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Salinity effects on five cultivars/lines of pearl millet (*Pennisetum americanum* [L] Leeke)

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Abstract

The effects of increased salinity [NaCl + $CaCl_2$] on seedlings of five accessions of pearl millet grown for 2 and 7 weeks, respectively, in salinised solution cultures at EC 0.6, 4, 8, 12, 16, and 20 ds m^{-1}, and sand cultures at EC 0.6 and 20 were assessed. There were no consistent relationships between seedlings characters at two and seven weeks in response to increased salinity, and no single character provided an acceptable means of differentiating cultivar/line response. The line having lower shoot mortality had a high root weight, a shoot:root ratio approaching 1, the greatest shoot water content, and the greatest plant height. No relationship was found between these whole-plant characters which suggest greater salinity tolerance, and the pattern of ion distribution, particularly Na^+ and Cl^-. Sufficient inter-cultivar/line variation in response to salinity was found to suggest that selection of individuals with increased salinity tolerance is possible within pearl millet.

Introduction

Considerable efforts are being made to develop salt-tolerant cultivars of crop species, which, integrated into appropriate management programmes may allow the exploitation of saline soils. If parallels are drawn with early selection and breeding programmes to improve crop species for non-saline environments, it is clear that very significant and rapid advancement in performance in a wide range of crop species was achieved thorough the exploitation of the considerable useful genetic variability present within those crops in their relatively unimproved state (Allard, 1960; Silvey, 1978) and in their wild relatives (Allard, 1960). Since most crops are unlikely to have been subjected to selection, whether natural or artificial, for improved salinity tolerance, it is possible that particularly in outbreeding species, variation in this character may exist although at relatively low frequency (Epstein *et al.*, 1980; Kingsbury and Epstein, 1984; Rush and Epstein, 1976; Malkin and Waisel, 1986; Ashraf *et al.*, 1986a).

The work reported here examines patterns of variation in response to salinity of two-and seven-week-old seedlings of live cultivars/lines of pearl millet (*Pennisetum americanum* [L] Leeke).

Materials and methods

Plant material

Both experiments used seed kindly supplied by the Agriculture Department of Pakistan, of the five cultivars/lines of millet, *Pennisetum americanum*, A1/3, 18BY, Exbourne, Giant Bajra, and WC9099.

Experiment 1

25 seeds of each cultivar were germinated on rafts of black alkathene beads, three layers of beads deep, floating on the surface of $\frac{1}{2}$ strength nutrient solution (Rorison, in Hewitt, 1966) in 300-cm^3 plastic beakers. Six salt concentrations having EC 0.6

(control), and 4.0, 8.0, 12.0, 16.0, and 20.0 ds m^{-1} were used. These were obtained by addition of NaCl and $CaCl_2$ (1:1 dry weight) to the nutrient solution. The experiment was set up as a completely randomised design having a total of 90 pots (5 cultivars/lines × 6 concentration × 3 replicates) in a growth cabinet at 22 °C, with 16 hours, daylength at an irradiance of 27 Wm^{-2} and relatived-humidity of 85%.

After two weeks growth, mean values of the following characters were determined for the seedlings of each cultivar/line at each EC value.

1) shoot length (to tip of longest leaf)
2) longest root length
3) shoot fresh and dry weights
4) root fresh and dry weights
5) total plant fresh and dry weights
6) shoot:root ratio
7) plant water content

From these data, values for different EC values were expressed as percentage of the control values (EC 0.6).

Experiment 2

Four plastic containers, 45 cm diameter and 35 cm deep were filled to a depth of 24 cm with thoroughly washed river sand in a glasshouse maintained at 18 °C with a 16 h daylength of natural daylight supplemented by 400 Watt mercury vapour lamps. The sand was thoroughly washed with tap water, followed by three washings with $\frac{1}{2}$ strength nutrient solution as used in the solution-culture experiment.

Seeds of the five cultivars/lines were pregerminated on moist filter paper in Petri dishes in a growth room at 22°C. Ten seedlings of each cultivar/line were transplanted as single rows 7 cm apart into the sand at a depth of 0.5 cm. Each container held 20 seedlings.

All seedlings were watered with a control solution having EC 0.6 dS m^{-1} for the first three weeks of the experiment. Salt treatments began after 4 weeks of the experiment for half the containers using a mixture of NaCl and $CaCl_2$ (1:1 by weight) in $\frac{1}{2}$-strength nutrient solution to give an EC of 3.0 dS m^{-1}. Salinity was increased stepwise by 3.0 dS m^{-1} every other day to give a final EC of 20.0 dS m^{-1}. Thereafter 9 l of the appropriate solutions were applied to control and salt-treatment containers every three days. E.C. of the leachate was thus maintained at that of the added solution. Once per three-day cycle, 2 l of tapwater was applied to each container to maintain sand moisture and to prevent salt accumulation. Each container was contained in a large plastic saucer 5 cm deep to retain solutions and maintain sand moisture.

After four weeks of salt treatments, plants were harvested and mean overall plant height and shoot- and root-fresh weights measured. Green and dead shoot material was separated, dried at 60°C for 7 days, and leaf-, shoot-, and root-dry weights measured.

Results

Experiment 1

The responses of the five cultivars/lines to increasing salinity are given in Fig. 1, presented as % of controls to allow comparison of the relative performance of the cultivars. Table 1 summarises analyses of variance of those data.

Increasing salinity caused overall significant reductions in shoot and root length, and fresh and dry weights. Cultivars/lines also differed significantly in all characters except shoot fresh and dry weight. Despite the variation in response of the cultivars/lines to increasing salinity (Fig. 1) differences were significant only for root fresh weight (cultivars (C) × treatment (T) interaction sig at $p < 0.01$), and water content (C × T interaction significant at $p < 0.001$). Differences in root fresh weight occurred mainly at EC 4, and EC 8, Exbourne being particularly sensitive to increasing EC, whereas A1/3 was markedly less sensitive. Percent water content differed at EC 12, 16, and 20. AC1/3 and Giant Bajra did not differ with increasing EC, WC 9099 and Exbourne declining to EC 16, but not thereafter. 18BY had a much lower water content at EC 20 than the other four cultivars/lines.

There appears to be some stimulation of root length in Exbourne, WC 9099, at EC 4, and in all cultivars/lines except A1/3 at EC 8 (Fig. 1). Root length is less than the controls only at EC values greater than 12. Root fresh and dry weights only show such stimulation in Exbourne at EC 4. Root

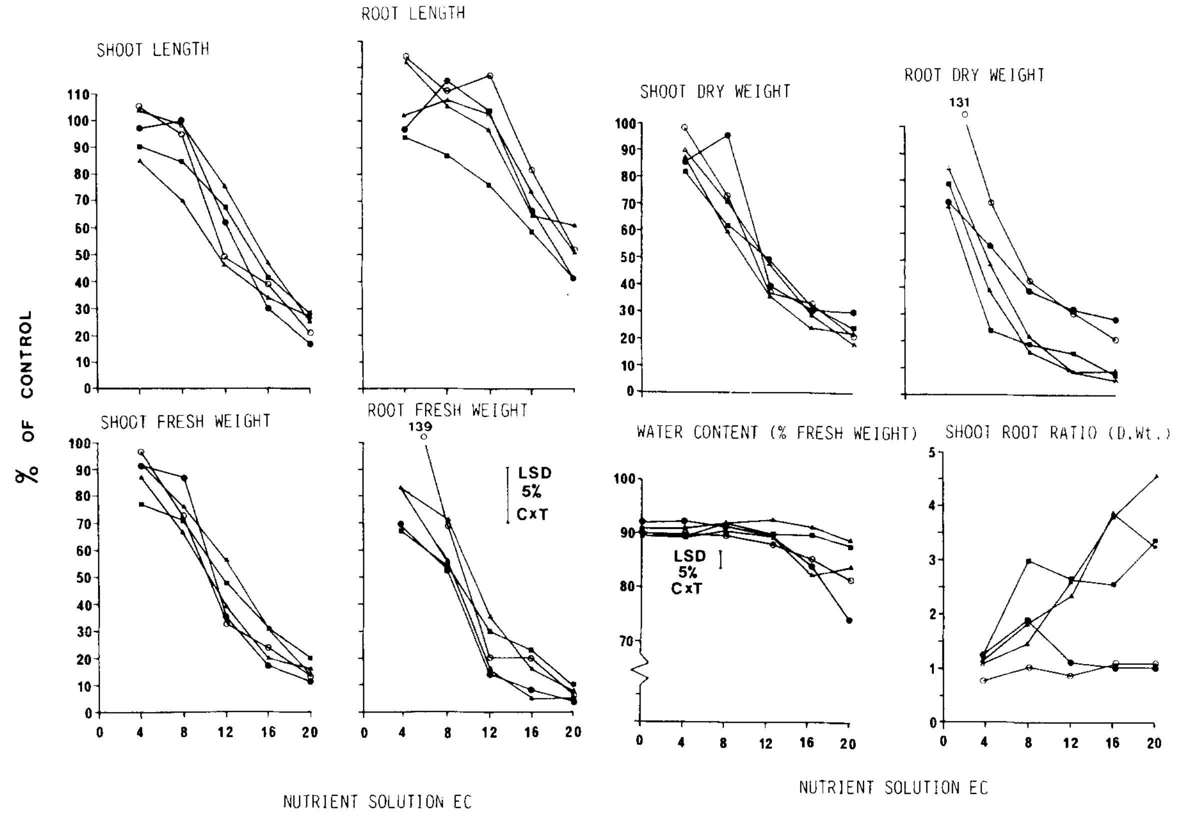

Fig. 1. The effects of increasing nutrient solution EC using NaCl + $CaCl_2$, on eight growth measurements in two-week old pearl millet seedlings. ■ AC 1/3; ● 18 BY; ○ Exbourne; △ Giant Bajra; ▲ WC 9099.

Table 1. Analyses of variance of the response of two-week-old seedlings of five cultivars/lines of pearl millet to increasing salinity in solution culture (Experiment 1)

Source of variation	df	Shoot length	Root length	Shoot F.wt	Root F.wt	Shoot D.wt	Root D.wt	Shoot/Root Ratio	Water Content
Cultivars (CV)	4	576.6***[a]	1289.6***	131.8 NS	1074.2***	217.0 NS	2614.0***	9.66**	13.95***
Treatments (T)	4	14953.0***	9935.8***	15270.5***	18143.0***	11983.8***	13219.0***	5.75**	35.16**
CV × T	16	186.6 NS	180.7 NS	161.6 NS	563.7**	172.4 NS	345.3 NS	1.78 NS	5.67***
Residual	50	100.0	198.3	104.0	168.9	92.0	233.4	1.44	2.41

[a] NS: differences between means not significant; **, ***: differences between means significant at $P \leqslant 0.1$, and 0.01, respectively.

fresh and dry weights of all other cultivars/lines at EC 4 were reduced by 15–30%. Greater reductions occurred as EC increased.

In Exbourne, and 18BY, shoot/root ratio remained at approximately 1 with increasing EC, but in the remaining three cultivars/lines it rose markedly with increasing EC.

Experiment 2

Plant height, root and shoot fresh-and dry weights, at EC 20 are given as a percentage of controls in Fig. 2 to provide relative cultivar/line comparisons. Figure 2 also gives shoot/root ratio, based upon dry weight measurements, shoot mortality, and water content. Differences occurred between cultivars/lines in all characters except water content.

Plant height was about 70% and 50% of controls in A1/3, 18BY, Exbourne, and Giant Bajra and WC9099, respectively.

Shoot dry weight at EC 20 in 18BY and Giant Bajra is about 50% of controls and about 60% in the other three cultivars/lines. Root fresh weights were 35–45% of control. Root dry weights however

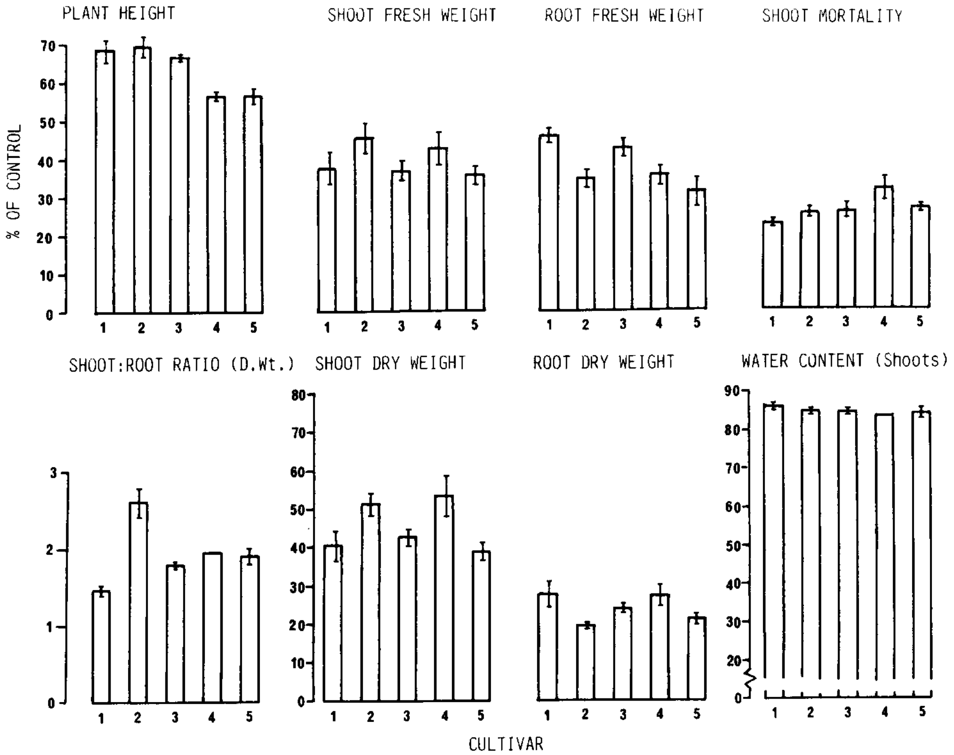

Fig. 2. Means of seven measures of growth of five lines/cultivars of pearl millet after 4 weeks growth in sand culture, (data expressed as percentages of control plants grown at EC 0.6), and of shoot:root ratio. Key to cultivars; 1, AC 1/3; 2, 18 BY; 3, Exbourne; 4, Giant Bajra; 5, WC 9099.

as percentage of controls varied from 20% in 18BY (2) and 28% to A1/3.

Data for shoot mortality allow good separation of the cultivars/lines in response to increased EC, ranging from 23% in A1/3 to 32% in Giant Bajra.

Overall A1/3 seems to be rather more tolerant to EC 20 than the other cultivars, with a shoot/root ratio of 1.5, a high water content, and the lowest shoot mortality.

Ion content. Data for Na^+, K^+, Ca^{2+} and Mg^{2+} and Cl^- are presented in Fig. 3.

Increasing EC caused a significant increase in all ions except Mg^{2+} in root, stem, and leaf of all cultivars. Significant differences also occur between ionic content of root-, stem-, and leaf fractions (except leaf Mg^{2+}) in different cultivars and in cultivar/line response to increased salinity, except for leaf-and stem Mg^{2+}.

Chloride. At EC 0.6 the greatest amounts of Cl^- occurred in stem fractions, least in leaves, and intermediate amounts in roots. There was considerable intercultivar/line variation (*e.g.* 2 *vs* 5). Much more Cl^- entered the plants at EC 20 particularly the proportion entering leaf tissue. The mean Cl^- content of leaf-, stem-, and root fractions at EC 0.6 was 12%, 53% and 35% of total plant Cl^-, whereas at EC 20, comparable figures were 37% in leaf, 42% in stem, and 21% in roots. Cultivar differences in response to increassed EC differed significantly for leaf- and stem Cl^-. Exbourne had 7% of total plant Cl^- in leaf tissues at EC 0.6, but 43% at EC 20; Giant Bajra had 12% total plant Cl^- in leaves at EC 0.6, but 26% at EC 20.

Sodium. Na^+ content of leaf-, stem-, and root fractions increased significantly with increase in EC, and cultivars differed significant in total Na^+ content.

At EC 0.6, 81% of total plant Na^+ was present in the roots, 5% in leaf, and 14% in stem tissues. At EC 20, leaf Na^+ was 26%, stem Na^+ was 40%, and root Na^+ was 34% of total plant Na^+. Cultivars/lines differed at both EC 0.6 and 20, and different cultivars/lines responded differently to increased EC. A1/3 had 36% of total Na^+ in leaf and stem and 28% in roots at EC 20, comparable figures for

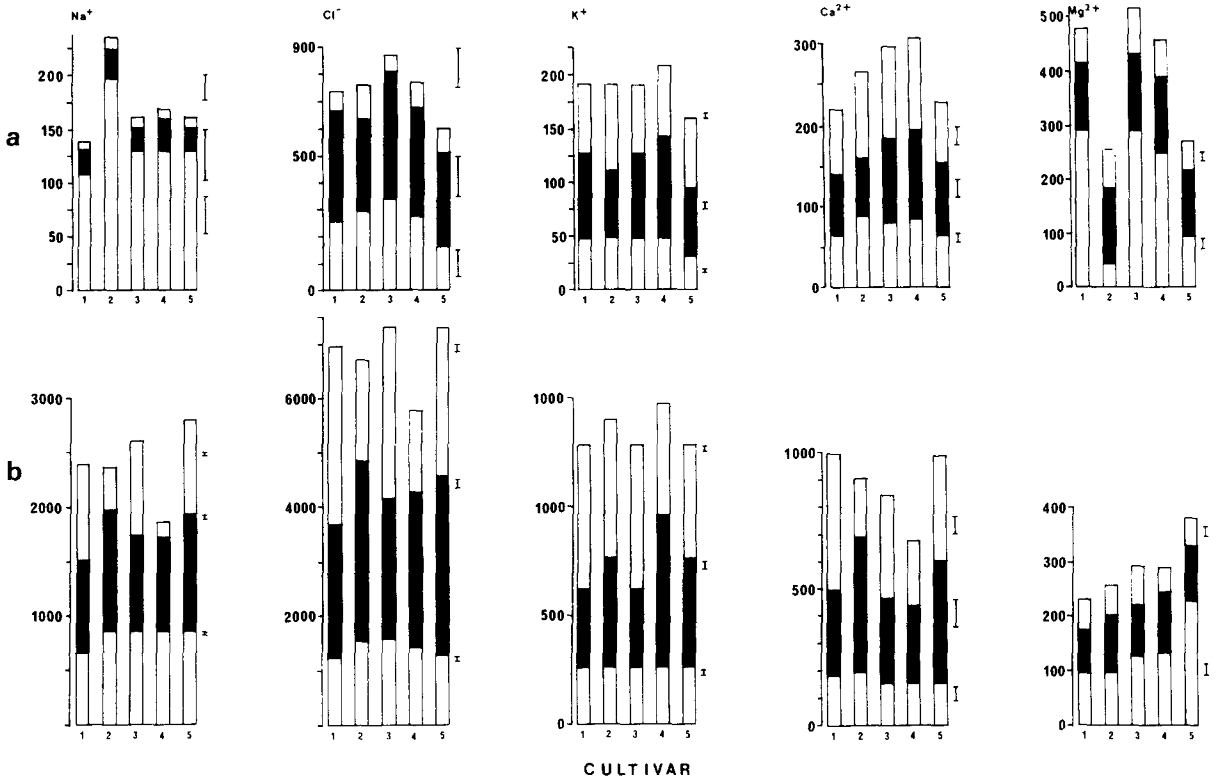

Fig. 3. Ion contents (from left to right: Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+}) of five cultivars of pearl millet after four weeks growth in sand culture, **a**) at EC 0.6, and **b**) at EC 20. Cultivars numbered as in Fig. 2. *Open columns* **top**: leaf tissue; *closed columns*: stems; *open columns* **bottom**: root tissue. L.S.D. 5% values for cultivar/line comparison given adjacent to columns.

Giant Bajra being 7%, 46% and 46%, respectively, for leaf-, stem-, and root tissues.

Potassium. At EC 0.6 there was less K^+ in root tissues than in stem- and leaf tissues. At EC 20, the proportion of total K^+ in roots was approximately 20%, with 36% in stem tissue. However the proportion in leaves rose to 45% of total K^+. There were differences between cultivars/lines in leaf-, stem-, and root fraction K^+, and cultivars/lines reacted differently to increased EC.

Calcium. Total plant Ca^+ was approximately three times greater at EC 20 than at EC 0.6, the increase being most marked in stem- and leaf tissues. There were marked differences in the reaction of the different cultivars/lines to increased EC, as can be seen by comparison of data for Giant Bajra (4) and WC9099 (5) in Fig. 3, and amounts in both stem- and leaf tissues differed significantly between cultivars/lines.

Magnesium. Total plant Mg^{2+} content of A1/3, Exbourne, and Giant Bajra were less at EC 20 than at EC 0.6. By contrast, WC9099 had higher total plant Mg^{2+} at EC 0.6, and no difference was found in 18BY due to changed EC. The Mg^{2+} content of root tissues was very variable at EC 0.6, and the pattern of variation at this EC was not related to Mg^{2+} content at EC 20. At EC 20, A1/3 and 18BY did not differ in root Mg^{2+}, but did differ from Exbourne and Giant Bajra which differed from WC9099, but did not differ among themselves. The Mg^{2+} content of stem tissues did not differ between cultivars/lines at the two EC levels.

Discussion

The data presented here allow an assessment of the effects of increased salinity [NaCl + $CaCl_2$] on five millet cultivars/lines after two weeks growth in solution culture, and after seven weeks growth in salinized sand culture.

Experiment 1 forms a parallel with the system for assessing the potential salt tolerance in wild collected plant material outlined by O'Leary (1979). It provides information on potential value in assessing the general response of millet at the post-germination stage to increasing salinity. The data suggest that pearl millet is moderately tolerant to salinity (Maas and Hoffman, 1977; Shannon, 1984) all cultivars growing at EC 16 and EC 20.

Correlations between seedling characters after two weeks growth (Experiment 1) and in seven-week-old plants (Experiment 2), are not evident from the data in Fig. 1 and Fig. 2. This confirms the findings of Akbar and Yabuno (1974) in rice, of Kinsbury and Epstein (1984) in wheat, and of Shannon (1984) in muskmelon, although the comparisons made here are based on data from a single EC for the seven-week-old plants.

Lack of a single readily assessed means of assessing salinity tolerance has been a severe constraint upon selection and breeding salinity tolerant crop species. Percentage of dead leaves may provide a good measure of salt injury in rice (Ponnamperuma, 1977), and in other crops resistance to leaf chlorosis has been successfully used as a selection criterion for increased salinity tolerance (Ream and Furr, 1976; Shannon, 1978). Good separation of the cultivars/lines examined here is given by the data (Fig. 2) for percentage shoot mortality which suggest that the line A1/3 is the least, and Giant Bajra the most affected by salinity. A1/3 also has high root dry weight, a shoot/root ratio approaching 1 which is lower than the other cultivars/lines, has one of the tallest mean shoot lengths, and has highest shoot water content. The combination of characters suggests that A1/3 is the most salinity-tolerant of the material examined. The complexity of individual whole-plant characters is however shown by data for Giant Bajra, which has the greatest shoot mortality, yet has the highest shoot dry weight, a high root dry weight, a high shoot/root ratio, but the lowest shoot water content. It thus again emphasises the difficulty in attempting to use any single growth character as a measure of the complexity of the reaction of the different cultivars/lines to increasing salinity, and therefore as a useful criterion for selection.

Analysis of plant material for specific ions has been suggested as a useful measure of salinity tolerance, and differences in the degree of ion exclusion has been cited as a major difference between salt-sensitive and salt-tolerant crop cultivars (Shannon, 1984). In rice for example, Na^+ uptake and plant survival (Flowers and Yeo 1981) have been shown to be negatively correlated, although in other crops the evidence is less clear, or conflicting (Dehan and Tal, 1978; Rush and Epstein, 1976; Tal, 1971). The data for millet are also not clear. For the reasons suggested above, A1/3 appears to be the most salinity-tolerant of the material examined, yet leaf and stem Na^+ at EC 20 are both significantly greater than in 18BY and Giant Bajra, and very similar to that in Exbourne and WC9099. Giant Bajra has by contrast the lowest leaf- and stem Na^+ and Cl^-, but the greatest % shoot mortality. Partitioning of ions between leaves of different ages may have provided more useful information, however.

The reaction of the material examined to increased salinity is complex, but it is nonetheless clear that cultivars/lines differ. However, their tolerance ranking varies with the character used for assessment. Nonetheless certain character combinations may provide useful selection criteria for increasing salinity tolerance through exploitation of inter- and intra-cultivar/line variation in response to high salinity.

Shannon (1984) considers salinity tolerance as the combined characteristic of many plant features, both morphological and physiological. Variation in whole-plant reaction to salinity would thus seem to provide the best means of initial isolation of salinity-tolerant genotypes through mass selection, such as has been successfully carried out recently in a number of forage species (Ashraf *et al.*, 1986a, b). Work to exploit such variability in pearl millet is currently in progress.

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