mM control, 120 mM of each NaCl, $MgCl_2$, $CaCl_2$, mixture of salts and natural salt concentrations. The overall osmotic potential increased by about 57% on 120 (mM) concentration of mixture of salts. Satisfactory K^+/Na^+ ratio was obtained from 120 mM of $CaCl_2$ treatment, sufficient Ca^{2+} (mM) content was noted in 120 mM of mixture of salts treatment in the cell sap of (*Panicum colonum* L.) leaves as compared to other treatments and control, respectively. However, increased osmotic potential about 58 and 88% on 120 mM of mixture of salts and natural salt, such condition of osmotic potential under various chloride stress conditions for little millet (*Panicum colonum* L.) claimed to be stress tolerant.

Keywords: chloride stress, cell sap, ion content, osmotic potential, physiology

Introduction

Salinity is an increasing problem of the world, an estimated 3.8 billion acres of land are salt-affected, with a third to a half of all irrigated land suffering from reduced production vice versa due to the excessive salts (Choukr-Alah, 1996). Increasing salinity threat to agriculture as it can substantially reduce plant growth, crop quality, crop productivity and soil condition (Munns *et al*., 2006; Ashraf, 1994; 2004; Flower, 2004). Salinization of soil may result from salt water intrusion, run-off from salt used during road de-icing and buildup of brine salts in irrigated regions (Merrill *et al*., 1990). Several researchers have suggested that salt accumulating halophytes could be used to revegetate and improve the quality of saline soils (Qadir *et al*., 1995; Zhao, 1991). Salinity produced by weathering in the form of large quantity of salts *viz* calcium, sodium, magnesium, chloride, potassium, carbonates, bicarbonates (Zaman and Al, 2005) and these salts gathered in solution to create osmotic pressure and water then uptake by plants. Millets are small grained cereals include finger, kodo, foxtail, proso, little millets and barnyard millets. They are the staple food of the million's inhabitation the arid and semi-arid tropics of the world. They are distributed in most of the Asian and African countries and part of Europe. The grains of little millets being nutritionally superior to rice and wheat, provide cheap source of proteins, minerals and vitamins to poorest of the poor where the need for such ingredients is the maximum (Weber, 1991). Millets are classified as glycophytes, having threshold to salinity resistant from 1-10 d S/m (Maas, 1993).

Salinity causes various effects in cell metabolism and inhibit plant growth, maximum salts create toxicity leads to disorder for nutrition uptake (Greenway and Munns, 1980). In high saline condition up to 14 d S/m

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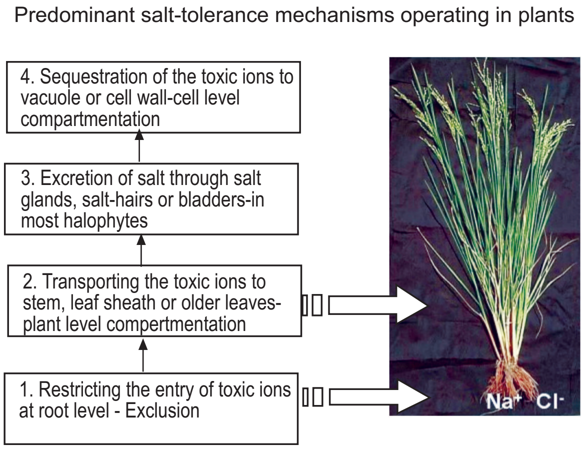

Fig. 1. Mechanism of salt tolerant in plants.

Ca^+, Na^+, Fe^{++}, Mn^{++} increased, while K^+ decreased in leaves of pearl millet (Alam and Naqvi, 1991). Traditionally little millets are the habitats of dry land location. However, they respond to irrigation and an immediate need to select genotypes for better water use efficiency (Zhu, 2001). The aim of this research to find out best treatment for adaptation of little millet (*Panicum colonum* L.) under adverse salinity environment.

Materials and Methods

Soil analysis. A plough layer fertile soil was taken, the pots were placed in a CRD design. The following parameters were analyzed:

- Soluble Na^+ and K^+ by flame photometery following USSL (1954) method.
- Ca^{2+} and Mg^{2+} (by titration method of USSL, 1954). The concentration of Na^+, Ca^{2+}, Mg^{2+} was in meq/L.

Method and time of sowing. Little millet (*Panicum colonum* L.) seeds was obtained from local market and sown in each pot. Ten seeds were placed in each pot 4 cm apart at 1 cm depth in summer season. After emergence, six seedlings were allowed to grow in each pot up to maturity.

Preparation of saline (NaCl, $MgCl_2$, $CaCl_2$, mixture of salts and natural salt extract) solutions. A range of concentrations (0 and 120) of each solution was prepared by dissolving NaCl, $MgCl_2$, $CaCl_2$, mixture of salts in distilled water and natural salt extract was separately prepared by collecting the soil having 120 mM concentration. In the control treatment, plants received only freshwater but in NaCl, $MgCl_2$, $CaCl_2$, mixture of salts in distilled water and natural salt extract treatments, plants were watered with 120 mM of NaCl, $MgCl_2$, $CaCl_2$, mixture of salts in distilled water and natural salt extract solutions, respectively. The volume of each solution and water applied to plants in each treatment was calculated on the basis of field capacity moisture.

Table 1. Methods used for cell sap analysis

Ions	Method and equipment used for analysis
Na^+ and K^+	Flame photometer.
Ca^{2+} and Mg^{2+}	Titration with standard EDTA solution.
Cl^-	Titration with standard silver nitrate ($AgNO_3$) solution

Flag leaf sampling and cell sap extraction for ion analysis. Fully expanded flag leaf of each plant was detached. The lamina of each leaf was removed. Leaf matter was then placed in micro centrifuge tubes and stored in a deep freezer at -10 °C. After thawing the leaf matter was crushed with blunt rod. The sap was then extracted through micro-centrifuge using the method of Gorham *et al*. (1997). The sap was diluted and analyzed for Na^+, Ca^{2+}, Mg^{2+}, K^+ and Cl^- in Table 1.

Ion analysis. Cell sap was obtained from each treatment replication wise for the following observation *viz* osmotic potential/plant (-Mpa), Na^+ content/plant, K^+ content/plant, K^+/Na^+ plant, Cl^- content/plant and Ca^{2+} content/plant).

Data analysis. The data was analyzed statistically using Minitab (11.12 32 Bit) software.

Results and Discussion

Osmotic potential (-Mpa) in little millet (*Panicum colonum* L.) under salt stress. The values in Fig. 2 reveal that when plants of (*Panicum colonum* L.) were treated with 120 (mM) natural salt solution the highest osmotic potential/plant was observed in relation to control and other treatments, respectively. Many studies revealed that due to osmotic stress the crop growth and yield usually affected and associated with nutrient uptake (Shrivastava and Kumar, 2015). Various physiological processes like, photosynthesis, respiration, carbohydrate metabolism and nitrogen fixation are disturbed during stress (Farooq *et al*., 2015). The conclusion was that salinity stress contain osmotic and ionic component related with accumulation of toxic elements on higher levels of (Na^+ and Cl^-) reported by (Lefevre *et al*., 2001).

For osmotic adjustment proline alleviates membranes, proteins, hunts free radicals and buffers at cellular level under stress conditions (Boscaiu *et al*., 2012).

Sodium content (mM) in little millet (*Panicum colonum* L.) under salt stress. The higher Na^+ (mM) concentration from cell sap (leaves) of (*Panicum colonum* L.) was obtained in 120 (mM) natural salt in Fig. 2. Similarly, 15, 5, 50, 17 and 62% of Na^+ (mM) was obtained on all the treatments of 120 (mM) of NaCl, $MgCl_2$, $CaCl_2$, mixture of salts and natural salt. However, on 120 (mM) of natural salt treatment the plants accumulate more Na^+ (mM) as compared to other treatments and control. Comparable finding were presented by Fatemi *et al*. (2019) that K^+ element influence the growth of plant. Elemental stress creates salinity stress for influx of Na^+ and Cl^- ions in tissues of plant can cause higher level of element imbalance which finally creates unfavorable conditions, while Shah *et al*. (2021) and James *et al*. (2011) recommended that higher Na^+ levels in plant tissues delays the uptake of ions which are crucial for growth and development, that is K^+ ions, which is principal source minimum yield.

Potassium (mM) in leaves of little millet (*Panicum colonium* L.) under salinity stress. The maximum K^+ (mM) content in leaves of (*Panicum colonum* L.) plants was obtained when irrigated with 120 (mM) of mixture of salts and natural salt treatments as compared to control and other treatments. However, 15, 9, 1, 29 and 31% K^+ reduction was observed in all the treatments of 120 (mM) of NaCl, $MgCl_2$, $CaCl_2$, mixture of salts and natural salt as compared to control and other treatments in Fig. 3. Similarly, higher Na^+ levels in plant tissues lowers the uptake of elements important for growth and development that is K^+ ions that is chief source of decreasing of production (James *et al*., 2011). The roots of transgenic plants accumulate lower amount of Na^+ and K^+ as compared to local plants when stressed with higher Na^+ and K^+ treatments (Mahalakshmi *et al*., 2006).

Level of K^+/Na^+ in little millet (*Panicum colonum* L.) leaves under salinity stress. The Fig. 4 indicating that maximum K/Na ratio was obtained from 120 (mM) of $CaCl_2$ in the leaves of (*Panicum colonum* L.) responded than other treatments and control. Likewise, halophytes usually revealed minimum root/shoot growth and overall growth of plant is also retarded, contain

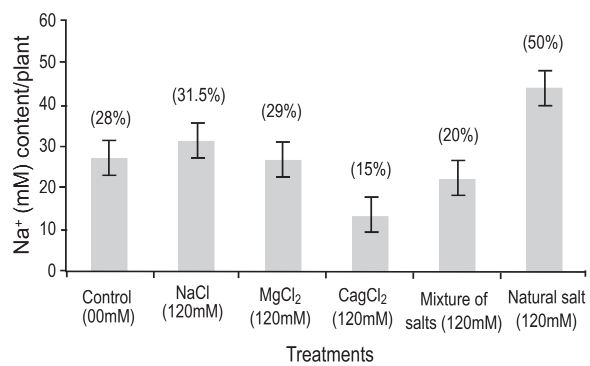

Fig. 3. Effect of different treatments of chloride salts of 120 mM on Na^+ content/plant of (*Panicum colonum* L.); SED = 0.35; LSD (0.05%) = 0.76.

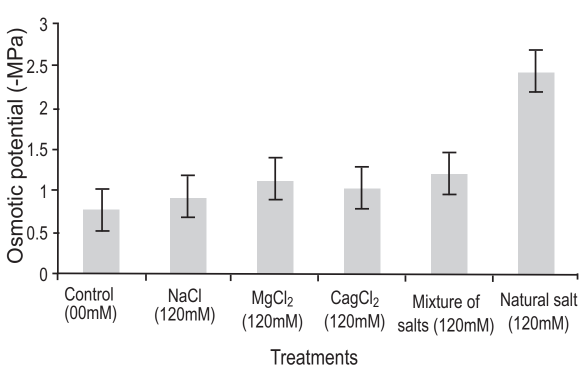

Fig. 2. Effect of different treatments of chloride salts of 120 mM on osmotic potential/plant of (*Panicum colonum* L.); SED = 3.40; LSD (0.05 %) = 7.41.

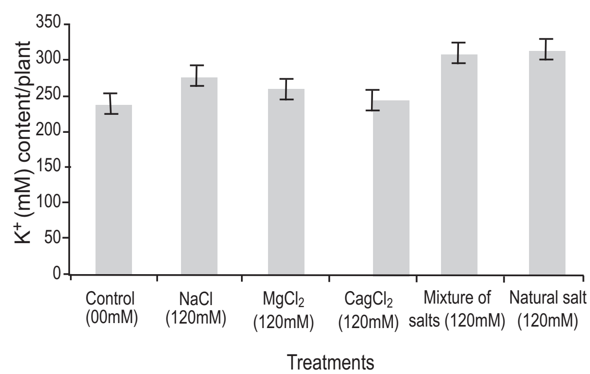

Fig. 4. Effect of different treatments of chloride salts of 120 mM on K^+ content/plant of (*Panicum colonum* L.); SED = 0.52; LSD (0.05%) = 1.12.

lower Na^+/K^+ ratio in leaves, while higher amount of total sugars retain in tissues as compared to glycophytes (Rahman *et al*., 2014). Under salinity stress different physiological and biochemical parameters such as biomass, Na^+ and K^+ ratio, membrane stability and chlorophyll content are not affected (Mukami *et al*., 2020; Ishikawa and Shabala, 2019).

Quantity of chloride (mM) in *Panicum colonum* plants under salinity stress. The higher Cl^- content in leaves of (*Panicum colonum* L.) was calculated when plants were watered with 120 (mM) of natural salt as compared to control and other treatments in Fig. 5. Concerning to this research other coworkers like Mariani and Ferrante (2017) declared that under drought/salinity conditions the cultivation of finger millet could be on high risk or can face critical conditions. Additionally, if calcium could use instead of nitrates, the $Cl^–$ make bonding with sodium ions to accumulation in the vacuoles. They also suggested that addition of calcium nitrate or chloride (Cl^-) can move the sodium elements from soil solution after application of irrigation.

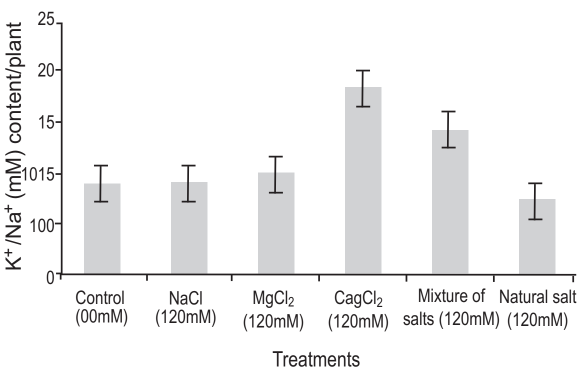

Fig. 5. Effect of different treatments of chloride salts of 120 mM on K^+/Na^+/plant of (*Panicum colonum* L.); SED = 3.33; LSD (0.05%) = 7.25.

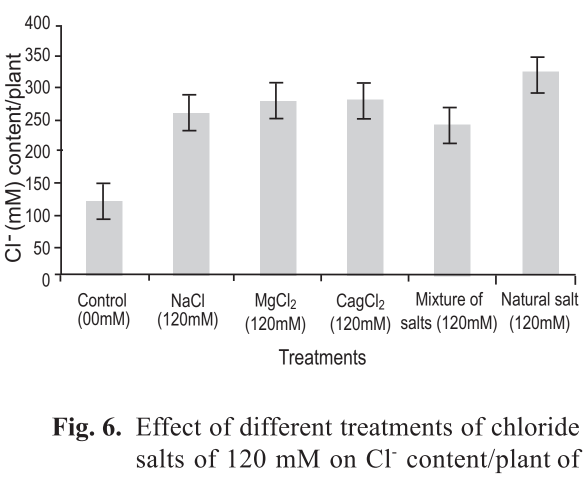

Fig. 6. Effect of different treatments of chloride salts of 120 mM on Cl^- content/plant of (*Panicum colonum* L.); SED = 0.38; LSD (0.05%) = 0.82.

Calcium content (mM) in *Panicum colonum* under salinity stress. The Fig. 6 shows the description regarding Ca^{2+} (mM) content in (*Panicum colonum* L.) plants. The highest Ca^{2+} (mM) content was noted in 120 (mM) of mixture of salts as compared to control and other treatments. Various other studies reveal that the application of fertilizers which contain maximum Ca^+ and Mg^+ can improve the soil condition and give suitable environment to root and plant growth. From physiological point of view calcium used is in the form of nitrates, the $Cl^–$ ions compete with Na^+ ions to accumulate in vacuoles. The application of nitrates to sodic soils may reduce salt uptake, although this aspect requires validation (Mariani and Ferrante, 2017).

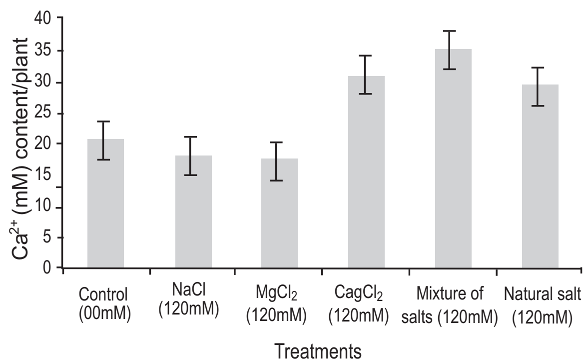

Fig. 7. Effect of different treatments of chloride salts of 120 mM on Ca^{2+} content/plant of (*Panicum colonum* L.); SED = 0.09; LSD (0.05%) = 0.20.

Conclusion

This research concluded that (*Panicum colonum* L.) greater osmotic potential/plant indicate a positive relationship and noticeable level of Na^+ on 120 (mM) of natural salt treatment when compete to other treatments and control. Similarly optimum K^+ was obtained on both treatments of 120 (mM) of mixture of salts and natural salt, adequate amount of K^+/Na^+ recorded on 120 (mM) of $CaCl_2$ treatment, higher Cl^- content was obtained on 120 (mM) of natural salt treatment, optimum Ca^{2+} content was noted under 120 (mM) of mixture of salts stress as compared to control and other treatments, respectively. Comparison of treatments showed that on an average osmotic potential increased by about 57% when the 120 (mM) concentration of mixture of salts.

Acknowledgement

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Conflict of Interest. The authors declare that they have no conflict of interest.

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