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Full Length Research Paper

Relationship between growth and ion relation in pearl millet (*Pennisetum glaucum* (L.) R. Br.) at different growth stages under salt stress

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Seedlings of two pearl millet (*Pennisetum glaucum* (L.) R. Br.) lines were exposed to 0 (control) and 100 mol m^{-3} NaCl salinity. Treatments were applied fourteen days after germination. Growth of the shoot and root system and ion contents were determined at seedlings (21 days after treatment) and vegetative stage (42 days after treatment). NaCl concentration caused reduction in the plant growth, particularly of the shoot, always with much intensity in the sensitive line. Results obtained indicated that the difference in growth between these two lines might be due to differences in ion transfer rates to the shoot and salt accumulation in the shoot. The sensitive line showed higher Na^+ plus Cl^- transfer rates to the shoot, especially in the beginning of the stress application and greater accumulation of these ions in the leaves The tolerant line, on the other hand, showed higher K^+ transfer rates and lower relative reduction in the Ca^{2+} transfer rates to the shoot under salt stress. So, these results suggest that tolerance to salt stress, in pearl millet lines studied may be related to plant ability to prevent accumulation of toxic ions like Na^+ and Cl^- and to maintain the shoot. Plant ability to make adequate osmotic adjustment, however, should not be ignored.

Keywords: Salinity, growth, ions, pearl millet.

INTRODUCTION

Many species of higher plants, including most crops, are subjected to growth inhibition under high-NaCl conditions. The salt-induced inhibition of plant growth, is caused not only by osmotic effects on water uptake but also by variable effects on plant cell metabolism. While the first component can bring about water deficit, the excess of a specific ion can cause toxicity and can induce nutritional disorders (Greenway and Munns, 1980).

Salinity is the process of accumulation of soluble slats, by which saline soils are produced. The salts in large amounts mostly are calcium, sodium, magnesium, chlo-ride and sulphate ions and in relatively small amounts are potassium, carbonates, bicarbonates, solution due to borate and lithium salts (Fitzpatick, 1980). Accumulation of these salts increases the osmotic pressure of the soil to which water intake by plants is restricted (Cramer et al., 1999).

Several repots appearing in the literature revealed that salinity causes many adverse effects on the morphology, anatomy and physiology of pearl millet (Maliwal and Paliwal, 1970; Bafana and Paikh, 1981). For instance, percent germination, height, grain and straw yield of pearl millet decreased with increasing concentration of salinity (Gundalia et al., 1992).

Salinity stress disturbs the uptake and accumulation of essential nutrients (Greenway and Munns, 1980; Shannon and Grieve, 1999; Zhu, 2001). Generally, Ca^{2+} and K^+ are decreased in plants under saline conditions (Khan, 1993; Al-Harbi, 1995) In contrast, Ashraf and Rauf (2001) reported that under saline conditions concentrations of Na^+, K^+ and Ca^{2+} increased significantly in all the parts of germinating from maize seeds primed with NaCl, KCl, and $CaCl_2 \cdot 2H_2O$, respectively. Alam and Naqvi (1991) observed that plant height and dry matter yield decreased with increase in salinity at 85-days old plants of pearl millet under salinity levels of 1.95, 4.69,

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Table 1. Effect of NaCl treatment on the shoot and root dry matter yield (g pot^{-1}) and shoot/roots ratio in two pearl millet lines

NaCl (mM)	Shoot	Root	Shoot/root-ratio
IC-8206			
0	0.074 aA	0.032 aA	1.8 aA
100	0.044 bA	0.024 bA	1.6 bA
18-BY			
0	0.058 aB	0.032 aA	1.9 aB
100	0.031 bB	0.022 bA	1.6 bB

Means, followed by the same small letter (between NaCl treatment, in each line) and by the same capital letter (between lines, in each NaCl treatment) for each plant part does not statistically at 5%.

9.38 and 14.06 dS m^{-1} NaCl. Salinity caused an increase in N, P, Ca^{++}, Na^{+}, Fe^{++} and Mn^{++} and decrease in K^{+} contents of the leaves.

The objective of this study was to assess the effects of NaCl concentration on the plant growth, solutes transfer from root to shoot accumulation and distribution of inorganic solutes (Cl^{-}, Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}) in order to better understand the ion transfer mechanism in pearl millet.

MATERIAL AND METHODS

Seeds of two pearl millet (*Pennisetum glaucum* (L.) R. Br.) lines, IC-8206 (salt tolerant) and 18-By (salt sensitive) were obtained from the Maize and Millet Research Station, Yousafwala, District Sahiwal, Pakistan. Seeds were surface sterilized by dipping in 10% sodium hypochlorite solution for 10 min, then rinsed with sterilized distilled water and air-dried at an ambient temperature of 32°C in the laboratory. Two levels of NaCl salt (0 and 100 molm^{-3} NaCl) were applied after 14 days of germination. The experiment was laid out as Completely Randomized Block Design (CRBD) with six replicates. Plants were harvested at seedlings stage (21- days after treatment) and vegetative stage (42 days after treatment).

Plants were uprooted carefully and washed in distilled water. Shoot, root and 1st internode length was measured with the help of scale meter in cm at final harvest (42 days after treatment). Plant samples were placed in oven at 75°C. After 4 days shoot and root dry weight (g/pot) was calculated with the help of electric balance at final harvest (42 days after treatment).

Dried plant material was finely ground and digested with a nitric-perchloric mixture. In stems plus sheaths, roots, young leaves (2nd leaf starting from the top) and old leaves (3rd leaf starting from the top) ion contents of Na^{+} and K^{+} were determined by emission spectrophotometry and of Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry (Allan, 1969). Data obtained was used to estimate the net ion transfer rates to the shoot according to the equation proposed by Salim and Pitman (1983) at both harvests (21 and 42 days after treatments). Chloride was extracted by stirring ground-dried samples with 0.1 M $NaNO_3$ for 30 min. After extract clarification with activated coal, it was added 13.2 mM $Hg(SCN)_2$ in methanol and 20.2% (w/v) $Fe(NO_3)_3$ (4 + 1) and the absorbance determined at 460 nm (Gaines et al., 1984).

Analysis of variance (ANOVA) technique was employed for carrying out statistical analysis of data collected (Steel and Torie, 1980). The means values were compared with Least Significant Difference (LSD) Test, following to Snedecor and Cochran (1980).

Table 2. Effect of NaCl treatment on the length (cm) of stem and roots of the stems and of the first internode in two pearl millet lines

NaCl (mM)	Root	Shoot	1st Internode
IC-8206			
0	19.6 aB	4.1 aA	1.4 aA
100	17.5 bB	3.4 bA	0.9 bA
18-BY			
0	28.6 aA	3.1 aB	1.2 aB
100	24.7 bA	2.0 bB	0.6 bB

Means, followed by the same small letter (between NaCl treatment, in each line) and by the same capital letter (between lines, in each NaCl treatment) for each plant part does not statistically at 5%.

RESULTS AND DISCUSSION

Shoot and root dry matter yield (g pot^{-1})

NaCl salt stress caused reductions of 28 and 35% in shoot and of 20 and 25% in root after 42-days of treatment dry matter yields in pearl millet lines IC- 8206 and 18-BY, respectively (Table 1). The tolerant line (IC-8206) yielded larger amount of shoot dry matter than the sensitive line (18-BY) . The shoot/roots dry matter ratio reduced 12 and 16% in the tolerant and sensitive lines, respectively. The high ratio of shoot/root dry matter (smallest reduction in the ratio) was noted in tolerant line in NaCl treated plants. It can be suggested that this plant characteristic could be an important tolerance indicator of salt stress in pearl millet.

Similarly, studies in rice varieties, had also shown a positive correlation between shoots/roots ratio for tolerance to NaCl salinity (Lutts et al., 1996). According to Shannon et al. (1994), the decrease in shoot/roots ratio under saline conditions, allows a better use of soil moisture and nutrients, which may have a beneficial effect on plant growth.

Shoot, root and internode length (cm)

After 42 days of treatment with 100 mol m^{-3} NaCl there was 8.6 and 10.8 % reduction in length of root system of in tolerant (IC-8206) and sensitive (18-BY) lines, respectively (Table 2). Similar, results were observed in barley seedlings by Cramer et al. (1989). In case of length of stem and first internode, sensitive line (18-BY) showed reductions much stronger than the tolerant line (IC-8206).

Ion transfer rates (m mol kg^{-1} dry mass day^{-1}) to shoot

The Na^{+} and Cl^{-} transfer rates to the shoot was six times higher in 100 mol m^{-3} NaCl treated plants as compared to control in both lines of pearl millet, during the period of 14 to 21 days (Table 3). The sensitive line (18-BY) under

Table 3. Effect of NaCl treatment on ion transfer rates (m mol kg^{-1} dry mass day^{-1}) to shoot in two pearl millet lines

Period: 14 to 21 days						
Lines	**NaCl (mM)**	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Cl^-**
IC-8206	0	58.1 bA	551.3 Aa	92.1 aB	62.7 aB	101.2 bA
	100	402.3 aB	262.1 bA	68.7 bB	48.5 bB	395.2 aB
18-BY	0	71.6 bA	532.4 aB	151.2 aA	91.2 aA	201.1 bA
	100	565.3 aA	142.5 bB	82.6 bA	77.7 bA	504.4 aA
Period: 21 to 42 days						
Lines	**NaCl (mM)**	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Cl^-**
IC-8206	0	54.8 bA	695.1 aA	144.2 aA	82.6 aA	307.4 bA
	100	244.1 aA	242.6 bA	62.7 bA	49.0 bA	444.8 aA
18-BY	0	35.8 bA	501.3 aB	184.2 aA	68.5 aB	185.1 bB
	100	165.5 aA	126.4 bB	62.3 bA	42.6 aA	311.3 aA

Means, followed by the same small letter (between NaCl treatment, in each line) and by the same capital letter (between lines, in each NaCl treatment) for each plant part does not statistically at 5%.

salt stress showed these ions transfer rates significantly higher than the tolerant line (IC-8206).

In the second experimental period, between 21 and 42 days, it was also observed a strong increase in ion transfer rates due to salt stress but there was no significant difference between lines, although the highest Na^+ and Cl^- concentrations were found in the shoot of sensitive line. The smaller Na^+ and Cl^- transfer rates shown by the tolerant line under salt stress at the first experimental period may be due to a better control of the root system absorption of these ions (Greenway and Munns, 1980), a higher salt retention in the roots (Salim and Pitman, 1983), a stronger retranslocation of these ions to the roots via phloem, a higher root efflux of these ions, particularly of Na^+, to the external solution (Lessani and Marschner, 1978; Moya et al., 1999).

The K^+ transfer rate to the shoot in the tolerant line was statistically higher at both experimental periods studied, regardless of the treatment applied (Table 3). The salt stress strongly reduced the K^+ transfer rate to the shoot in both lines, in two studied periods, especially in the sensitive line. The smaller K^+ transfer to the shoot under saline conditions is probably due to a reduced K^+ absorption by the roots. This is may be due to the competition of ion Na^+ with K^+ for the same absorption site in the plasma membrane (Marschner, 1995; Taleisnik and Grunberg, 1994).

In control plants, the Ca^{2+} and Mg^{2+} transfer rates to the shoot in the first experimental period were smaller in tolerant line (Table 3). After salt treatment, both ions transfer rates to the shoot were reduced in two lines Ca^{2+} transfer rate reduction in the sensitive line (35%) was much higher than in tolerant one (20%). This is may be due to the absorption and translocation of various mineral ions (specially Ca^{2+}) in shoot depend upon the transpiration flux, due to reduced transpiration rates of NaCl stressed plants, as suggested by Wolf et al., (1990).

These reductions in transfer rates of ions could also be due to a lowering of the root pressure in salt stressed plants (Marschner, 1995). In spite of that, however, one cannot reject the possibility of an inhibition of the root absorption of these ions by an excess of Na+ in the external solution (Cramer et al., 1985).

Inorganic solutes accumulation and distribution

The Na^+ and Cl^- contents greatly increased in NaCl treated plants with 100 mol m^{-3} as compared to control plants (0 mol m^{-3} of NaCl) in both lines. Na^+ contents increased significantly in 18-BY line (Table 4). The highest contents of these ions were found by order in stems *plus* sheaths, roots, young leaves (2nd leaf starting from the top) and old leaves (3^{rd} leaf starting from the top). This salt accumulation in the stems *plus* sheaths region was taken as indicative of a pearl millet salt tolerance mechanism quite common in most of glycophytes (Greenway and Munns, 1980).

According to this suggestion tolerant plants would be able to avoid salt accumulation in the leaves by removing it from xylem and retaining or accumulating it in stems, sheaths or petioles before reaching the leaves (Jeschke and Wolf, 1988). Besides, these toxic ions could be compartmentalized in specific tissue or cell of the leaf blade (Boursier and Läuchli, 1989). Therefore, it is feasible that salt tolerance could be associated to the synchronization between rate of ion transport to the shoot and the capacity to compartmentalize them in different tissues and cells.

The K^+ contents in control plants were much higher than that of Na^+ and Cl^- and reduced after NaCl treatment (Table 4). The salt stimulate reductions in K^+ contents were more severe in sensitive line (18-BY), except in the oldest leaves. These results indicate that the sensitive line under salts tress transfers lower amounts of K^+ to the

Table 4. Effect of NaCl treatment on inorganic solutes (m mol kg^{-1} dry mass) contents in different plant parts of two pearl millet lines

Solute contents	NaCl (mM)	2nd leaf	3rd leaf	Stem plus sheath	Roots
IC-8206					
Na^+	0	12.4 bA	13.5 bA	24.6 bA	48.6 bA
	100	412.4 aB	87.2 aB	954.1 aB	6575.4 aB
K^+	0	965.2 aA	654.6 aA	1033.2 aA	987.7 aB
	100	548.6 bA	488.8 bA	721.6 bA	525.5 bA
Ca^{2+}	0	65.5 aB	125.2 aB	132.5 aB	50.0 aA
	100	54.4 aA	140.0 aA	45.5 bA	39.6 bA
Mg^{2+}	0	77.7 bB	95.6 bB	199.8 aB	136.4 aB
	100	122.2 aB	121.5 aB	131.1 bA	123.4 bB
Cl^-	0	181.4 bA	173.5 bA	330.6 bA	126.7 bA
	100	472.8 aB	358.6 aB	978.3 aB	528.2 aB
18-BY					
Na^+	0	28.3 bA	24.6 bA	36.6 bA	59.9 bA
	100	632.3 aA	462.6 aA	879.6 aA	735.6 aA
K^+	0	874.6 aB	446.6 aB	886.1 aB	698.8 aA
	100	420.5 bB	391.8 bA	305.5 bB	356.6 bA
Ca^{2+}	0	132.6 aA	189.9 aA	155.5 aA	69.6 aA
	100	68.6 bA	126.7 bB	44.4 bA	41.1 aA
Mg^{2+}	0	98.9 bA	156.6 bA	260.0 aA	184.9 bA
	100	123.3 aA	165.2 aA	145.6 bA	202.5 aA
Cl^-	0	165.6 bA	198.8 bA	442.2 bA	174.8 bA
	100	470.7 aA	502.6 aA	853.3 aA	870.1 aA

shoot and it is not capable to maintain an adequate K^+ concentration in leaves, as does the tolerant line. Part of the salt tolerance differences between these lines, therefore, may reside in their differences in K^+/ Na^+ selectivity and in the transfer of K^+ to the shoot, as suggested by Marschner (1995).

The Ca^{2+} contents in the shoot of the control plants were higher in sensitive line than tolerant line (Table 4). The application of salt stress, however, resulted in a strong reduction of Ca^{2+} contents mainly in the shoot of plants in the sensitive line but only in the stems *plus* sheaths of the tolerant line. On average, the sensitive line experienced a reduction in Ca^{2+} contents twice as much than the tolerant line. The greatest reductions in the contents of this ion were observed in the stems *plus* sheaths in both lines, especially in the sensitive one. A great decrease in Ca^{2+} contents were also observed in the young leaves of the sensitive line and this could contribute to higher leaf growth inhibition in this line (Läuchli et al., 1994). The Ca^{2+} contents in the roots did not differ between lines and salt stress reduced them only in the tolerant line.

In general, Mg^{2+} contents in the sensitive line were higher than in the tolerant line, regardless of the treatment applied (Table 4). The salt stress caused reduction in Mg^{2+} contents in the stems *plus* sheaths in both lines, especially in the sensitive one. On the contrary, in the leaf blades Mg^{2+} contents increased in salt stressed plants of both lines. Increase or reduction in Mg^{2+} con-tents in leaves caused by excess of salts has been observed in other species (Araújo, 1994; Lutts et al., 1996; Meloni, 1999) . In spite of that, the significance of the change in Mg^{2+} contents during salt stress to plant tolerance is not clear yet (Araújo, 1994; Lacerda, 1995).

The Na^+/K^+ ratio, which was very small in the control plants, increased in plants exposed to NaCl in the two lines, particularly in the sensitive one. This increase took place in all plant parts studied, but mainly in the stems plus sheaths. In this plant part the Na^+/K^+ ratio in sensitive line became 1.8 times higher than in the tolerant. It is interesting to observe that, at percent basis, the tolerant line under salt stress not only retained more Na^+ in the stem plus sheaths (Table 4) but also exhibited a smaller reduction in K^+ transfer to shoot (Table 3) and maintained higher K^+ contents in the leaves (Table 4) than the sensitive line.

Apparently, due these factors, the tolerant line was able to maintain a leaf metabolic activity higher than that of the sensitive one. The total concentration of Na+ plus Cl-, which was low in the control plants, increased about 5.5 and 8.2 times, on average, in the tolerant and sensitive lines, respectively, with the application of the salt stress.

CONCLUSIONS

NaCl concentration caused reduction in the plant growth, particularly of the shoot, always with much intensity in the sensitive line. Results obtained indicated that the difference in growth between these two lines might be due to differences in ion transfer rates to the shoot and salt accumulation in the shoot. The sensitive line showed higher Na^+ plus Cl^- transfer rates to the shoot, especially in the beginning of the stress application and greater accumulation of these ions in the leaves. The tolerant line, on the other hand, showed higher K^+ transfer rates and lower relative reduction in the Ca^{2+} transfer rates to the shoot under salt stress. So, these results suggest that tolerance to salt stress, in two pearl millet lines studied may be related to plant ability to prevent accumulation of toxic ions like Na^+ and Cl^- and to maintain the shoot. Plant ability to make adequate osmotic adjustment, however, should not be ignored.

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