Grain Yield and Ionic Relations of four Sorghum Accessions Grown in NaCl Salinity

FAQIR MUHAMMAD AZHAR AND THOMAS MCNEILLY†
Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad–38040, Pakistan
†School of Biological Sciences, The University of Liverpool, L69 3BX, Liverpool, UK

ABSTRACT

Relationship between grain yield and ion concentration in four sorghum accessions, Double TX, Giza 114, INRA 133 and INRA 353 was studied in sand culture. Ion contents of leaves of different ages differed, but not consistently among accessions. Double TX and Giza 114 had the lowest Cl^- concentration in young leaves, and these increased with leaf age. In INRA 133 and INRA 353 leaf Cl^- decreased in older leaves. Pseudostems contained the highest plant concentration of Cl^- and were similar in the four accessions. Root Cl^- concentrations were similar to those in leaves, and were similar in the four accessions. $Na^+:K^+$ ratios were lower in leaves and Pseudostems of Double TX and Giza 114 than in INRA 133 and INRA 353. Ca^{2+} concentrations progressively decreased in younger leaves. Pseudostems contained the lowest concentrations. The concentration of these ions in all plant parts were generally lower at harvest-2 than harvest-1, except for increases in root $Na^+:K^+$ ratio. There were also marked differences in accession leaf and Pseudostem concentration of Cl^- and $Na^+:K^+$ ratio but smaller differences in Ca^{2+} content. On the basis of pattern of ionic accumulation in plant parts, Double TX and Giza 114 may be regarded salt tolerant accessions. By contrast INRA 133 and INRA 353 which contained more Cl^- in all part parts, and yet revealed better performance than Double TX Giza 114 in terms of relative grain yield. The data suggest that accumulation of low Cl^- ions and high K^+ selectivity against Na^+ is by no means a general phenomenon in salt-tolerant varieties.

Key Words: Sorghum; Growth stages; Ion distribution; Grain yield

INTRODUCTION

The soils of Pakistan present the best illustration of the advance of salination, and according to an estimate about 6.3×10^6 hectares of arable land have been adversely affected by moderate to severe salinity (Malik & Shah, 1996). The presence of salts of different species near the soil surface develop highly stressful conditions for plant growth, and ultimately limit yield or result in total plant death. For economic utilization of these saline areas, cultivation of salt-tolerant varieties of conventional crops has been suggested to be useful (Qureshi *et al.*, 1980). Clearly this strategy necessitates the study of physiological and genetic mechanisms controlling salt tolerance in different plant species, to facilitate selection of tolerant plant material.

In general, physiological mechanism that potentiates a plant to cope with stressful condition due to salinity are extremely complex (Wyn Jones, 1981). In some glycophytes variation in salinity tolerance is associated with the efficiency with which they exclude excess of Na^+/ or Cl^- from leaves (Greenway & Munns, 1980). For example, resistant varieties of cotton maintain low concentration of Cl^- in stem and leaves (Khan *et al.*, 1994). When plants are grown in salinity, it effects accumulation of K^+ cation in leaves, and the concentration may decline with an increase in salinity, as has been observed in *Lycopersicon pimpinellifolium* and *L. esculentum* (Guerrier, 1996). Calcium is also an

important nutrient in plants, and high Na^+/Ca^{2+} ratios in plant tissues may be one cause of salt toxicity (Plaut & Grieve, 1988). The work described here examines compartmentation of Cl^-, Na^+/K^+ ratio and Ca^{2+} in leaves of different ages, stems and roots, and their relation with grain yield of four accessions of sorghum.

MATERIALS AND METHODS

Four sorghum accessions/cultivars, Double TX, Giza 114, INRA 133 and INRA 353 were assessed in the salinised sand-culture. Plants of the accessions were grown in plastic containers 50 cm diameter x 35 cm deep, each containing 70 kg washed dried sand. Each container was provided with holes at its base to allow quick drainage of treatment solutions which were retained in plastic saucers placed beneath each container. Seven-day-old seedlings of each of the four cultivars were transplanted in each container in rows 10 cm apart with 6 cm between plants within a row.

Seedlings were watered with half strength nutrient solution containing zero, 100 mM NaCl (EC=10 dSm^{-1}), and 150 mM NaCl (EC=13 dSm^{-1}). All seedlings were watered with the nutrient solution every two days for the first six weeks of the experiment. After this period, salinized solution containing 50 mM NaCl was applied to all saline treatment-units. The NaCl concentration of the nutrient solution was progressively increased by adding 25 mM NaCl every other day until the desired

levels of 100 mM and 150 mM NaCl were attained five and seven days after the application of the first treatment. Thereafter, five litres of each salinized solution, and the control solution were applied every other day to each container. This was sufficient to thoroughly irrigate the plants and keep the sand moist for two days. Treatments continued until the plants were harvested at the appropriate growth stage. To avoid salt accumulation in the root zone of the plants, all the containers were flushed copiously once weekly with unsalinized base nutrient solution, following which the containers were immediately watered with their respective solutions until the EC of the leachate was equal to that of the solution added. The experiment had three replicates, and was carried out in a glasshouse in which the temperature ranged from 18 to 38°C, and relative humidity from 39 - 73%. Natural daylight of 16 hours was provided by additional lighting using 400 Watt mercury vapour lamps.

The plants were harvested at three growth stages (GS); the GS-1 being when plants reached six-leaf stage and leaves were fully expanded, and GS-2 when floral initiation in leaf sheath became apparent, and the final harvest (GS-3) being at plant maturity. At each harvest, whole plants were harvested. Six leaves (top leaves at

for 12 hour in 1 mL concentrated HNO_3. Samples were then digested at 70°C on a hot plate and 5 mL of double distilled deionized water was added to each vial. The concentration of Na^+, K^+ and Ca^{2+} was assayed by Flame photometer (Varian 1275). For Cl^- analysis, 5 mL of double distilled deionized water was added to 20 mg of each of the three plant parts, and digested for one hour on a hot plate maintained at 70-80°C. The content of Cl^- in the sample were determined using CMT Chloride Titrator (Radiometer).

RESULTS AND DISCUSSION

Differences in Cl^- concentrations at the two growth stages were significant (P ≤ 0.001, Table I) and similarly the four accessions differed (P ≤ 0.001) for total Cl^- concentration. Total Cl^- concentration differed significantly with increasing NaCl concentration when data were pooled across growth stages, accessions and plant parts. Total Cl^- accumulation in plant parts also differed significantly (P ≤ 0.001). Interaction, growth stages X accessions X concentrations X plant parts, accessions X concentrations X plant parts, and that growth stages X accessions X plant parts were non-

Table I. Mean squares from analysis of variance of ion accumulation in different plant parts of four sorghum accessions assessed at two growth stages using three NaCl concentrations

Variation due to	Degrees of freedom	Na^+	Cl^-	$Na^+{:}K^+$	Ca^{2+}
Blocks	2	6131385***	1308**	129***	1973 [NS]
Growth stages (GS)	1	18766 [NS]	5621***	7 [NS]	238212***
Accessions (Acs)	3	3185397***	2645***	41***	12615***
NaCl conc. (Conc)	2	53177424***	26255***	511***	333588***
Plant parts (P)	7	4602262***	3438***	151**	191031***
GS x Acs	3	696656**	332**	26**	1717 [NS]
GS x Conc	2	3537443***	1498***	7 [NS]	51911***
Acs x Conc	6	1146948***	401***	18**	2365 [NS]
GS x P	7	395037*	246**	14**	13732***
Acs x P	21	123531 [NS]	85 [NS]	3 [NS]	1263 [NS]
Conc x p	14	886558***	235**	32***	25745***
GS x Acs x Conc	6	1018959***	397***	22***	1526 [NS]
GS x Acs x P	21	80690 [NS]	63 [NS]	2 [NS]	2835***
GS x Conc x P	14	205771 [NS]	89 [NS]	6 [NS]	4201***
Acs x Conc x P	42	103283 [NS]	53 [NS]	3 [NS]	1239 [NS]
GS x Acs x Conc x P	42	128174 [NS]	65 [NS]	3 [NS]	659 [NS]
Residual	382	163430	71	5	1362

GS-1, and flag leaf at GS-2, being leaf 1), psuedostem, and roots of all plants of each accession were collected separately, and a bulk sample of each fraction made subsequently. However, at GS-3 grains were obtained from the plants when they attained maximum dry weight.

Chemical analysis of plant material. The material was oven dried at 70°C for at least seven days, and a small randomly chosen portion of the different plant parts were cut into small pieces. A 20 mg sub-sample was soaked

significant (P ≤ 0.005, Table I). However the interaction term, growth stages X accessions X concentrations was significant (P ≤ 0.001) reflecting different amounts of Cl^- being accumulated in the four accessions at the two growth stages.

$Na^+{:}K^+$ ratio at the two growth stages did not differ (Table I). When data was pooled across growth stages, NaCl concentrations, and plant parts, accessions-differences for Na^+/K^+ ratio were significant (P ≤ 0.001).

Total $Na^+:K^+$ ratios in the three NaCl solutions also differed significantly ($P \leq 0.001$). The interaction terms, growth stages X accessions X concentrations X plant parts, accessions X concentrations X plant parts, and that between growth stages X accessions and plant parts were non-significant ($P \leq 0.05$, Table I). The interaction, growth stages X accessions X concentrations was, however, significant ($P \leq 0.001$), which reveals that $Na^+:K^+$ ratios in the four accessions grown at three NaCl levels differed significantly between growth stages.

Table II. Mean squares from analysis of variance of grain yield per plant using actual values and relative values measured in sand culture

Variation due to	Degrees of freedom	Absolute grain yield	Relative grain yield
Blocks	2	1.34	159.0[NS]
NaCl (conc)	2(1)	55.25***	631.08[NS]
Accessions (Acs)	3	5.64**	2091.9**
Cons x Acs	6(3)	5.98**	45.8[NS]
Residual	22(14)	1.06	324.6

*, **, ***, denote differences significant at P 0.05, 0.01, 0.001, respectively, whilst NS shows non-significant differences

Differences in Ca^{2+} concentrations at the two growth stages were significant ($P \leq 0.001$, Table I), when data were pooled across accessions, NaCl concentration, and plant parts. The accessions differed significantly in Ca^{2+} concentrations. When data were pooled across growth stages, accessions, and concentrations, Ca^{2+} accumulation in the three plant parts differed significantly ($P \leq 0.001$). Mean squares in Table I show that the interaction, growth stages X accessions X concentrations X plant parts, accessions X concentrations X plant parts, and growth stages X accessions X concentrations were non-significant ($P \leq 0.05$). However the interaction, growth stages X accessions X plant parts, was significant ($P \leq 0.01$), which suggests that Ca^{2+} concentration in leaves, stems, and roots of four accessions grown at two growth stages were significantly different.

Fig. 1a. Grain yild per plant (g) of four accessions at three NaCl levels

Mean squares obtained from analysis of variance of absolute grain yield revealed that mean grain yield per plant of all accessions was significantly decreased ($P \leq 0.01$) at 100 and 150 mM NaCl when compared with the control yield (Table II). Grain yields of accessions at 100 and 150 mM NaCl however, did not differ significantly (Fig. 1a). When data of relative grain yield of accessions were analysed, the interaction term concentration X accessions appeared to be non-significant which showed that the four accessions did not differ in their response to NaCl ($P \leq 0.05$, Table II). However, significant differences in relative grain yield between accessions

Fig. 1b. Relative grain yield per plant of four accessions

were found ($P \leq 0.01$, Fig. 1b). INRA 353 with an overall relative grain yield of 60% performed significantly better than Double TX and Giza 114 (which did not differ themselves), but the difference from INRA 133 was not significant.

Fig. 2a. Cl⁻ concentration in different plant parts of four accessions at GS-1

The Cl⁻ concentration in root and pseudostems at GS-1 was very consistent in all accessions (Fig. 2a), and pseudostem contained more Cl⁻ than leaves and roots. In Double TX and Giza 114 remarkably lower concentration of Cl⁻ in young leaves and comparatively higher concentration in stem and roots suggested that there was exclusion of Cl⁻ through leaves. This trend of

Fig. 2b. Cl⁻ concentration in different plant parts of four accessions at GS-2

Fig. 3a. Na⁺:K⁺ ratio in different plant parts of four accessions at GC-1

accumulation of Cl⁻ did not occur in INRA 133 and INRA 353 .At GS-2 uptake of Cl⁻ decreased in pseudostem and leaves of all accessions, but overall response of accessions was similar to that observed at GS-1 (Fig. 2b). Accession INRA 353 was again high Cl⁻ accumulator, and leaves contained considerably low Cl⁻ than pseudostem and roots. On whole plant basis (Fig. 2c), INRA 353 at GS-1 accumulated significantly more Cl⁻ when grown in 100 mM NaCl than the other three accessions, whilst at 150 mM NaCl Double TX, INRA 133 and INRA 353 contained similar amounts of Cl⁻, but significantly more than Giza 114. At GS-2, Cl⁻ concentrations in all the four accessions were significantly lower in 100 mM NaCl than at GS-1, although INRA 353 had significantly greater amount of Cl⁻ than the other three accessions. At 150 mM, Giza 114 accumulated much Cl⁻ as at GS-1, but significantly lower than INRA 133 and INRA 353. These data indicate that a major difference in the responses of the accessions to the salinity stress was the varying concentration of Cl⁻ in stem and leaves. Thus Double TX and Giza 114 may be regarded as the most salinity tolerant accessions, as suggested by Khan *et al*. (1994), while INRA 133 and

Fig. 2c. Total plant Cl⁻ concentration of four accessions at three NaCl levels a two growth stages

INRA 353 which contained more Cl⁻, a characteristic of sensitive genotypes (Hajbagheri *et al.,* 1987). However, in terms of relative grain yield per plant INRA 133 and INRA 353 showed superior performance than Double TX and Giza 114 (Fig. 1b). Such differential salt tolerance of cultivars of other cereals like maize, triticale and barley have been reported in the literature (Yeo *et al*., 1977; Salim, 1988, 1991). Thus the observations of Kingsbury *et al*. (1984), that higher concentration of Cl⁻ ions occur in salt-sensitive varieties were not substantiated here. These data suggest that accumulation of low concentration of Cl⁻ ions is by no means a general phenomenon in salt tolerant species. Although accumulation of Cl⁻ ionHs decreased with ageing in Double TX, Giza 114, and INRA 133, it increased significantly in INRA 353 (Fig. 2c). Nonetheless, differences in the responses of all accessions to Cl⁻

Fig. 3b. Na⁺:K⁺ ratio in different plant parts of four accessions at GC-2

accumulation were maintained wholly or partially, at GS-1 and GS-2.

The concentrations of K⁺ in leaves were considerably less than those in roots, possibly the result

of Na^+ interfering with K^+ uptake by the roots (Munns *et al*., 1983). At GS-1, INRA 133 and INRA 353 had much greater Na^+/K^+ ratios than Double TX and Giza 114 showing K^+ selectivity in the latter two accessions (Fig. 3a). By contrast, with increasing plant age, uptake of K^+ was reduced to a low level in Double TX and Giza 114 (Fig. 3b). On whole plant basis, the Na^+/K^+ ratio for

Fi. 3c. Total Na:K ratio in four accessions at here NaCl levels at two growh stages

Double TX and Giza 114 increased at GS-1 but non-significantly so, between 100 and 150 mM NaCl (Fig. 3c). In INRA 133 and INRA 353, however, Na^+/K^+ ratio was significantly higher at 150 mM NaCl. Accession INRA 353 at 100 mM and INRA 133 at 150 mM NaCl had significantly higher Na^+/K^+ ratios than the other three accessions. At GS-2, there were no significant differences between accessions at 100 mM NaCl. In 150 mM NaCl, Double TX, Giza 114 and INRA 133 did not differ, but INRA 353 showed a marked increase in Na^+/K^+ ratios. All accessions had significantly a greater Na^+/K^+ ratio at 150 mM than 100 mM NaCl. The higher

Fig. 4a. Ca^{++} concentration in different plant parts of four accessions at GS-1

Fig. 4b. Ca^{++} concentration in different plant parts of four accessions at GS-2

concentration of Na^+ and relatively low K^+ concentration at GS-2 may have reached a level which affected protein synthesis (Flowers & Lauchli, 1983) and synthetic activity (Huber, 1985) which may possibly have accounted for poor grain development in Double TX and Giza 144 (Fig. 1b).

Fig. 4c. Total Ca^{++} concentration of four accessions at three NaCl levels at two growth stages

In contrast to K^+ concentration, Ca^{2+} concentration was markedly lower in the stem (Fig. 4a,b), perhaps the result of Na^+ and Ca^{2+} ions competing more successfully for common uptake sites. On whole plant basis Ca^{2+} concentration of all accessions in both 100 mM and 150 mM NaCl was less than in control at both GS-1 and GS-1 (Fig. 4c). However, differences in Ca^{2+} concentration between NaCl level were not great. The Ca^{2+} concentration of all the four accessions at GS-2 was lower in control plant, as well as in the low NaCl treatments. There were no consistent trends in accession-responses. Competition between the two species of cations can lead to Ca^{2+} deficiency, and can be an

important factor in NaCl toxicity, as noted by Plaut and Grieve (1988). Compared to INRA 353, the Ca^{2+} concentration of Double TX, Giza 114 and INRA 133 were much lower, and the decreases at GS-2 were also great. These data are in accord with those of Maas *et al.* (1986) who noted a similar decrease in Ca^{2+} concentration in sorghum at GS-2. Data discussed above indicate the existence of differential salt tolerance in the four sorghum accessions of different origins like many other plant species. Although Double TX has been recognized as a salinity tolerant cultivar (Maas *et al.*, 1986), the assessment carried out here has revealed that, at least under the limits of this experiment, INRA 133 and INRA 353 also showed appreciable salinity tolerance as suggested by Maas (1986). Comparatively higher salt tolerance of these two cultivars seemed to be due to relatively less K^+ selectivity against Na^+ in their shoots and their ability to control Cl^- flows. Thus from these data, it would appear to question the possibility, at least in this species, of using patterns of ion distribution as a selection criterion for improved salinity tolerance.

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