EFFECT OF SALINITY ON GERMINATION, GROWTH, YIELD, IONIC BALANCE AND SOLUTE COMPOSITION OF PIGEON PEA (*CAJANUS CAJAN* (L.) MILLSP)

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Abstract

Salt tolerance of Pigeon pea (*Cajanus cajan* (L.) Millsp) was determined at three growth stages since it has already been observed by a number of workers that degree of salt tolerance of different crops varies with their ontogeny. Therefore, salt tolerance of three accessions, Local Arhar, ICPL-151 and ICPL-850014 of pigeon pea was assessed at germination, seedling and adult stage.

It is clearly evident from this study that there was no positive correlation between the tolerance at the early growth stages and at the adult stage of pigeon pea, since no clear difference in salt tolerance of three accessions was observed at the germination and the seedling stage, whereas accessions differed considerably at the adult stage.

Although increasing salt concentrations adversely affected the growth of all three accessions, ICPL-151 excelled the other two accessions in fresh and dry biomass, yield and yield components when tested at the adult stage. The tolerant accession, ICPL-151 accumulated significantly lower shoot and root Na^+ and shoot Cl^-. By contrast it was higher in shoot and root K^+, K/ Na ratios K versus Na selectivity, soluble sugars, root starch, free amino acids and proline compared with the other two accessions.

The better performance of ICPL-151 under saline conditions seems apparently due to accumulation of less Na^+ and more K^+ and K/Na ratio and higher concentration of proline, free amino acids and soluble sugars than the other two accessions. However, relatively greater accumulation of organic osmotica was probably not enough to decrease the osmotic potential of the tolerant accession.

Introduction

High levels of salts in the soil can often cause serious limitations to agricultural production and land development. The main factors that contribute to this problem are the arid and semi-arid climates and the salt load in the water used for irrigation. The soil salinity may cause several deleterious effects on growth and development of plants at physiological and biochemical level (Gorham *et al*., 1985; Munns, 2002). These effects can be due to low osmotic potential of soil solution, specific ion effects, nutritional imbalance or a combined effect of all these factors (Ashraf, 1994a; Marschner, 1995).

Pigeon pea (*Cajanus cajan* (L.) Millsp) is widely grown as a pulse crop in many parts of the Indian subcontinent (Bokhari & Ashraf, 1990). It is largely eaten in the form of split pulse as dal, while its tender green pod constitute a very favorite vegetable. It is often grown for green manure or for cover crop. The roots also aerate the soil and vegetative parts add a lot of organic matter to the soil. Despite its great economic importance little

information regarding its degree of salt tolerance is available in literature, although yield losses are considerable when subjected to saline conditions (Subbarao *et al*., 1990). Pulses in general are highly sensitive to salinity (Ashraf & Waheed, 1990). But a great amount of intra specific variation for salt tolerance does exist in various pulse crops e.g. lentil (Ashraf & Waheed, 1990), chickpea (Ashraf & Waheed, 1992) and mung bean (Ashraf *et al*., 1990).

It is now well evident that salt tolerance of most crops varies with the change in stage of their life cycle (Kingsbury & Epstein, 1984; Shannon & Grieve, 1999; Ashraf & Harris, 2004). By contrast other studies have revealed that in some species degree of salt tolerance is consistently maintained at different growth stages e.g. alfalfa (Noble *et al*., 1984), three leguminous forage species (Ashraf *et al*., 1986 a), four grass species (Ashraf *et al*., 1986 b). It has been argued (Blum, 1985) that if a species maintains its degree of salt tolerance consistently at all developmental stages, selection at any growth stage provides tolerant individuals. However, in the former case selection procedure becomes tedious in view of its imposition at each stage.

In addition a better understanding of physiological / biochemical responses under salt stress may also help to improve the salt tolerance of the crop species (Chinnusamy *et al*., 2005). A primary response of plants exposed to salinity stress is a decrease in plant water potential, which reduces plants water use efficiency (Cha-um, *et al.*, 2004). The salt tolerant species possess a high capacity to resist salt stress through the biosynthesis and accumulation of compatible solutes. These substances raise the overall osmotic pressure within the cells, thereby enabling plant cells to maintain both turgor and the driving gradient for water uptake (Hasegawa, *et al.,* 2000; Cha-um, *et al.,* 2004). Thus compatible solutes like proteins, carbohydrates, amino acids and quaternary ammonium compounds serve important roles as osmotic balancing agents as well as plant cell stabilizers (Rhodes and Hanson, 1993; Holmstrom *et al.,* 2000; Ashraf & Harris, 2004)

The present study, therefore, was undertaken to ascertain whether there is a positive relationship between the degrees of salt tolerance at different growth stages in pigeon pea. Parallels between the growth performance of different accessions under saline conditions and some important inorganic and organic osmotica have also been drawn.

Materials and Methods

Seeds of   pigeon pea (*Cajanus cajan* (L.) Millsp) accessions, ICPL-151 and ICPL-850014 were obtained from NARC (National Agriculture Research Centre, Islamabad), whereas those of a local line was obtained from a local seed supplier.

Germination experiment: The seeds of three pigeon pea accessions, ICPL-151, ICPL-850014 and Local arhar were sterilized in 5 % sodium hypochlorite solution for 5 minutes before experimentation. Twenty-seven seeds of each accession were sown in plastic Petri dishes with internal diameter of 6cm. Plastic Petri dishes were arranged in a completely randomized design with three replicates, three treatments and three accessions. Different NaCl treatments used were 0 (control), 50 and 100 mol m^{-3} in full strength Rorison nutrient solution (Rorison in Hewitt, 1966). Seven ml of appropriate treatment solution was applied daily to each Petri dish. Germination experiment was performed in a green house at 26 ± 3^0C day temperature and 12 h day length, and light intensity of 40 Wm^{-2}. Numbers of seeds germinated were counted daily and data were recorded for 14 days. A seed was considered germinated when both plumule and radicle had emerged $\geq$ 0.05cm. Total germination was expressed as percent of that in control

treatment for each accession and then data were arcsine transformed for statistical analysis. Rate of germination was determined on the basis of days to 50 % germination, calculated from the untransformed data.

Seedling experiment: Ordinary river sand was washed with tap water, distilled water and finally with full strength Rorison nutrient solution. Plastic beakers of 500 cm^3 size were filled with the washed and dry sand. The experiment was conducted in a green house at 25 ± 3^0C day temperatures and 16 ± 2^0C night temperature. The concentrations of NaCl used were 0 (Control), 50 and 100 mol m^{-3} NaCl in full strength nutrient solution. The experiment was arranged in a completely randomized design with three replicates, three salt treatments and three accessions. Four seedlings of seven days old of each accession were transplanted equidistant from each other into each beaker. All the beakers were irrigated for two weeks with full strength nutrient solution every other day. Salt treatments in full strength nutrient solution were begun three weeks after the start of the experiment. The salt concentration was increased in increments of 25 mol m^{-3} on alternative days until the appropriate salt treatments were reached. Treatments continued with the addition of 500 ml of the appropriate solution to each beaker after every two days.

The plants were harvested four weeks after the start of salt treatment. Plant roots were removed carefully from the sand, shoots and roots were washed with distilled water and separated. Fresh weights of all samples were recorded. Plant material was dried at 70 ^{0}C for 7 days and dry weights measured. Plant water content was calculated as follows:

$$\text{Percent plant water content} = \frac{\text{Plant fresh weight} - \text{plant dry weight}}{\text{Plant fresh weight}} \times 100$$

Adult experiment: Seeds of three accessions of pigeon pea, Local Arhar, ICPL-151 and ICPL-850014 were sown in Petri dishes. Three one week old seedlings of each accession were transplanted equidistant from each other into each plastic pot of 24 cm diameter. The pots were filled with 5 Kg washed and dried sand. The experiment was conducted in a green house at 28 ± 3^0C day temperature and 20 ± 3^0C night temperature and 12 h day length. The experiment was placed in a randomized complete block design with three blocks, each block containing three accessions and three salt treatments. All the pots were irrigated for four weeks with full strength Rorison nutrient solution (Hewitt, 1966).

NaCl treatments in full strength nutrient solution were begun four weeks after the start of the experiment. The different concentrations of NaCl used were 0, (control), 40 and 80 mol m^{-3} in nutrient solution. The salt concentration was increased in aliquots of 20 mol m^{-3} on alternate days until appropriate salt treatments were reached. Treatments continued with the addition of two liter of appropriate solution once a week. One plant from each pot was harvested eight weeks after the start of salt treatments i.e., at the flowering stage. The roots were removed carefully from the sand and were washed in cold LiNO$_3$ solution isotonic with corresponding treatment in which the plants were growing. 2 mol m^{-3} Ca (NO$_3$)$_2$ 4H$_2$O was added in LiNO$_3$ solution to maintain membrane integrity, while Li+ was used to exchange in cations from exchange sites in the cell walls. The LiNO3 was made isotonic with treatment to avoid osmotic shock during washing of roots. Fresh and dry weights of shoots and roots of all the samples were recorded and dry matter was used for the estimation of ions, shoots and root soluble sugars and starch. Eight weeks after the start of the treatment, data for following biochemical/physiological parameters were also recorded.

Cations (Na$^+$, K$^+$, Ca^{2+}): 100 mg well ground leaf samples were digested in 2 ml concentrated HNO$_3$. After digestion, the volume of the sample was made up to 100 ml with distilled deionized water. All cations (Na$^+$, K$^+$, and Ca^{2+}) were estimated with an Atomic Absorption Spectrophotometer.

Chloride (Cl$^-$): 50 mg of leaf sample was extracted by boiling in 10 ml of distilled deionized water for three hours. The chloride content of the extract was determined with a chloride analyzer (Corning, Chloride Analyzer 925).

Leaf osmotic potential: About 1 g of fully expanded youngest leaves was excised from each pot at 0800 hr. The leaf material was frozen in 2cm^{-3} polypropylene tubes for two weeks, thawed and frozen sap was extracted. After centrifugation the sap was used directly for osmotic potential determination in an osmometer (Wescor, 5500).

Determination of soluble proteins: Total soluble proteins were determined as described by Lowry *et al*., (1951). 0.2 g of fresh leaf material was homogenized in 4 ml of Sodium Phosphate buffer solution (PH 7.0) and the extract was treated with an appropriate reagent. The optical densities were read at 620 nm using spectrophotometer (Hitachi, U. 2000).

Determination of free amino acids: Total free amino acids were determined following the ninhydrin method (Hamilton & Vanslyke, 1943)**.** For the determination of total free amino acids 1ml of each sample which was extracted during the soluble protein estimation was reacted with 1ml of 10% pyridine and 1ml of 2% ninhydrin solution. The optical densities of the colored solutions were then read at 570 nm using spectrophotometer (Hitachi, U 2000).

Proline estimation: Proline was estimated spectrophotometrically following the method described by Bates *et al*., (1973). 0.5 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered. 2 ml of filtrate was reacted with 2.0 ml of acid ninhydrin and 2 ml of glacial acetic acid. The reaction mixture was extracted with 4 ml toluene. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance was read at 520 nm.

Total soluble sugars and starch: Total soluble sugars and starch were estimated following Malik & Srivastava (1979). For the total soluble sugars and starch, 0.1 g of well ground dry material was homogenized in 80 % ethanol and centrifuged at 2900 X g. The residue was retained which was repeatedly washed with 80 % ethanol to remove all the traces of soluble sugars. The filtrate thus obtained was used for the determination of soluble sugars. The residue was used for the determination of starch. Five ml of the distilled water and 6.5 ml of 52% perchloric acid were added to the residue. Extraction of starch with perchloric acid was carried out at 0°C for 20 minutes, then centrifuged at 2900 X g and the extract was retained. With the residue the above step was repeated using fresh perchloric acid and the extract of this step was combined with extract of first step and then volume of each of sugar and starch extracts were made upto 100 ml by the addition of distilled water. The extracts for both soluble sugars and starch were treated with the anthrone reagent and the optical densities were read at 625 nm using a Spectrophotometer (Hitachi, U- 2000)

Results

Germination and seedling experiment: Data for mean germination percentage and rate of germination (days to 50% germination) of three accessions of pigeon pea are presented in Fig. 1. The increasing salt concentrations had no significant effect on total germination percentage but there was a significant effect of salt on rate of germination ($p \leq 0.01$). Rate of germination of ICPL- 151 and ICPL-850014 decreased (greater days taken for germination) consistently with the increase in salt concentration, whereas that of Local Arhar remained unaffected at all salt treatments. Fresh and dry weights of shoots and roots of all accessions (Fig. 2) decreased significantly ($p \leq 0.01$) due to the effect of addition of NaCl in the growth medium. Local Arhar produced significantly greater ($p \leq 0.05$) shoot fresh matter than IPCL 850014 at 100 mol m-3 NaCl. However, the accessions did not differ significantly for shoot dry weight at both the salt treatments. ICPL – 151 had significantly greater ($p \leq 0.05$) root fresh and dry biomass than the other two lines at 50 mol m^{-3} NaCl. At 100 mol m^{-3} all the three accessions did not differ significantly. The data for shoot and root percent moisture content and shoot/root ratios (Fig. 3) show that all the three accessions did not differ significantly for percent moisture content and shoot/root ratios.

Adult experiment: The results for mean shoot and root fresh and dry weights of three accessions of pigeon pea grown in 0, 40 and 80 mol m^{-3} NaCl are presented in Fig. 4. The shoot and root fresh and dry biomass of all three accessions reduced significantly with increasing NaCl concentrations in the rooting medium. Accessions x treatment interaction was also highly significant ($p \leq 0.01$). ICPL-151 was the highest in shoot biomass production at all salt treatments and Local Arhar was the lowest of all accessions at the heighest salt treatment. ICPL-151 and ICPL-850014 had significantly greater ($p \leq 0.05$) root fresh and dry matter at 80 mol m^{-3} NaCl. At 40 mol m^{-3} NaCl all three accessions did not differ significantly for root dry weight.

Mean data for Na^+ and Cl^- concentrations of shoots and roots of three accessions are presented in Fig. 5. Shoot and root Na^+ concentrations of all three accessions increased significantly with addition of NaCl in the rooting medium except ICPL-151, which did not accumulated significantly higher shoot Na^+ at 80 mol m^{-3} NaCl. Local Arhar and ICPL-850014 had significantly greater shoot Na^+ concentrations compared with ICPL-151 at both salt treatments. The accessions did not differ significantly for root Na^+ at both salt treatments. ICPL-850014 and Local Arhar had significantly greater ($p \leq 0.05$) shoot Cl^- than ICPL-151 at both the salt treatments. At 40-mol m^{-3} NaCl ICPL-151 contained significantly greater Cl^- in the roots than those in the other two accessions, whereas, at 80-mol m^{-3} NaCl ICPL-850014 was the highest and Local Arhar the lowest in root Cl^- of all accessions.

Shoot and root K^+ concentrations (Fig. 6) of Local Arhar and ICPL-850014 decreased significantly with increase in salt treatments, whereas those of ICPL- 151 remained unaffected. At 40-mol m^{-3} NaCl ICPL- 151 had significantly greater ($p \leq 0.05$) and ICPL-850014 lower shoot K^+ concentrations than the other accessions, whereas at the highest NaCl treatment ICPL-151 accumulated relatively greater and Local Arhar lower shoot K^+ concentrations. ICPL-151 was the highest in root K^+ concentration of all accessions at 80mol m^{-3} NaCl. By contrast at 40 mol m^{-3} NaCl the three accessions did not differ significantly.
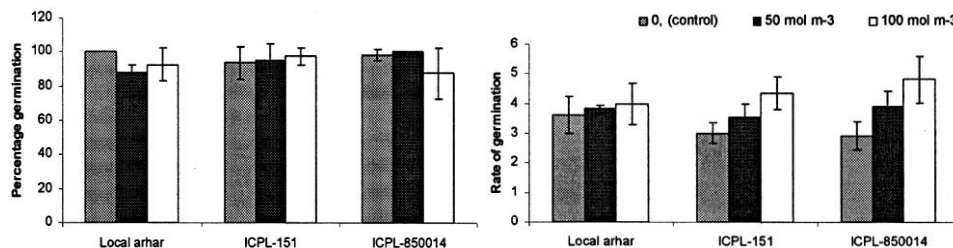
Fig. 1. Mean germination percentage and rate of germination (days to 50% germination) of three accessions of pigeon pea after two weeks growth in solution culture salinized with 0, (cont.), 50 and 100 mol m^{-3} NaCl in full strength Rorison nutrient solution.
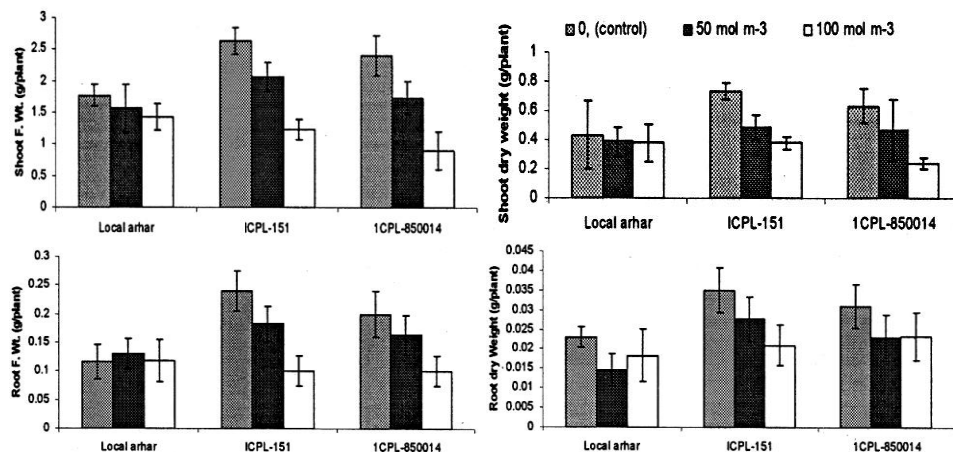

Fig. 2. Mean fresh and dry weights (g/plant) of shoots and roots of three accessions of pigeon pea after 28 days growth in sand culture salinized with 0, (cont.), 50 and 100 mol m^{-3} NaCl in full strength Rorison nutrient solution.
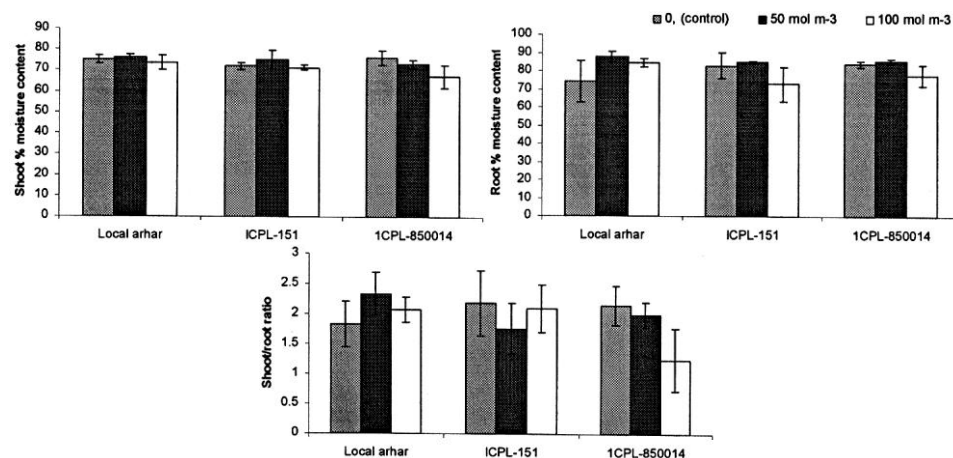

Fig. 3. Mean shoot and root percent moisture contents and shoot/root ratio of three accessions of pigeon pea after 28 days growth in sand culture salinized with 0, (cont.), 50 and 100 mol m^{3} NaCl in full strength Rorison nutrient solution.
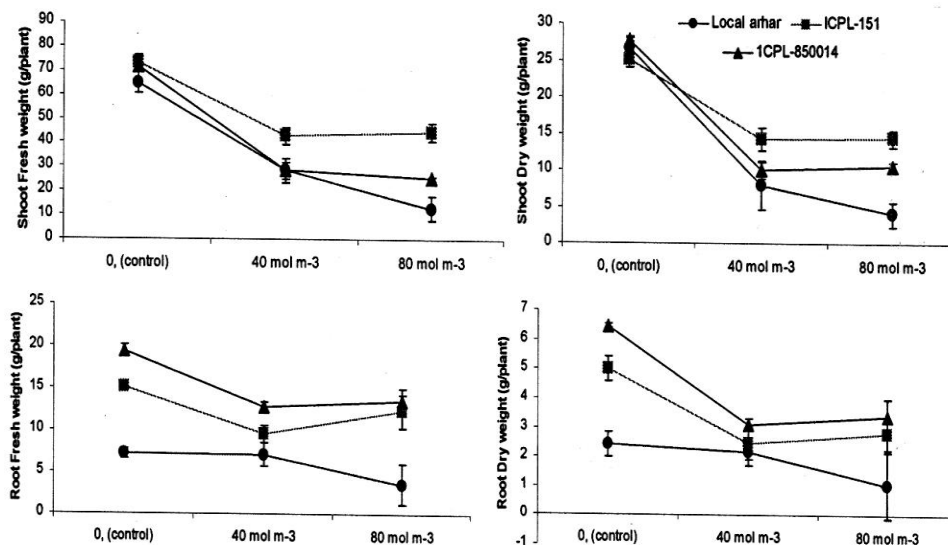
Fig. 4. Mean fresh and dry weights (g/plant) of shoots and roots of three accessions of pigeon pea, when grown for 56 days in sand culture salinized with 0, (cont.), 40 and 80 mol m^{-3} NaCl in full strength Rorison nutrient solution.
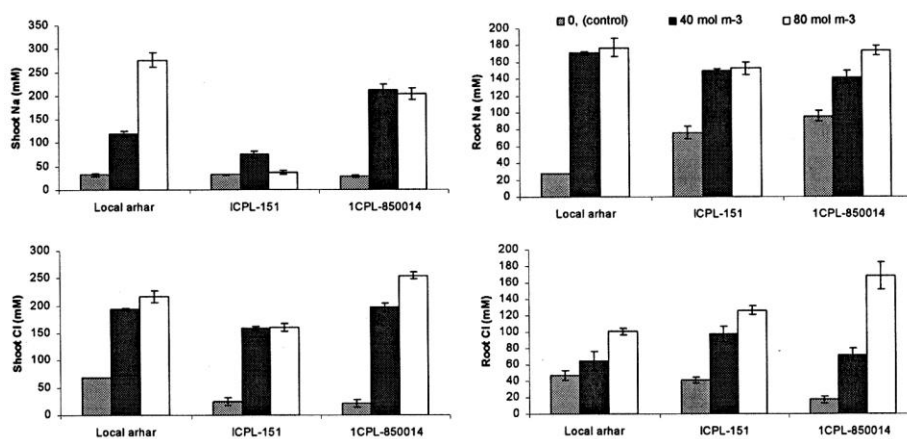

Fig. 5. Mean Na$^+$ and Cl$^-$ concentrations (mol m^{-3} Kg^{-1} fresh weight) of shoots and roots of three accessions of pigeon pea, when grown for 56 days in sand culture salinized with 0, (cont.), 40 and 80 mol m^{-3} NaCl in full strength Rorison nutrient solution.

Shoot Ca^{+2} (Fig. 6) of Local Arhar decreased, whereas that of ICPL-151 remained unaffected with the increase in NaCl concentration of the rooting medium. By contrast the shoot Ca^{+2} of ICPL-850014 were increased at the highest salt treatment. ICPL-850014 had greater shoot Ca^{+2} than the remaining two accessions at 80 mol m^{-3} NaCl, which did not differ significantly from one another. The accessions did not differ significantly for shoot Ca^{+2} at 40mol m^{-3} NaCl. The root Ca^{+2} concentrations of all the three accessions decreased at both NaCl concentrations. But all the three accessions did not differ significantly for root Ca^{+2} concentrations.

Shoot and root K/Na ratios (Fig. 7) of all accessions except those of ICPL-151 decreased significantly with the increase in NaCl concentration in the rooting medium. ICPL-151 had significantly greater shoot K/Na ratios than the other two accessions at both salt treatments. At 40 and 80 mol m^{-3} NaCl the three accessions did not differ significantly for root K/Na ratios. ICPL-151 had significantly greater shoot selectivity (S $_{k, Na}$) at both salt treatments and root selectivity at the highest salt treatment than the other two accessions (Fig. 7).

Data for total soluble proteins (Fig. 8) show that increasing NaCl concentrations had no significant effect on all the three accessions and response of all accessions to increasing salt concentrations was also non-significant.
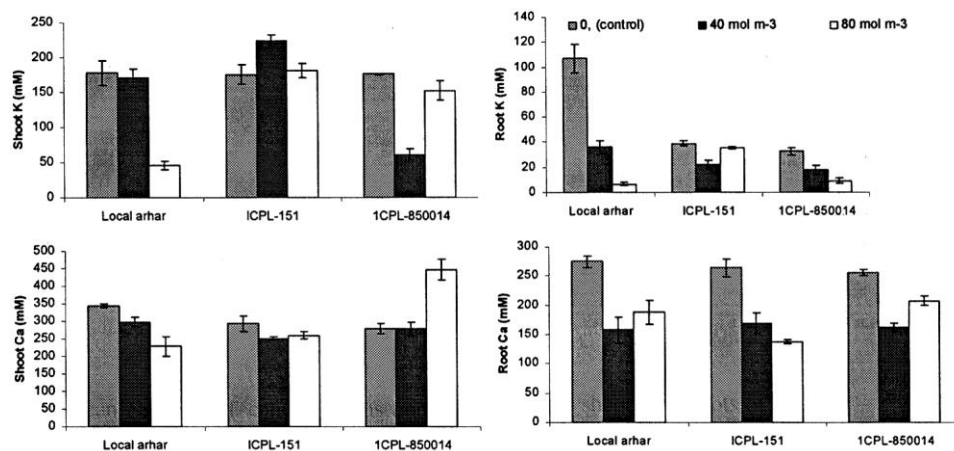

Fig. 6. Mean K$^+$ and Ca^{2+} concentrations (mol m^{-3} Kg^{-1} fresh weight) of shoots and roots of three accessions of pigeon pea, when grown for 56 days in sand culture salinized with 0, (cont.), 40 and 80 mol m^{-3} NaCl in full strength Rorison nutrient solution.

Fig. 7. Mean shoot and root K$^+$/Na$^+$ ratio and K$^+$ versus Na$^+$ Selectivity (S $_{K, Na}$) of three accessions of pigeon pea, when grown for 56 days in sand culture salinized with 0, (cont.), 40 and 80 mol m^{-3} NaCl in full strength Rorison nutrient solution.
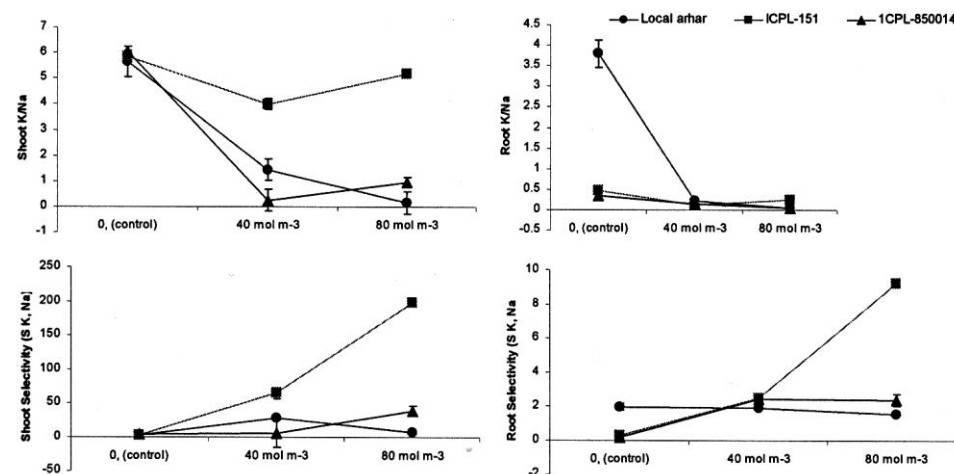
Fig. 8. Mean total soluble proteins (mg/g fresh tissue), total free amino acids (μg/g fresh tissue), proline content (μ mol/g fresh tissue) and osmotic potential (-MPa) of three accessions of pigeon pea, when grown for 56 days in sand culture salinized with 0, (cont.), 40 and 80 mol m^{-3} NaCl in full strength Rorison nutrient solution.

Fig. 9. Mean total soluble sugars (mg/g dry weight and starch (mg/g dry weight) of shoots and roots of three accessions of pigeon pea, when grown for 56 days in sand culture salinized with 0, (cont.), 40 and 80 mol m^{-3} NaCl in full strength Rorison nutrient solution.
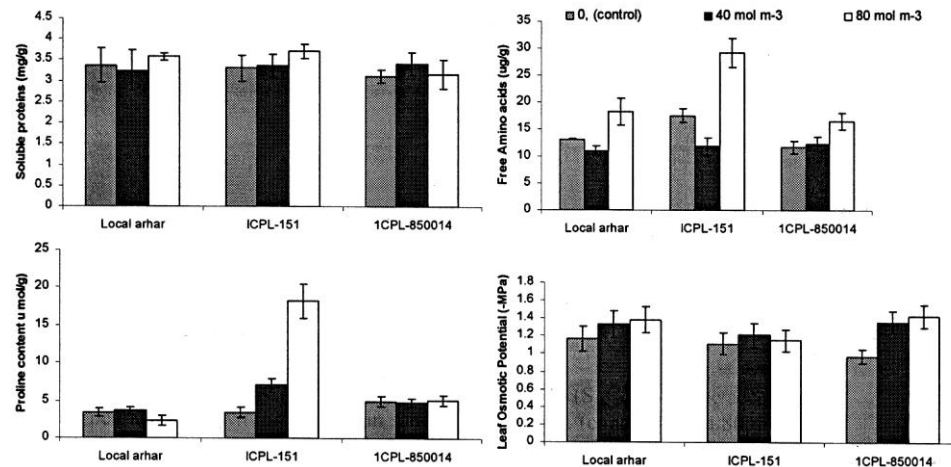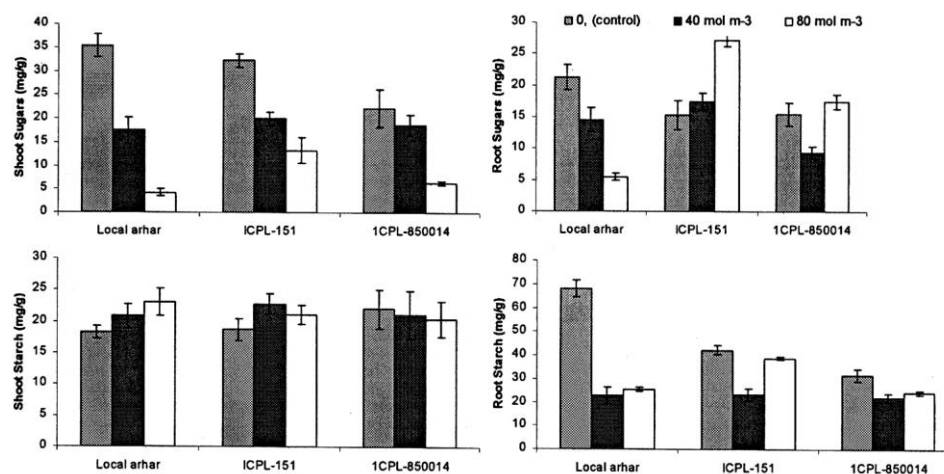
Leaf free amino acids of all three accessions (Fig. 8) increased significantly (p≤ 0.001) at the highest salt treatment and at the same treatment ICPL-151 was the highest in the accumulation of free amino acids of all accessions.

Leaf proline content of only ICPL-151 increased significantly (p≤ 0.05) with the increase in salt concentration in the growth medium(Fig. 8).whereas proline content of Local Arhar and ICPL-850014 remained unchanged. ICPL-151 had significantly (p≤ 0.001) greater amount of proline at both salt treatments.
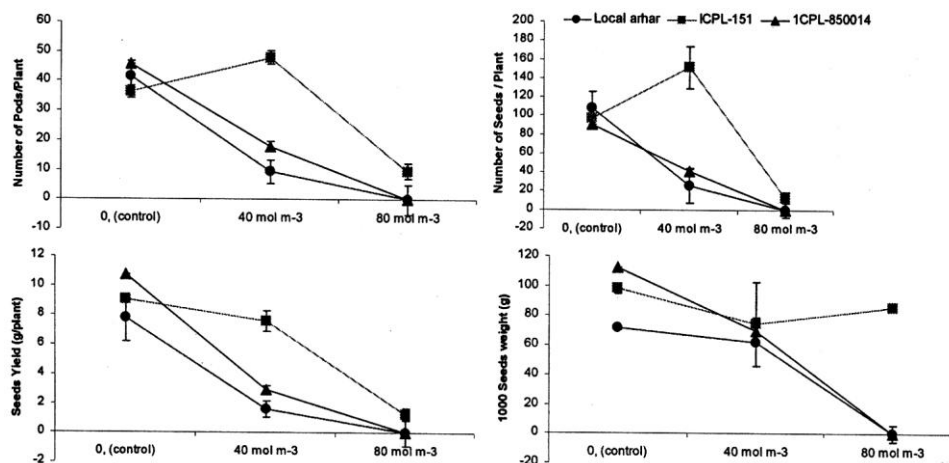
Fig.10. Mean number of pods/plant, number of seeds/plant, seed yield (g/plant) and 1000 seeds weight of three accessions of pigeon pea, when grown till maturity in sand culture salinized with 0, (cont.), 40 and 80 mol m^{-3} NaCl in full strength Rorison nutrient solution.

Increasing NaCl concentration of the rooting medium markedly reduced the leaf osmotic potential of all the accessions except ICPL-151 (Fig. 8). ICPL-151 had significantly greater (p≤0.05) leaf osmotic potential than the remaining two accessions at both salt treatments.

Total soluble sugars and starch of shoots and roots are presented in Fig. 9. With the increase in salt concentration the shot sugars of all three accessions decreased significantly (p≤0.001). ICPL-151 produced significantly greater amount of shoot sugars than the other two accessions at the highest salt treatment. At 40 mol m^{-3} NaCl three accessions did not differ significantly. The root sugars of Local Arhar decreased, whereas those of ICPL-151 increased but those of ICPL-850014 remained unchanged. ICPL-151 had significantly (p≤0.05) greater amount of root sugars at both the salt treatments compared with the other two accessions.

Increasing salt concentration had no significant effect on shoot starch of all the three accessions. Accessions also did not differ significantly. The salt treatments had an overall significant effect (p≤0.01) on root starch of all three accessions (Fig. 9).The root starch of Local Arhar and ICPL-850014 decreased, whereas no consistent pattern of increase or decrease in root starch was observed in ICPL-151. The accession ICPL-151 synthesized significantly greater (p≤0.05) root starch than the remaining two accessions at 80mol m^{-3} NaCl. At 40mol m^{-3} the accessions did not differ significantly for root starch.

The mean data for number of pods/plant, number of seeds/plant, seed yield (g)/plant and 1000 seed weight of all three accessions (Fig. 10) show that increasing salt concentrations had significantly adverse effect on all the yield parameters (all p≤0.001). Of the three accessions only ICPL-151 survived at higher salt concentration i.e., 80mol m^{-3} NaCl till seed setting. At 40 mol m^{-3} NaCl ICPL-151 was the highest, ICPL-850014 moderate and Local Arhar lowest in all the yield parameters.

Discussion

It is now well evident that salt tolerance in many crop species varies with the change in their growth stage e.g., in wheat and barley (Ayers *et al*., 1952), in rice (Akbar & Yabuno, 1974), in barley (Norlyn, 1980), in wheat (Kingsbury & Epstein, 1984; Shannon, 1984), in sugar beet (Bernstein & Hayward, 1958). The results for the degrees of salt tolerance observed in pigeon pea in this study show that there is no positive correlation between the tolerance at early growth stages and that at the adult stage, since no clear difference in salt tolerance of the three accessions was observed at the germination and the seedling stage, whereas accessions differed considerably at the adult stage.

Considering yield and yield components it is clear that ICPL-151 is relatively tolerant, Local Arhar sensitive and ICPL-850014 is moderately tolerant. Although a few accessions of pigeon pea were examined, there is still a great amount of variation in them for salt tolerance. This confirms the early evidence from a number of studies that genetic variation for salt tolerance exists in many crops (Lacerda *et al*., 2003; Ashraf, 2004; Misra & Gupta, 2005). The availability of genetic variation for salt tolerance in pigeon pea is of considerable importance for the improvement of this trait.

The results for ion uptake show that the high biomass producing accession ICPL-151 contained significantly lower Na^+ and Cl^- but higher K^+ in the shoots compared with the other two accessions. The results for this tolerant accession can be explained in the light of early findings of many scientists that salt tolerant mesophytes generally exclude either Na^+ and/or Cl^- from their shoots (Lauchli *et al*., 1994; Ashraf, 2004; Saqib *et al*., 2005) because Na^+ is the primary cause of ion specific damage, resulting due to a range of disorders in enzyme activation and protein synthesis (Tester & Davenport, 2003). Therefore, exclusion of Na^+ at root level and maintenance of high K^+ at shoot level are vital for the plants to grow under saline conditions (Munns *et al*., 2000; Tester & Davenport, 2003). This accession also maintained considerably high K/Na ratio in both shoots and roots. This trait has also potential value as selection criterion for salt tolerance (Greenway & Munns, 1980; Ashraf, 2004).

The salt tolerant accession ICPL-151 maintained significantly higher shoot and root selectivity ($S_{K, Na}$) compared with sensitive and intermediate accessions. These results are in conformity with the early findings of several workers (Pitman, 1976; Taleisnik & Grunberg, 1994; Saqib *et al*., 2005) that selectivity is a possible factor which partly contributes to salt tolerance of crops.

The reduction in the osmotic potential of plants subjected to salt stress may be due to water loss or an increased uptake of dissolved solutes or enhanced synthesis of organic osmotica or a combination of all of these (Mansour, 2000). The high leaf osmotic potential of ICPL-151 can be related to its relatively low uptake of Na^+ and Cl^-. By contrast the salt sensitive accession Local Arhar and moderately tolerant ICPL-850014 accumulated high amounts of both Na^+ and Cl^- in their shoots and contained relatively low amounts of K^+ thus maintaining low K/Na ratios. Their low leaf osmotic potential may have been due to high Na^+ and Cl^- uptake.

Calcium plays an important role in regulating ion transfer into plant sells growing in saline medium (Ashraf & Naqvi, 1992; Soussi *et al*., 2001). Ca^{2+} can also affect membrane stability (Rengel, 1992; Marschner, 1995), and ion translocations (Maas & Grieve, 1987; Cramer, 1992; Unno *et al*., 2002). Calcium was also shown to inhibit Na^+

absorption in beans (Lahaye & Epstein, 1971; Awada *et al*., 1995), soybeans (Wieneke & Lauchli, 1980) and pigeon pea (Subbarao *et al*., 1990b) and thus may be an important factor in controlling salinity response of legumes. The accumulation of moderate amounts of Ca^{+2} by ICPL-151 in its shoots and roots is less easy to explain in view of the early findings of various workers.

The crucial role of organic solutes in plants subjected to salinity stress has been greatly emphasized (Lacerda *et al*., 2003; Ashraf & Harris, 2004; Misra & Gupta, 2005; Ashraf & Foolad, 2006; Niknam *et al*., 2006). Osmoregulation in almost all types of plants involves synthesis and accumulation of organic solutes which reduce the cell osmotic potential to a level to provide high turgor potential for maintaining growth (Lacerda *et al*., 2003; Ashraf & Harris, 2004; Cha-um *et al*., 2004). Accumulation of proline can enable the plants to maintain low water potentials and effectively regulate the accumulation of essential nitrogen and it is osmotically very active (Rains, 1981; Wyn Jones & Gorham, 1983; Misra & Gupta, 2005). It is also compatible with other cytoplasmic components and can be easily converted to glutamate that takes part in the synthesis of other essential amino acids (Rains, 1981). Recently, it has also been reported that proline accumulation protects plants against free radical induced damage by quenching of singlet oxygen (Matysik *et al*., 2002) Thus high proline content of the salt tolerant accession confirms the early findings (Fougere *et al*., 1991; Petrusa & Winicov, 1997) and may have been due to any or all of these phenomena. Accumulation of soluble carbohydrates has been widely reported as organic osmotica in plants in response to salinity and the magnitude of change in their concentration is related to the degree of salt tolerance (Ashraf, 1994b; Murakeozy *et al*., 2003). Although the salt tolerant accession accumulated significantly higher amount of soluble sugars compared with the other two accessions, shoot sugars of all three accession decreased with increase in salinity.

Although the three accessions did not differ significantly for soluble proteins accumulation at all levels yet the tolerant line i.e., ICPL-151 synthesized greater amounts of free amino acids particularly at the highest salt treatment; this might have contributed partially to enhance the salt tolerance of the accession through osmoregulation as suggested by Martino *et al*., (2003).

In general the tolerant accession ICPL-151 accumulated higher amounts of proline, free amino acids and sugars compared with the remaining two accessions but were not so high to significantly lower its osmotic potential. The accumulation of high shoot and root starch content suggests that it may have been a source for the synthesis of soluble sugars under saline conditions.

From this study it is clear that the underlying mechanisms for the salt tolerance of pigeon pea are exclusion of Na^+ and Cl^- from shoots, high uptake of K^+, maintaining high K/Na ratios in the shoots and high accumulation of proline, free amino acids and soluble sugars.

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