Euphytica (2007) 158:179–188
DOI 10.1007/s10681-007-9441-3

Identification of pearl millet [*Pennisetum glaucum* (L.) R. Br.] lines tolerant to soil salinity

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Received: 7 March 2007 / Accepted: 17 April 2007 / Published online: 18 May 2007

Abstract Crop tolerance to salinity is of high importance due to the extent and the constant increase in salt-affected areas in arid and semi-arid regions. Pearl millet (*Pennistum glaucum*), generally considered as fairly tolerant to salinity, could be an alternative crop option for salt affected areas. To explore the genotypic variability of vegetative-stage salinity tolerance, 100 pearl millet lines from ICRISAT breeding programs were first screened in a pot culture containing Alfisol with 250 mM NaCl solution as basal application. Subsequently, 31 lines including many parents of commercial hybrids, selected from the first trial were re-tested for confirmation of the initial salinity responses. Substantial variation for salinity tolerance was found on the basis of shoot biomass ratio (shoot biomass under salinity/ non-saline control) and 22 lines with a wide range of tolerance varying from highly tolerant to sensitive entries were identified. The performance of the genotypes was largely consistent across experiments. In a separate seed germination and seedling growth study, the seed germination was found to be adversely affected (more than 70% decrease) in more than half of the genotypes with 250 mM concentration of NaCl. The root growth ratio (root growth under salinity/control) as well as shoot growth ratio was measured at 6 DAS and this did not reflect the whole plant performance at 39 DAS. In general, the whole plant salinity tolerance was associated with reduced shoot N content, increased K^+ and Na^+ contents. The K^+/Na^+ and Ca^{++}/Na^+ ratios were also positively related to the tolerance but not as closely as the Na^+ content. Therefore, it is concluded that a large scope exists for improving salt tolerance in pearl millet and that shoot Na^+ concentration could be considered as a potential non-destructive selection criterion for vegetative-stage screening. The usefulness of this criterion for salinity response with respect to grain and stover yield remains to be investigated.

Keywords Ionic distribution · K/Na ratio · *Pennisetum glaucum* · Salinity tolerance · Shoot biomass ratio · Shoot Na Content

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Introduction

Salinity is a major constraint to crop production, especially in the arid and semi-arid areas of the

world, where low precipitation, high surface evaporation, irrigation with saline water, rising water tables and poor irrigation practices generally increase the level of soluble salts (Ashraf 1994; Hollington 1998; Houshmand et al. 2005). As an example, soil salinity levels measured as an EC of 3.3–3.8 dS m^{-1} were shown to reduce durum wheat yields by 58–81% (Houshmand et al. 2005). Salinity management options through soil reclamation and/or improved irrigation techniques in the arid and semi-arid tropics are viable but often prohibitively expensive in poor developing countries. On the other hand, crop improvement could be a less expensive and more sustainable solution for agricultural use of salt-affected areas. Most of the pearl millet is grown as grain and fodder crop in the arid and semi-arid zones of south Asia and west Africa (Blummel et al. 2003), where the soils are often prone to salinity problems which affect the crop productivity. Pearl millet is also a potential crop to grow in the rice fallows of saline areas in south Asia, where typical increases of salinity levels during post-rainy season prevent crop production. Therefore, improved tolerance could help intensify the production under this environment (Bidinger and Hash 2003). Crops species vary in their sensitivity to salinity (Francois and Maas 1994; Serraj et al. 1998; Munns et al. 2002). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] and its wild relatives are rated to be fairly tolerant to salinity (Mass and Hoffman 1977; Shannon 1984; Ashraf and McNeilly 1987; http://www.biosalinity.org/salt-tolerant_plants.htm) and provide an option while selecting crops that can be more profitably grown in saline soils (Chopra and Chopra 1993).

Lack of a single reproducible screening protocol and lack of knowledge on trait(s) that confer yield under salinity is a great limitation to breeding tolerant varieties. Field screening under salinity stress may not be effective because of the extent of variability in salinity experienced within a single field and among plots even at shorter distances (Richards and Dennet 1980). Pearl millet seems to be sensitive at germination stage in ECe of 16 dS m^{-1} and beyond but this sensitivity is to some extent compensated by the tillering capability (Dua 1989). However, it seems that salinity response estimated at germination stage does not correlate well with plant performance at later stages (Munns and James 2003). Na^+ exclusion and grain K/Na ratios were suggested to be reliable traits for selection. However, their usefulness as selection criteria (Munns and James 2003; Poustini and Siosemardeh 2004) was not demonstrated with five cultivars in pearl millet (Ashraf and McNeilly 1987) and therefore this relationship needs to be evaluated with a wider range of genotypes. Overall, it seems that although various aspects have been related to tolerance, the variation in whole plant reaction to salinity has been suggested to provide the best means of initial isolation of salinity tolerant genotypes (Shannon 1984; Ashraf and McNeilly 1987).

Large genotypic variation was reported to exist in pearl millet for salinity response in terms of whole plant response (Ashraf and McNeilly 1987, 1992; Dua 1989). Moreover, availability of high levels of tolerance in other species of *Pennisetum* (Ashraf and McNeilly 1987, 1992; Muscolo et al. 2003) and within the *P. glaucum* (Dua 1989) offers a scope for understanding the traits related to tolerance and to integrate these tolerant crop species/genotypes into appropriate management programs to improve the productivity of the saline soils.

Quantitative trait loci (QTLs) for salt tolerance have been mapped in several cereals including rice (Flowers et al. 2000; Koyama et al. 2001; Takehisa et al. 2004; Ren et al. 2005), barley (Ellis et al. 1997; Mano and Takeda 1997) and bread wheat (Quarrie et al. 2005; Ma et al. 2007) with markers not adequately robust enough to use across a range of germplasm and a range of salinity conditions. The limited success of these studies was suggested to be likely due to limited amount of diversity available within the modern cultivars which were used as parents (Munns et al. 2002). Therefore, it seems necessary to identify traits that are highly related to salinity tolerance through a simple and repeatable screening method and to select genotypes with high levels of polymorphism for use in molecular studies.

The objectives of the present study were to identify the extent of genotypic variation for salinity tolerance measured as a proportion of shoot biomass production under saline condition as that of non-saline control during the early vegetative stage among the range of currently used breeding lines at ICRISAT, to identify physiological traits that could be used as potential screening criteria and to evaluate the potential use of seed germination and seedling growth responses for predicting the whole plant responses of genotypes to salinity.

Materials and methods

Pot culture screening

In the first pot experiment, 100 entries comprising 35 hybrid parental lines, 61 population progenies, 2 popular open-pollinated varieties and 2 germplasm accessions were exposed to NaCl salinity using a randomized complete block design with three replications. Pots of 12.5-cm diameter were filled with 1.2 kg of Alfisol mixed with di-ammonium phosphate at the equivalent rate of 200 kg ha^{-1} on 29 Mar 2003, and sealed at the bottom to avoid salt loss. Two levels of salinity were applied prior to sowing through a one-time application of deionized water with and without 250 mM NaCl. The amount of water added to bring the soil to field capacity was determined on a soil weight basis (23.2% w/w). The resulting solution EC was 23.4 dS m^{-1} and the NaCl-treated soil ECe was 18.1 ± 0.19 dS m^{-1}, compared to 2.9 ± 0.26 without NaCl. Irrigation was provided on alternate days up to 20 days after sowing (DAS) and every day at later stages of growth to replace evapotranspirational losses and bring soil moisture levels to field capacity. The water needed for these subsequent irrigations was determined by daily weighing of 10 representative pots, to avoid either water logging or water deficit in the pots. Sixteen seeds of each genotype were sown in each pot in four equally spaced hills. A maximum of four plants pot^{-1} were retained after thinning at 10 DAS. One plant per pot was sampled at 18, 25, 32 and 39 DAS. In case a pot had less than four plants, the plants were reserved for the later sampling stage(s), and earlier sampling was skipped. The harvested plants were separated into root (extractable) and shoot, dried in hot air draught oven at 60°C for 3 days and the dry weights were recorded. A ratio of shoot biomass measured under salinity to that of control, used as a proxy for estimating the salinity tolerance for biomass production at vegetative stage, was calculated replicate-wise for each sampling time.

A second pot experiment (Experiment 2) was conducted only with 31 hybrid parental lines tested in the first experiment and was sown on 17 Sep 2003. The experimental procedure was the same as in experiment 1, except that the pot size was 15-cm diameter, contained 2-kg Alfisol, and all plants were harvested at the same time at 35 DAS.

Soil and plant assessment

Ionic contents of shoots were estimated using the sample harvested at 39 days after sowing from experiment 1. The pooled shoots (stem + leaves) of all the three replications were used for the determination of N, P, K, Na and Ca. One hundred and fifty milligrams of finely ground shoot sample was digested in 4 ml of concentrated sulfuric acid with 0.5% selenium powder at 360°C for 75 min on a block digester and the digest was diluted to 75 ml. Using this digest, total N was estimated using SKALAR Auto Analyzer, Netherlands (Krom 1980) to determine whether N absorption has any role in reducing plant growth under saline conditions. Exchangeable K, Na and Ca were estimated (Sahrawat et al. 2002) using an atomic absorption spectrophotometer (Varion model 1200, Australia).

The EC (electrical conductivity) of the NaCl solutions was measured directly using a conductivity meter (Model 1481-50, Cole-Parmer Instrument Company, Chicago). The soil EC was measured using a 1:2 (soil: water; w/v) extract.

Germination studies

Twenty seeds of each of the 100 entries were surface sterilized with 1% sodium hypochlorite solution for 10 min, and germinated on filter paper in closed petri dishes for 6 days in 15 ml deionized water (control) or in 15 ml of a 250 mM NaCl solution in a randomized complete block design with three replications in a growth chamber at 28/25°C day/night temperature with 12-h light. Five representative seedlings from each petri dish were used for the measurement of root and shoot length. Relative seed germination (RSG) was calculated as the ratio of the number of seeds germinated under saline conditions to the number of those germinated in control, relative root length (RRL) as the ratio of root length under saline conditions to the mean root length of control, and relative shoot length (RSL) as the ratio of shoot length under saline conditions to the mean shoot length of control. These variables were subjected to statistical analysis as outlined in the next section. The resulting best linear unbiased predictors (BLUPs) for each trait were used to estimate correlations and regressions among RSG, RRL, RSL, and shoot

biomass ratio observed under different stages of vegetative growth.

Statistical analysis

The data on each variate from individual experiments were analyzed using the following linear additive mixed effects model

$$Y_{ik} = \mu + r + g_k + e_{ik}$$

where Y_{ik} is the observation on genotype k in block i, μ is the general mean, r_i is the effect of block i, g_k is the effect of genotype k, and e_{ik} is the plot error. The general mean μ and block effect r_i were considered as fixed. The genotype effect g_k, and the error term e_{ik}, were assumed as random effects, each with mean zero and constant variances σ_g^2 and σ_e^2 respectively. Using the above model, residual maximum likelihood (ReML) was used to obtain the unbiased estimates of the variance components σ_g^2 and σ_e^2, and the BLUPs of the performance of the 100 genotypes in the first and 31 genotypes in the second experiment. Heritability was estimated as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$. The significance of genetic variability among genotypes was assessed from the standard error of the estimate of genetic variance σ_g^2, assuming the ratio $\sigma_g^2/\mathrm{SE}(\sigma_g^2)$ to follow normal distribution asymptotically.

Geometric mean (n^{th} root of the product of n observations) of shoot biomass ratios was calculated from the four BLUPs of the four DAS for each genotype for the first experiment. These geometric means of the first experiment and the BLUPs of second experiment for 31 common genotypes were used for grouping them into a few distinct groups by a hierarchical cluster analysis using Ward's incremental sum of squares method. All the statistical analyses were carried out using GenStat, Release 6.1 (Payne 2002).

Results

Pot culture screening

In the current study the genotypic variability for salinity tolerance was assessed, based on the ratio of shoot (stem + leaf) biomass produced under salinity as that of control. Large genotypic variation was found for the shoot biomass ratio at all stages of crop growth both in experiment 1 and 2 (Table 1). The heritability values observed for the four samples of experiment 1 and the single one-time sample of experiment 2 ranged from 0.33 to 0.45.

The hierarchical cluster analysis had yielded five distinct groups at a similarity index of 0.90 and the entries in groups with top one (highly tolerant) and two (moderately tolerant) and the bottom (highly sensitive) shoot biomass ratios were identified (Table 2).

Ion distribution

Shoot Na^+ content under saline condition was negatively correlated with shoot biomass ratio (Fig. 1A; $r^2 = 0.39$; $P = < 0.001$). This relationship improved further with the mean shoot biomass under salinity (Fig. 1B; $r^2 = 0.43$; $P = <0.001$). Shoot Na^+ content

Table 1 Trial means, range of predicted means, genetic variance and heritability for shoot biomass ratio (shoot biomass under salinity/shoot biomass under control) for pearl millet genotypes at 18, 25, 32 and 39 DAS in experiment 1 and shoot biomass ratio at 35 DAS in experiment 2

Sampling time	Ratio of shoot biomass			
	Trial mean	Range of predicted means	σ_g^2 (SE)	Heritability (h^2)
Experiment 1 (n = 100)				
18 DAS	0.048	0.020–0.198	0.0027 (0.0007)	0.33
25 DAS	0.080	0.023–0.344	0.0083 (0.0017)	0.45
32 DAS	0.127	0.047–0.390	0.0112 (0.0028)	0.36
39 DAS	0.313	0.107–0.633	0.0292 (0.0065)	0.39
Experiment 2 (n = 31)				
35 DAS	0.049	0.014–0.133	0.0013 (0.0005)	0.45 m

Table 2 The shoot biomass ratio of pearl millet genotypes that clustered into highly tolerant, tolerant and sensitive groups based on hierarchical cluster analysis (Ward's ISS method) using the data of experiment 1 (geometric mean of 18, 25, 32 and 39 day ratios) and the 35 day ratio of experiment 2

Genotypes	Mean shoot biomass ratio	
	Experiment 1	Experiment 2
Highly tolerant		
HTP 94/54 (HHB 146 pollinator)	0.234	0.083
CZI 9621	0.206	0.074
ICMP 451 (ICMH 451 pollinator)	0.151	0.118
IP 3757	0.128	0.133
Moderately tolerant		
863-B	0.097	0.068
ICMB 02111	0.104	0.073
ICMB 94555	0.079	0.062
ICMB 95333	0.080	0.061
ICMB 00888	0.112	0.041
PRLT 2/89-33	0.130	0.051
ICMB 01222	0.135	0.068
CZI 98-11	0.170	0.069
IP 3732	0.149	0.095
Highly sensitive		
ICMB 95111	0.069	0.037
ICMB 95222	0.059	0.030
ICMB 96333	0.067	0.030
ICML 22	0.074	0.035
Tift 23D2B1-P5	0.051	0.047
H 77/833-2 (HHB 67 pollinator)	0.060	0.014
81-B	0.039	0.014
MIR 220	0.046	0.014
J 104 Selection	0.077	0.016

under control did not show any such relationship with the actual shoot biomass under control (data not shown). The overall average shoot Na^+ content under salinity (0.99%) was about four times higher than that under control conditions (0.24%) and ranged from 0.35 to 2.66%

Shoot K^+ content under saline conditions was also positively related to the shoot biomass ratio (r^2 = 0.18, P = <0.001). The strength of this relationship improved further with the shoot biomass under salinity (r^2 = 0.24, P = <0.001). Unlike the Na^+ content, the mean change in overall mean K^+ content

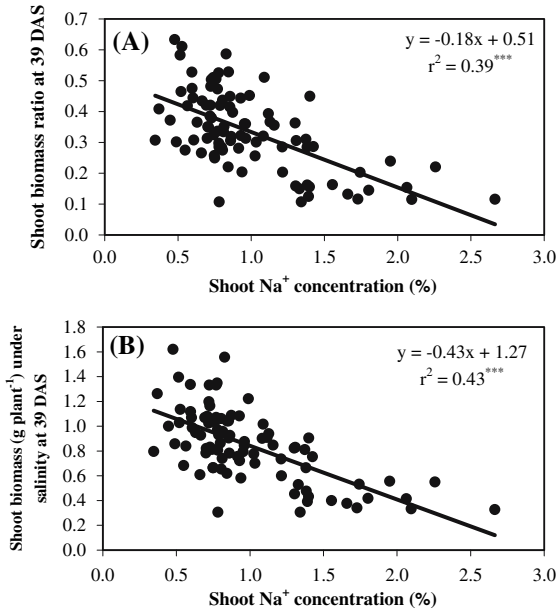

Fig. 1 Relationship of shoot Na^+ concentration (%) with (**A**) shoot biomass ratio at 39 DAS and (**B**) with shoot biomass under salinity (g $plant^{-1}$) at 39 DAS in pearl millet entries. (*** denotes significance at a probability level of 0.001)

under salinity (1.27%) was not that different from that of the one under control (1.55%). The K^+/Na^+ ratio was positively associated with the shoot biomass ratio at 39 DAS (r^2 = 0.21, P = <0.001; Fig. 2A). Also this relationship was much higher with the shoot biomass under salinity (r^2 = 0.28, P = <0.001). The overall mean of K^+/Na^+ ratio was about 1.7 under saline conditions, substantially lower than that under the non-saline control (about 6.6).

Ca^{++} content was not correlated either to the shoot biomass ratio or to the shoot biomass under salinity. By contrast, the Ca^{++}/Na^+ ratio was positively correlated to both shoot biomass ratio (r^2 = 0.17, P < 0.001; Fig. 2B) as well as the shoot biomass under salinity (r^2 = 0.23, P < 0.001).

Under saline condition, the N concentration of shoots was negatively correlated with shoot biomass ratio (r^2 = 0.32, P = <0.001; Fig. 3) as well as the shoot biomass under salinity (r^2 = 0.35, P = <0.001), whereas under control conditions this correlation was not significant. This result also indicated that the salinity-tolerant entries had relatively lower N concentration, varying from 0.3 to about 1.3% (Fig. 3). This was likely due to the fact that tolerant plants maintained relatively higher growth rates and thus

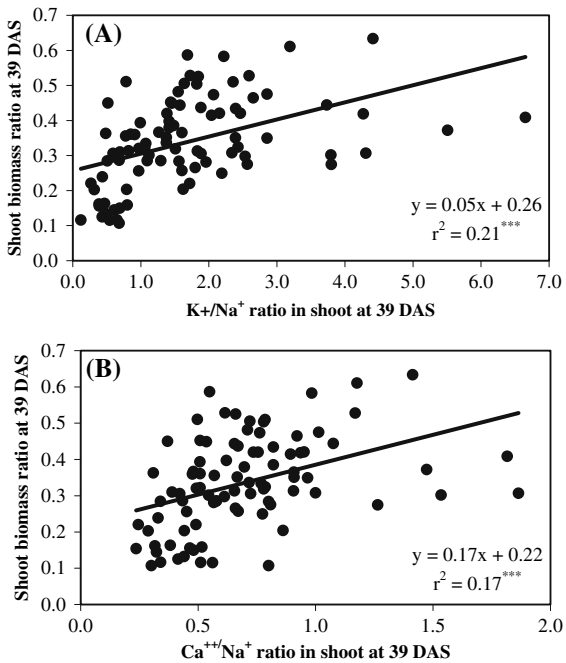

Fig. 2 Relationship of (**A**) shoot K^+/Na^+ ratio and (**B**) shoot Ca^{++}/Na^+ ratio with the total dry matter plant^{-1} of pearl millet entries under salinity at 39 days after sowing. (*** denotes significance at a probability level of 0.001)

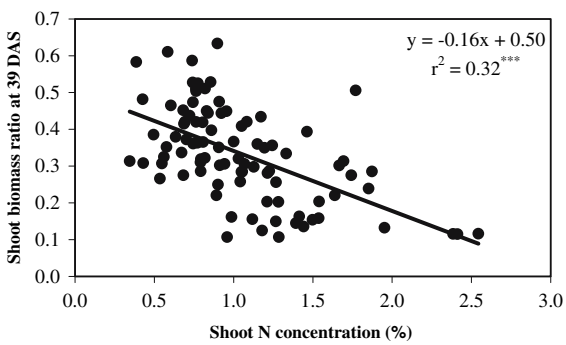

Fig. 3 Relationship of shoot N concentration (%) with the shoot biomass ratio at 39 DAS of pearl millet entries. (*** denotes significance at a probability level of 0.001)

''diluting'' the amount of N taken up, while reduced growth in sensitive entries resulted in higher N concentrations in the shoot. In general, N acquisition by plants seems to have been affected under salinity, as indicated by the overall environmental means. The overall mean N concentration under saline conditions was 1.05%, compared to 1.42% in the non-saline control.

Germination studies

Twenty one entries that showed <80% germination under control conditions were excluded from the study of the variation in seed germination under salinity and in subsequent evaluation of its relationship with root and shoot growth under salinity at seedling stage with the shoot biomass ratios at different stages of growth, to avoid confusion between poor seed germination and salt effects on early vegetative growth. There was significant genotypic variation in the response of germination to salinity as measured by the ratio of germination under salinity to that of control (RSG) (Table 3). There was a large range of variation for the RSG among the entries tested (Table 3). RSG was 0.1 to 0.4 in 11 progenies and in one genotype RIB 3135-18 (Pollinator of hybrid RHB 12) indicating that these entries are highly sensitive to seed germination under salinity. It was between 0.4 and 0.7 in 12 progenies and in some of the B-lines such as ICMB 94111, ICMB 89111, ICMB 96444, 843B, ICMB 95222, ICMB 98111, ICMB 00888 and 863B indicating that these were moderately sensitive. Thirty-three entries, including 15 progenies and 18 parental lines had more than 0.8 RSG. It is notable that all the highly tolerant entries based on the shoot biomass ratio listed in Table 2 possessed an RSG of >0.8.

Besides seed germination, the ratio of root and shoot growth of the seedlings, estimated as length

Table 3 Trial means, range of predicted means, genetic variance and heritability for the ratio of seeds germinated in 250 mM saline solution as that of control (%) (RSG), ratio of root length under salinity as that of control (RRL) and the ratio of shoot length under salinity as that of control (RSL) in the 79 pearl millet entries that showed >80% germination under control

Trait	Trial mean	Range of predicted means	σ_g^2 (SE)	Heritability (h^2)
RSG	0.684	0.11–1.07	0.0602 (0.0106)	0.77
RRL	0.198	0.054–0.461	0.0100 (0.0017)	0.80
RSL	0.273	0.045–0.480	0.0092 (0.0017)	0.70

under salinity to that of control, also varied greatly across entries (Table 3). Root growth was relatively more affected by salinity than shoot growth as shown by the overall means and the ranges of these two traits (Table 3). The significance pattern of the genetic correlations, while relating RSG, RRL and RSL of the seedlings with the shoot biomass ratio at 18, 25, 32 and 39 DAS, was largely the same as that of the phenotypic correlation (Table 4). RSG exhibited high levels of positive correlation, both genetic as well as phenotypic, with the shoot biomass ratios recorded at all the stages of growth. Genetic correlation showed that RRL was related to shoot biomass ratio at 39 DAS and the phenotypic correlation showed both RRL and RSL was correlated with the shoot biomass ratio at 39 DAS (Table 4). The correlation coefficients obtained with shoot biomass under salinity instead of shoot biomass ratio were also largely of similar values (data not shown).

Discussion

The main purpose of this study was to assess the range of variation for salinity tolerance with respect to biomass yield, an important consideration in breeding forage cultivars. Entries with contrasting relative shoot biomass ratio (shoot biomass yield under salinity/shoot biomass yield under non-saline control), a measure of salinity tolerance, were identified. The most tolerant entries included some of the restorers such as HTP 94/54 (pollinator of a released hybrid HHP 146) and ICMP 451, an open-pollinated variety CZI 9621 and a germplasm accession, IP 3757. The moderately tolerant entries included some of the B-lines, which are also parental lines of released hybrids such as 863 B and ICMB 94555. Similarly, the highly sensitive entries also included B-lines (81B, ICMB 95111, ICMB 96333) and restorer lines (H 77/833-2 and MIR 220). The poor value of using salinity tolerance at seedling stage was also confirmed. Further shoot Na^+ concentration emerged as an indirect non-destructive selection criterion.

Measuring the biomass production at 39 days after sowing following saturation of the soil to field capacity with a 250 mM NaCl solution has provided a reasonably good screening method to identify tolerant sources in relative biomass production in the early vegetative stages under saline conditions, and has revealed substantial variation among entries. The salt concentration (250 mM NaCl resulting in a soil ECe of 18.1 ± 0.19 dS m^{-1}) chosen for screening, was similar to that in some previous studies on

Table 4 Genetic and phenotypic correlations of the shoot biomass ratios (salinity/control) (SBR) observed at 18, 25, 32 and 39 days after sowing with the relative seed germination (%) (RSG), relative root length ratio (RRL) and the relative shoot length ratio (RSL) in 79 pearl millet entries

Traits	SBR (18DAS)	SBR (25 DAS)	SBR (32 DAS)	SBR 39 DAS	RSG	RRL
Genetic correlation						
SBR (25DAS)	0.675***					
SBR (32DAS)	0.648***	0.791***				
SBR (39DAS)	0.464*	0.516**	0.519**			
RSG	0.560***	0.386**	0.527***	0.469**		
RRL	0.067	0.237	0.089	0.335*	0.398***	
RSL	0.247	0.036	0.113	0.211	0.415***	0.649***
Phenotypic correlation						
SBR (25DAS)	0.366***					
SBR (32DAS)	0.323***	0.488***				
SBR (39DAS)	0.191**	0.336***	0.411***			
RSG	0.218**	0.242**	0.292***	0.267***		
RRL	0.038	0.114	0.059	0.173*	0.374***	
RSL	0.108	0.030	0.077	0.153*	0.374***	0.613***

*, ** and *** denotes significance at probability levels 0.05, 0.01 and 0.001, respectively

screening of pearl millet (e.g. Dua 1989; Ashraf and McNeilly 1987, 1992; Muscolo et al. 2003). However, few others have also used lower concentrations in their study (Dua 1989; Albassam 2001). In the present study, this level of salinity was used to cover the salinity-affected soil levels that occur in most pearl millet growing areas globally as a large number of previous workers have chosen 15–20 dS m^{-1} as medium concentration for screening large number of pearl millet entries. The level of salt concentration used in the present study seemed suitable for screening this crop species as only few entries could reach a ratio of 0.50 at 39 DAS in this study under salinity as that of control.

The shoot Na^+ concentration under saline conditions appeared to be most closely related to the shoot biomass ratio ($r^2 = 0.39$, $P \geq 0.001$) or shoot biomass production under salinity ($r^2 = 0.43$, $P \geq 0.001$). The use of shoot Na^+ concentration to predict the shoot biomass ratio would certainly deserve more investigation for consideration as a trait for screening plants grown under saline conditions. If compared with sorghum, that has been found to be an efficient excluder of Na^+, restricting its accumulation in the roots (Weinberg et al. 1984; Grieve and Mass 1988) and stem but excluding most of it from the top leaves (Netondo et al. 2004) pearl millet does not seem to be as efficient excluder of Na^+ from the shoot. The mean Na^+ concentration in the shoots of all the 100 entries of pearl millet in the present study was 1.0%, four times higher than that observed under control (0.24%) and twice higher than that observed in sorghum under the same saline environment (Krishnamurthy et al. 2007). However, there was a large range of variation available (0.35 to 2.66%) for Na^+ concentration among pearl millet entries for possible exploitation as a selection criterion. Occurrence of similar range of variation in Na^+ concentration in wheat had lead to suggestion of using this trait for use in screening (Omielan et al. 1991; Poustini and Siosemardeh 2004; Munns and James 2003).This trait would also have the advantage of being non-destructive. The K^+/ Na^+ and Ca^{++}/ Na^+ ratios were also well correlated with biomass production under salinity (r^2 close to 0.2) but the relationship was not that strong to serve as a screen. The relative seed germination under salinity as that of control was largely well correlated with the shoot biomass ratios at all vegetative stages. In addition, all the top tolerant entries for shoot biomass had the highest relative seed germination. However in sorghum, relative seed germination has been found not to be related to the shoot biomass ratios at later vegetative stages (Krishnamurthy et al. 2007) indicating that the seed germination in itself was affected at a solution EC of 23 dS m^{-1} in some entries of pearl millet. This germination differences can be of use for discarding large number of genotypes in the preliminary screening and thereby improve the efficiency of the advanced screening. However, root and shoot growth observations at seedling stage are likely to be less important in pearl millet though seedling relative shoot growth vigor had been found to be both genetically and phenotypically correlated to some extent to the shoot biomass ratios 1t 18, 25, 32 and 39 DAS in sorghum (Krishnamurthy et al. 2007).

Heritability values for shoot biomass ratios ranged from 0.33 to 0.45 showing that selection for this trait would be fairly effective. There may be a scope to further improve the screening efficiency for shoot biomass ratio and its operational heritability values by sampling larger numbers of plants at one time. In relatively more sensitive crop like rice, for the trait K^+/Na^+ ratio measured at 12 dS m^{-1} culture medium, the heritability values reported were low (narrow sense = 0.198 and broad sense = 0.367) (Gregorio and Senadhira 1993).

Overall, it can be concluded that substantial variation in early vegetative stage salinity tolerance among pearl millet entries was found in this study and several relatively salinity tolerant and sensitive pearl millet entries for shoot biomass production were identified. The Na^+ concentration in the shoot was proposed as a potential proxy for phenotyping pearl millet genotypes for salinity tolerance as this trait was found to be well related to the ratio of shoot biomass. However, further investigation would be needed before using this trait as a screening measure. Seed germination under salinity as that of control has proved to be a potential trait for discarding sensitive entries initially. Early root or shoot growth of seedlings in response to salinity may not be useful as traits for selection as they were not related to biomass productivity at anthesis. Further work is under progress to elucidate the physiological and genetic mechanisms of salinity response and to implement a marker-assisted selection program for salinity tolerance in pearl millet.

Acknowledgments This research was initially supported by core funds of ICRISAT and later by OPEC Fund for International Development assigned to biotechnology-assisted improvement in salinity tolerance through the GT-Biotechnology of ICRISAT. The authors gratefully acknowledge the guidance on statistical analysis provided by Dr S Chandra, Principal Scientist (Biometrics), ICRISAT.

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