

ION UPTAKE AND DISTRIBUTION IN *PANICUM ANTIDOTALE* RETZ. UNDER SALT STRESS

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Abstract

In the present project, distribution of K^+ , Na^+ , Mg^{2+} Ca^{2+} and inorganic phosphate between the leaves of different age of *Panicum antidotale* was determined. The plant exposed to high level of salinity experiences a severe reduction in shoot fresh and dry matter yield. Accumulation of Na^+ in leaves increased with decrease in potassium. However, this antagonistic relationship between Na and K was more in older leaves. Hence lowest K^+/Na^+ ratio in the oldest lamina and highest was observed in youngest. As Na^+ is translocated mainly through xylem and K^+ through phloem, the younger leaves close to shoot apex would derive their mineral requirements initially from Phloem. Inflorescence accumulated low Na^+ and high K^+ resulting in highest K^+/Na^+ ratio under both non-saline and saline conditions. This could be one of adaptive features for acclimatizing salt stress. Accumulation of Ca^{2+} and Mg^{2+} also decreased in older leaves particularly under saline conditions. However, accumulation of Ca^{2+} and Mg^{2+} was higher in older leaves indicating both elements were phloem-immobile. Concentration of phosphate increased with the decreasing age both in the lamina and leaf sheath. Inorganic phosphate was phloem mobile, higher external salinity led to lower phosphate concentration. Overall, the degree of salt-induced inhibition in leaf growth of varying ages, or inflorescence of *Panicum antidotale* has been correlated with pattern ion distribution, and maintenance of K^+/Na^+ ratio in plant parts. Thus, nutritional distribution in leaves of various ages under salt stress is closely linked with their growth.

Keywords: ion distribution, grass, K^+/Na^+ ratio, leaf age, lamina, leaf sheath, inflorescence, salt tolerance

Introduction

Salinity adversely affects various biochemical and physiological processes resulted in reduced growth. Salt induced reduction in growth mainly occurs due to salt induced osmotic and toxic effects which reduces uptake of other mineral nutrients, such as N, K^+ , and Ca^{2+} (Ashraf, 2004). Both halophytes and glycophytes maintain shoot ion concentrations by regulating ion transport to acclimatize salt stress (Tester & Davenport, 2003). Regulation of K^+ uptake and/or prevention of Na^+ entry, efflux of Na^+ from cell and utilization of Na for osmotic adjustment are strategies commonly used by plants to maintain desirable K^+/Na^+ ratios in the cytosole. Osmotic homeostasis is established either by Na^+ compartmentation into the vacuole or by biosynthesis of accumulation of compatible solutes (Ashraf, 2004; Ashraf & Harris, 2004) However, differences in nutrient uptake and metabolism have been recognized among plant species at different

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stages of growth (Grattan & Grieve, 1999; Ashraf, 2004). Thus, various salinity-nutrient interactions, and salinity-biochemical interactions are occurring at the same time resulting in reduced crop growth and/or productivity; however, it depends upon the level of salt stress, composition of salts in saline growth medium, type of species, and a number of environmental factors such as relative humidity, temperature etc.

Although traits relating to salt tolerance of plants are generally associated with lower accumulation of Na^+ and higher accumulation of K^+ of the plants grown in the presence of salts (Flowers, 2004), tolerance to salt stress is not related to the concentration of sodium in the shoot in all cases (Cramer *et al.*, 1994; Ashraf, 2004). Thus, considerably more research is needed to find out the basis of salt tolerance in plants, particularly in those naturally adapted to salt affected soils. Of various plant species naturally adapted to salt affected soils, *Panicum antidotale* a perennial grass, is of great importance as a fodder grass. It has also been introduced into many countries as a pasture grass and cultivated in some countries (Skerman & Riverose, 1990). It is widely accepted that salt tolerance in cereals is related to Na^+ exclusion at root and leaf level (Tester & Davenport, 2003; Ashraf, 2004). Furthermore, Na^+ is generally accumulated in older leaves while allowing transport of K^+ to young leaves (Munns & James, 2003; Ashraf, 2004). However, little information is available on how distribution of nutrients in various plant organs plays a role in salt tolerance of *Panicum antidotale* Retz. Thus, the present study was aimed to determine distribution of K^+ relative to Na^+ and Mg^{2+} relative to Ca^{2+} among leaves of different age in drought tolerance species *Panicum antidotale* Retz., which could be used for exploiting this species for the rehabilitation of salt affected area.

Material and Methods

The present study was conducted to investigate the tissue concentration and distribution of ions in different parts of *P. antidotale* Retz. accession RI 8/6 under salt stress. The work was carried out at the plant physiological laboratory of Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan where the average PAR measured at noon ranged from 848 to 1254 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$. The seeds of *Panicum antidotale* Retz. were obtained from Cholistan Institute of Desert Studies (CHIDS) Bahawalpur. Before experimentation, all the seed samples were surface sterilized with 10 % sodium hypochlorite solution for five minutes and washed three times with sterilized distilled water.

Seeds of *P. antidotale* were sown in plastic pots filled with 500 g of fine, sieved soil. The soil was kept moist with distilled water. Fifty days after sowing, basal nutrient N and P were applied at the rate corresponding to 40 kg N ha^{-1} as urea and 60 kg P_2O_5 ha^{-1} as $\text{NH}_4\text{H}_2\text{PO}_4$ and adding the dissolved nutrients in 50 ml solution per pot. In salinized plants, 125 mM NaCl was applied in nutrient solution. Soil moisture was maintained at field capacity by weighing and addition of the amount of H_2O lost daily. Plants were grown in natural light. Each treatment replicated three times. After four weeks of imposition of salt stress, plants were harvested. Main branches were harvested along with the inflorescences while the youngest tillers on the main branch were harvested separately. The leaves were detached and separated into laminae and sheath from main branch as well as from the youngest tillers. Inflorescence was also separated. The oldest laminae and sheath were designated as No. 1 and youngest laminae and leaf sheath as no. 6. Leaves sustaining the youngest tiller were included in the main branch. Fresh weight of

laminae and leaf sheath were recorded and then oven dried at 65 °C and dry weights were recorded.

Determination of different nutrients in laminae, leaf sheath and inflorescence: The oven-dried ground material (0.1 g) of leaves and roots was digested with 2 ml of sulfuric acid-hydrogen per oxide mixture according to the method of Wolf (1982). Potassium and sodium in the digests were determined with a flame photometer (PFP-7, Jenway Ltd. Felsted, Dunmow, Essex, U.K.). P, Ca²⁺ and Mg²⁺ in the digests were determined following Allen *et al.*, (1986).

Statistical analysis: Analysis of variance technique was employed for carrying out statistical analysis of the data collected using a Costat 6.33 computer package (Cohort Software, California, USA). The mean values were compared with the least significant difference test (LSD) following Snedecor & Cochran (1980).

Results

Salt stress reduced the fresh weight of both leaf laminae and sheath of leaves of all ages. However, salt induced reduction in fresh weight was more in leaf lamina of leaf No.3 and 4. Furthermore, leaf lamina is more adversely affected by salt stress compared with leaf sheath. Fresh weights of both leaf lamina and leaf sheath were higher in fully developed and younger leaves as compared with the youngest or the oldest leaf. In contrast, dry matter production of sheath was more reduced compared with leaf lamina. However, maximum reduction in sheath dry matter was observed in oldest leaf, while no change in dry matter of the youngest sheath due to salt stress (Fig. 1). Youngest tiller on the main branch had three leaves on it. Salt stress also reduced both leaf lamina and sheath of all three leaves; however, salt induced reduction was more in leaf lamina as compared with leaf sheath (Fig. 1).

Imposition of salt stress caused increase in Na⁺ accumulation in both leaf lamina and sheath of all leaves on main branch (Fig 2). However, accumulation of Na⁺ was higher in leaf lamina compared with corresponding leaf sheaths of all leaves. Furthermore, the youngest leaf lamina or leaf sheath had lower Na⁺ compared with those of older leaves. Although the lowest accumulation of Na⁺ was observed in inflorescence under both non-saline and saline conditions, the difference in Na⁺ accumulation in inflorescence was negligible.

K⁺ concentration was highest in the laminae of the youngest leaf but decreased strongly in the older leaves. Salt induced reduction in K⁺ was negligible in the youngest leaf; however, this reduction in K was higher in older leaves. Furthermore, adverse effect of salt stress on lamina K was less compared to those of leaf sheaths. Although salt stress reduced K⁺/Na⁺ ratio both in leaf lamina and leaf sheath, leaf sheath showed higher K/Na ratio particularly in the youngest leaf. In addition, inflorescence exhibited maximum value for K/Na ratio both under non-saline and saline conditions (Fig. 2).

Likewise, Na⁺ in the laminae and leaf sheaths of the youngest tiller showed similar pattern to that of main branch (Fig 3). Concentration of K⁺ in the youngest leaf of the side branch was remarkably high as shown in Fig. 3. This indicated that K⁺ might had been derived by phloem import from the supporting leaf, since a side branch originates from the axillary bud and constitutes a substantial sink.

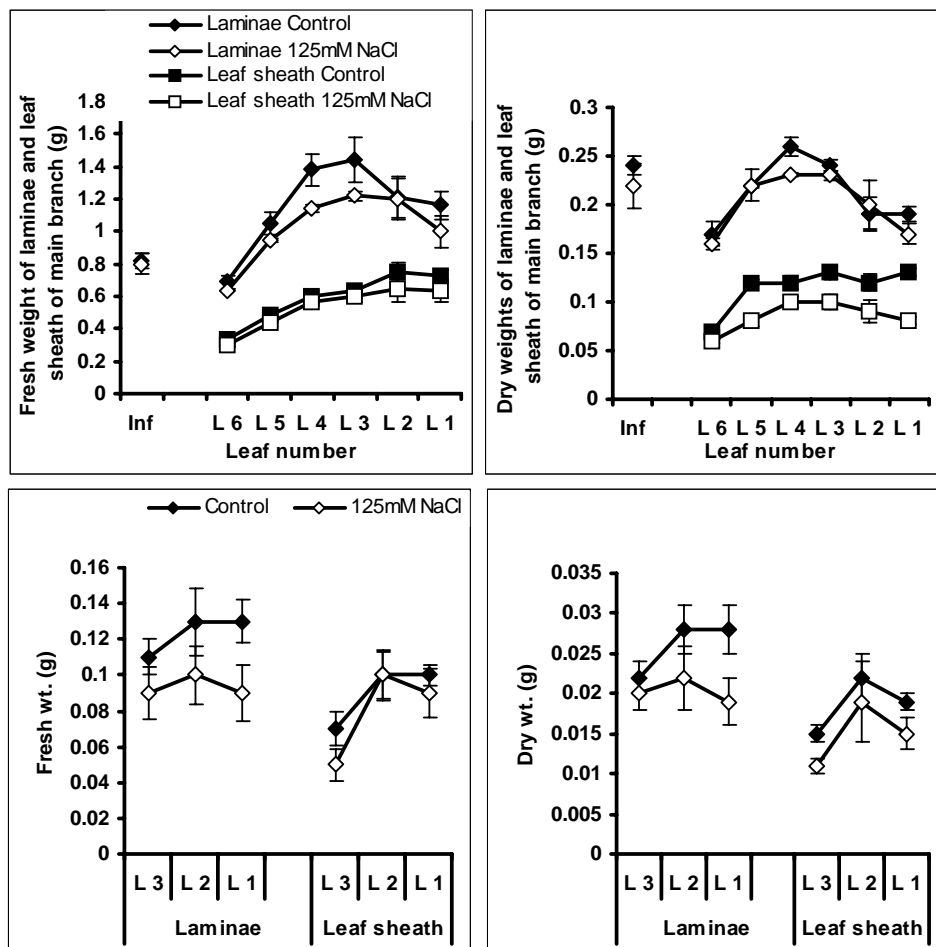


Fig. 1. Fresh and dry weights (g) of laminae and leaf sheath of main branch and freshly born tillers of *Panicum antidotale* Retz. under normal and saline conditions [Inf = Inflorescence; L = Leaf; L 6, L 3 = Youngest Leaf; L1 = Oldest leaf].

Growth medium saline conditions reduced Ca^{2+} both in lamina and sheaths of all leaves. Furthermore, this salt-induced reduction in Ca^{2+} was more in older lamina and sheaths. However, salt-induced reduction in Ca^{2+} was higher in lamina compared with those of leaf sheaths, particularly in younger leaves (Fig 2). Mg^{2+} was higher in lamina and sheath of older leaves but it was lower in inflorescence of main branch (Fig. 2). Reduction in leaf lamina and leaf sheath Mg^{2+} was higher in older leaves. However, Mg^{2+} was higher in leaf lamina in comparison with those of leaf sheaths both under saline and non-saline conditions (Fig. 2). Likewise, Ca^{2+} and Mg^{2+} were reduced both in the laminae and leaf sheaths of leaves of youngest tiller due to salt stress. However, both Ca^{2+} and Mg^{2+} were higher in older leaves under saline or non-saline conditions. Furthermore, both Ca^{2+} and Mg^{2+} were higher in lamina compared with leaf sheaths of all leaves (Fig. 3).

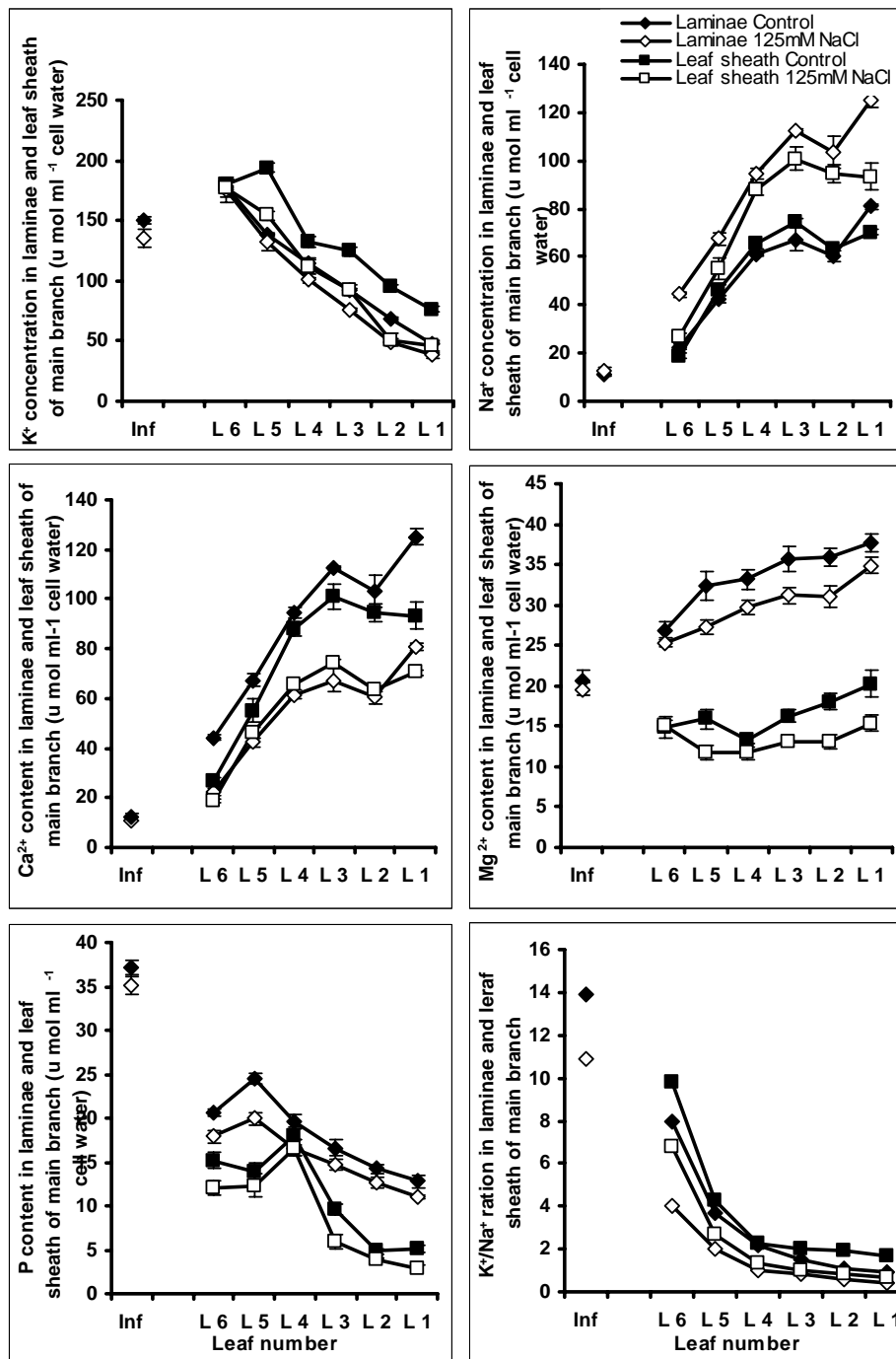


Fig. 2. Concentration of nutrients in leaf sheath and laminae of main branch of *Panicum antidotale* Retz. under normal and saline conditions.

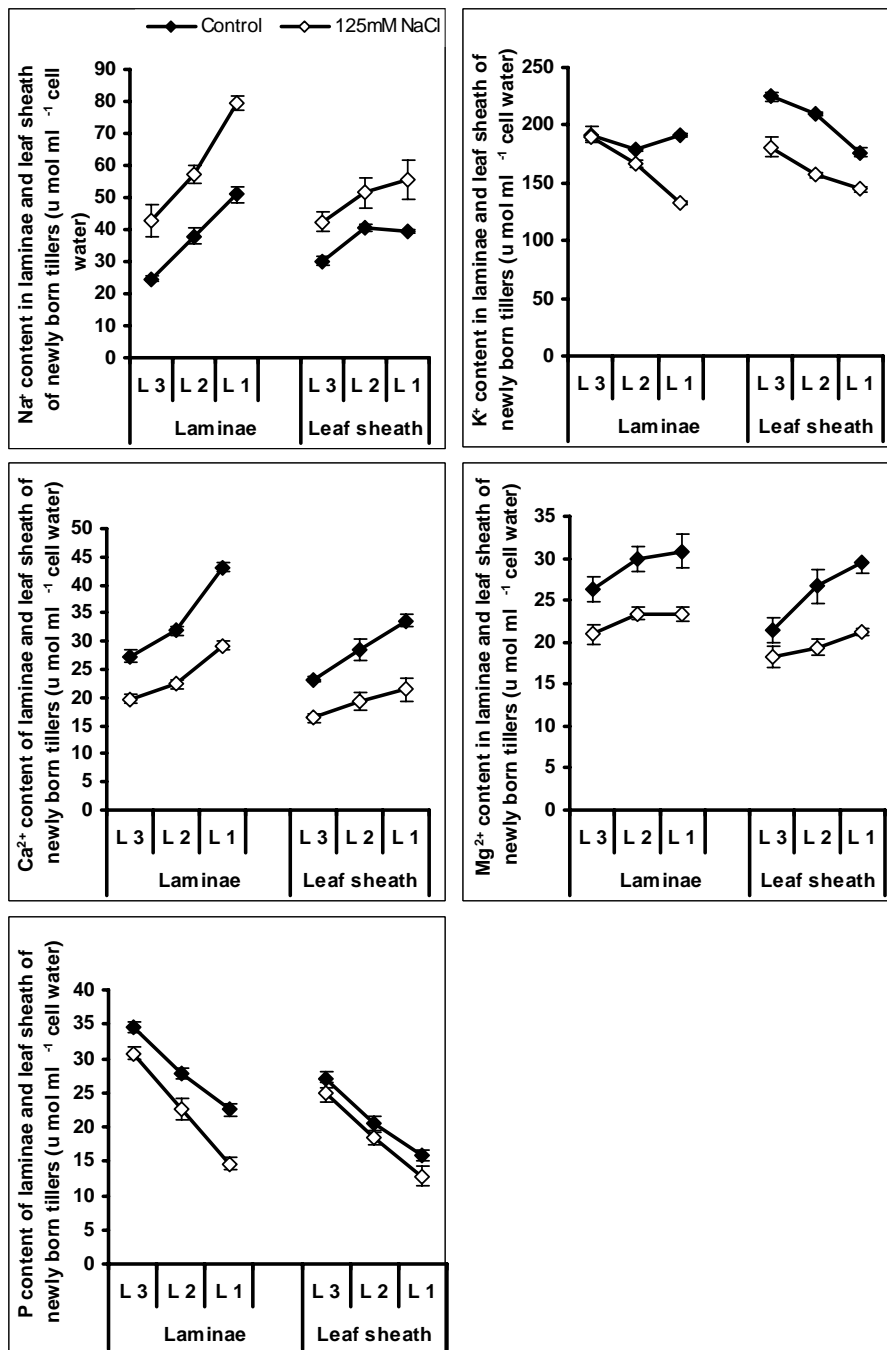


Fig. 3. Concentration of nutrients in leaf sheath and laminae of freshly born tillers of *Panicum antidotale* Retz. under normal and saline conditions [Inf = Inflorescence; L = Leaf; L 6, L 3 = Youngest Leaf; L1 = Oldest leaf].

Imposition of salt stress also reduced P accumulation in all parts of plant, i.e., inflorescence, leaf lamina, leaf sheaths of varying ages of leaves. However, accumulation of P was more in inflorescence and leaf lamina of the main branch or the youngest tiller (Fig 2, 3). Furthermore, P accumulation was more in younger leaves of salt stressed or non-stressed plants (Fig. 3).

Discussion

From the results of the present study, it is clear that salt stress reduced growth of both leaf sheath and leaf lamina of leaves of different age. Although leaf sheath and leaf lamina of younger leaves had lower biomass, adverse effect of salt stress was less on younger leaves. Thus, *Panicum antidotale* maintained healthier young actively growing tissues under salt stress conditions. But fresh and dry weights of laminae in the *Panicum antidotale* exceeded those of the corresponding sheath because length of the sheath in *Panicum antidotale* was much smaller than that of blade. However, in kallar grass, leaf sheaths and leaf blades have similar length but sheaths had higher biomass than that of leaf lamina which is attributable to higher relative water contents in leaf sheath than the corresponding laminae (Bhatti *et al.*, 1993). In view of large number of studies, it is widely accepted that Na^+ exclusion from photosynthesizing tissues has been shown to be an important mechanism in salt tolerance in glycophytes (Ashraf, 1994; 2004; Munns *et al.*, 2006). In cereals, older leaves or leaf base have been shown to act as ion sinks that restrict ion entry into meristematic and actively growing photosynthesizing tissue (Yeo & Flowers 1982; Tester & Davenport, 2003). Thus, more salt-induced reduction in both older leaves and leaf sheaths compared with the corresponding leaf lamina may be one of adaptive features in *Panicum antidotale* to acclimatize salt stress.

Since high salt stress induces ion toxicity and nutritional imbalance at both the cellular and the whole-plant levels, differential effects of salt stress on leaf sheath and leaf lamina of different leaf ages may have been due to differences in ion distribution. The results of the present study showed that both leaf sheath and leaf lamina of the oldest leaf accumulated maximum Na^+ , while accumulation of K^+ was the lowest. However, the pattern of ion accumulation in younger leaves was reverse. In addition, K^+/Na^+ ratio was higher in leaf sheath and leaf lamina of younger leaves compared with those of older leaves. These results are similar with those of Aslam *et al.* (1986) who found that K^+/Na^+ ratio is 2.5-3 times greater in younger leaves than the fully expanded leaves of *Atriplex amnicola*. While working with salt sensitive and salt tolerant barley cultivars Wei *et al.* (2003) found that the enhanced salt tolerance of salt tolerant cultivar Golden Promise was associated with restriction of Na^+ accumulation in young actively growing tissues, the selective partitioning of Cl^- within leaf tissues and the maintenance of high K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratios in young tissues. Likewise, salt tolerance in *Hordeum vulgare* has been found to be related to the ability to selectively partition Na^+ into old leaves and sheaths, while K^+ into growing tissues (Boursier *et al.*, 1987). It is widely accepted that Na^+ translocates mainly through xylem and K^+ through phloem (Marschner, 1995). Thus, younger leaves accumulated more K^+ but less Na^+ because younger leaves receive their mineral nutrients from phloem (Webb & Gorham, 1964). However, under saline conditions the preferential import of K^+ to young leaf laminae from phloem or from older leaves also contributed higher accumulation of K^+ in parts of younger leaf that resulted in higher K^+/Na^+ ratio as has earlier been observed in *Leptochloa fusca* (Bhatti *et al.*, 1993) and in barley (Jeschke & Wolf, 1988).

Although accumulation of Ca^{2+} and Mg^{2+} reduced in leaf sheaths and leaf lamina of salt stressed plants, their accumulation was maximal in older leaves (Fig 2). Concentration of Ca^{2+} and Mg^{2+} in the laminae and sheath of *Panicum antidotale* increased with the age. Low Ca^{2+} concentration in the younger leaves might be due to limited capability of xylem to supply Ca^{2+} for growing tissues, which were low-transpiring (Marschner, 1995). However, lower accumulation of Mg^{2+} in younger leaves was due to its import through xylem exceeded the phloem export (Jeschke & Pate, 1991).

As the results indicate that the concentration of phosphate increased with the decreasing age both in the laminae and leaf sheath in the *Panicum antidotale* and followed the pattern of phosphate distribution reported by Bhatti *et al.* (1993) in kallar grass. The leaf sheath had low phosphate contents as compared to corresponding laminae in the *Panicum antidotale* as in *Leptochloa fusca* (Klagges *et al.*, 1993). Higher external salinity led to lower phosphate concentration in laminae and sheath (Klagges *et al.*, 1993). The pattern of distribution of inorganic phosphate in *Panicum antidotale* agreed with *Leptochloa fusca* and *Ricinus communis* and suggested the substantial recycling of stored phosphate and its mobilization which was well documented (Greenway *et al.*, 1966; Hill, 1980).

In conclusion, the degree of salt-induced inhibition in leaf growth of varying ages of *Panicum antidotale* has been correlated with pattern of Na^+ and K^+ distribution. Large number of reports has already shown that macronutrients (N, P, K^+ , Ca^{2+} and Mg^{2+}) are spatially distributed along the grass leaves (Bhatti *et al.*, 1993; Bernstein *et al.*, 1995; Hu *et al.*, 2000; De Lacerda *et al.*, 2003; Neves-Piestun & Bernstein, 2005); however, young actively growing tissues are a strong sink for nutrients. Thus, nutritional distribution in leaves of various ages under salt stress is closely linked with their growth.

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