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### Large variation for salinity tolerance in the core collection of foxtail millet (*Setaria italica* (L.) P. Beauv.) germplasm

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**Abstract.** Foxtail millet (*Setaria italica* (L.) P. Beauv.) is an ideal crop for changing climate and stressed environments due to its short duration, high photosynthetic efficiency and a good level of resistance to pest and diseases. Soil salinization is on the rise with 23% of the global cultivated land already being affected. Foxtail millet can be a potential crop for salt affected soils with its high level of tolerance to salinity. The foxtail millet core collection (n=156) was screened in a soil saturated once with 100 mM NaCl and a non-saline control in 2008 and a subset (n=84) in 2009 in a partly-controlled environment using Alfisol (clayey-skeletal, mixed, iso-hyperthermic family of Udic Rhodustalfs with sandy clay loam to clay type neutral soils) to identify the best salt tolerant germplasm. Plants were grown in pots and protected from rains. The salinity response was measured as grain yield per pot. Genotype and salinity  $\times$  genotype interaction effects were significant for most traits, and there was a large range of yield and biomass variation across the accessions. Salinity delayed panicle emergence and maturity, and reduced shoot biomass by 24 to 41% and grain yield by 7 to 30%. Salinity did not reduce harvest index. Among the plant components stem biomass was reduced most by salinity. There was a large range of variation in grain yield and other traits among the genotypes in the saline pots. The yield loss by salinity was associated with duration of crop growth and grain yield loss was highest in the early maturing accessions. All the accessions were grouped into five groups based on grain yield under saline conditions, and the top, most highly tolerant, group had 13 accessions. The salinity tolerant accessions can be useful parents once their performance is confirmed under saline field conditions.

**Additional keywords:** abiotic stress, grain yield, salinity, shoot biomass, panicle harvest index

## Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is one of the world's most important ancient crops with its domestication in China dating back to 8700 years (Lu *et al.* 2009). It is widely cultivated in Asia, Europe, North America, Australia and North Africa for grain or forage (Austin 2006). Poor seedling establishment, need for hand weeding, inadequate breeding effort for improvement (Ahanchede *et al.* 2004) and limited utilization of genetic variability for the development of improved cultivar are the causes for its low yield (Vetriventhan *et al.* 2012). Foxtail millet has attracted international research attention due to its high drought tolerance, photosynthetic efficiency, nutritional values and health benefits (Liu *et al.* 2011). With a small diploid genome (400 Mb; Bennetzen *et al.* 2012), C<sub>4</sub> carboxylase pathway and short duration, foxtail millet has become a model system for studying biofuel crops and comparative genomics among the grasses (Wang *et al.* 2010; Bennetzen *et al.* 2012). Foxtail millet is considered to be an ideal crop for the changing climate due to its short duration, high photosynthetic efficiency, nutritional richness and low incidence of pest and diseases (Vetriventhan *et al.* 2012). The changes foreseen under climate change scenarios are the changes in the pattern of rainfall, rather than the quantum, leading to long spells of drought and spells of water-logging of the soils. The advantage of this crop species is that it can mature and yield with a single pre-sowing precipitation (Dwivedi *et al.* 2012).

Global estimates, dating back two decades, indicate a constant increase in salt affected soils. It was estimated that the cultivated area, have already been affected by about 23% by salinity and 37% by sodicity (Tanji *et al.* 1990). Also the usual course of salinity increase in intensely irrigated cropping systems and the transient dry land salinity threatens crop production; necessitating identification of both crop species that can tolerate the soil salinity/alkalinity the best and an understanding of the genetic variation within each species for tolerance to saline conditions. Foxtail millet can be a potential crop for salt affected soils due to its high level of tolerance to salinity (Maas 1985) and the salt 'escape' potential due to its short growing duration.

To understand the extent of variation in the germplasm, core collections were considered ideal as these are subsets representing the diversity of the entire collection consisting of about 10% of the entire collection. A core collection of foxtail millet with 155 accessions representing the entire collection of 1474 accessions using the data on

taxonomic and qualitative traits was developed by Upadhyaya *et al.* (2008).

The objectives of this study were to characterize the core collection of foxtail millet germplasm for its response to salinity stress and to identify a few highly tolerant and sensitive accessions for use in improvement of salinity adaptation of foxtail millet.

## **Materials and Methods**

### *Plant growth, treatment conditions, sowing dates and genetic material*

Plants were grown in pots filled with soil that was either left untreated (non-saline treatment) or treated with NaCl (saline treatment) in an open-air facility that was protected only when necessary from rain by a movable rain-out shelter. Experiments were undertaken over two years at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) headquarters in Patancheru, Andhra Pradesh, India (17°32' N, 78°16' E, altitude: 546 m above sea level). The plants were sown on 12 May 2008 and 2 June 2009, and harvested as and when matured.

The pots (27 cm diameter), containing 11 kg of Alfisol, were buried in plots spaced 0.45 × 0.35 m apart such that the pot rim was in level with the outside soil surface to avoid direct solar heating of the pots. These Alfisols were clayey-skeletal, mixed, iso-hyperthermic family of Udic Rhodustalfs with sandy clay loam to clay type with neutral soils with a CEC of 160-440 mmol kg<sup>-1</sup> dry soil, pH of 6.9, CEC:clay ratio of 0.29 and EC of 0.1 mM (El-Swaify *et al.* 1984). The soil was taken from the top 10 cm at the ICRISAT farm and was fertilized with di-ammonium phosphate (DAP) and muriate of potash at a rate of 200 mg kg<sup>-1</sup> soil each. In both 2008 and 2009, half of the pots were artificially salinized by applying a dose of 1.08 g NaCl kg<sup>-1</sup>, equivalent to applying a 100 mM solution of NaCl in sufficient volume (2.035 L) to wet the Alfisol precisely to field capacity (19.7% w/w). A salt concentration of 100 mM NaCl was chosen for this screening as this was similar to the one used in some successful previous studies with sorghum (Maas 1985; Francois *et al.* 1984; El-Haddad and O'Leary 1994; Igartua *et al.* 1995) and recently for foxtail millet causing a plant biomass loss of 37% (Islam *et al.* 2011). The remaining pots received tap water containing no significant amount of NaCl in the same quantities to bring them to field capacity.

The saline treatment was applied as two half doses at sowing and 12 days after

sowing to more realistically represent a field situation than a single application (Krishnamurthy *et al.* 2011). After the salt application and for the remaining crop cycle, pots were watered with tap water and maintained close to a range of 60-90% field capacity (determined gravimetrically using a subset of pots with a gradient of plant canopy size) to avoid an increase in the salt concentration in the soil solution. The base of the saline treatment pots were sealed to avoid salt leakage, while the pots of the non-saline treatment had holes to allow drainage. Availability of initial pot plus soil weight and the water it held helped to cross check, if required, and thus over-watering of all pots was avoided. This method had yielded consistently good results both in pulses and cereals (Krishnamurthy *et al.* 2011; Srivastava *et al.* 2006, 2008).

In 2008 about 12 and in 2009 about 20 seeds were planted in each pot and were thinned to two plants per pot before 12 days after sowing. This accommodated 12.7 plants m<sup>-2</sup>. The experiments were planted in a 40 × 4 alpha lattice (incomplete block design) with three replications in 2008, and in a 42 × 2 alpha lattice with five replications in 2009 with two salinity levels (saline and non-saline). In 2008, 155 entries of the foxtail millet accessions of the core collection along with five more (ISE 375, -376, -1468, -1470 and -1541) control accessions known to be agronomically superior among the whole germplasm collection were tested. But in 2009, only 80 accessions and four checks that were tested in 2008 were included. The selection of 80 accessions was on the basis of extreme contrasts in salinity yields as top (n=10), bottom (n=5) and a whole spectrum of reaction (n=35) as well as for their early flowering (n=10) and high yields in non-saline field trials (n=20) in 2008,

## *Weather*

There was a constant decrease in maximum daily temperature from near 40°C at 2008 sowing to near 30°C at the end of the experiment (Table 1). The minimum temperature also showed similar pattern of decrease. The first four weeks of 2008 was relatively warm with less precipitation, high pan evaporation and low relative humidity compared to 2009 as this was sown 20 days early in the season (12 May). Also, there were major and frequent rains in 2009 after the 2 June sowing. Maximum temperature range in the growing season was 23.0-40.1°C in 2008 and 25.9-38.8°C in 2009, and minimum temperature range was from 19.8-27.4°C in 2008 and 20.4-27.6°C in 2009.

## *Measurements*

Days to panicle emergence, days to maturity, shoot biomass at maturity (g pot<sup>-1</sup>) including grains and grain yield at maturity (g pot<sup>-1</sup>) were measured in each year. Salt tolerance was primarily measured through the grain yield productivity under salinity. The harvest index was estimated as the percent ratio of grain yield as that of the total shoot biomass and the panicle harvest index as the percent ratio of grain biomass as that of the panicle biomass. Also the relative ratio of grain yield under salinity to that of control was used for assessing the salinity tolerance.

## *Statistical analysis*

The replication-wise values of various traits in each salt environment were used for statistical analysis using ReML considering genotypes as random. Variance components due to genotypes ( $\sigma^2_g$ ) and error ( $\sigma^2_e$ ) and their standard errors, were determined. Environment wise best linear unbiased predictors (BLUPs) for the mini core accessions and controls were calculated. Heritability in a broad sense was estimated as  $h^2 = \sigma^2_g / (\sigma^2_g + (\sigma^2_e / r))$ . The significance of genetic variability among accessions was assessed from the standard error of the estimate of genetic variance  $\sigma^2_g$ , assuming the ratio  $\sigma^2_g / SE(\sigma^2_g)$  to follow normal distribution asymptotically.

For the pooled analysis, homogeneity of variance was tested using Bartlett's test (Bartlett 1937) with year (environment) fixed, and the genotype (G) × environment (E) interaction as random. The variance due to genotypes ( $\sigma^2_g$ ) and (G) × (E) interaction ( $\sigma^2_{gE}$ ) and their standard errors were determined. The significance of the fixed effect of the year or saline treatment was assessed using the Wald statistic that asymptotically follows a  $\chi^2$

distribution.

As seed yield of germplasm accessions under salinity across years had a significant interaction, their BLUPs were further grouped into various response groups for salt reaction by a hierarchical cluster analysis following Ward (1963). All statistical analyses were carried out using Genstat, Release 14.1 software (VSN International, 2011).

## Results

Seedling emergence was delayed by two days under salinity (data not shown). It was observed visually that salinity delayed the development of nodal roots and the inability of seminal root to anchor the shoot leading to the lodging and the death of the seedlings. In previous pilot trials, it was observed that majority of the lodged plants never recovered and died leading to a single plant per pot or none in many cases. Despite this realization, in 2008 trial, there were a few single plant cases (n=12) that were not considered for the ANOVA and the means were presented only for 148 accessions. During the second year the targeted two plants were fully achieved by using about 20 seeds per pot and later thinning to two.

Salt treatment significantly influenced all the traits except the harvest index and the panicle harvest index during 2008. Salinity  $\times$  genotype interaction was also significant for all the traits in both the years (Table 2). Salinity delayed phenology. The delay was two days in panicle emergence and four days in maturity during 2008 and by four days for both panicle emergence and maturity in 2009 (Table 3). Total shoot biomass was reduced by 24% and 41% in 2008 and 2009, respectively. Similarly the grain yield was reduced by 7% in 2008 and by 30% in 2009. Salinity increased the harvest index by 7% only during 2009. Panicle harvest index was reduced by salinity substantially in 2008.

The biomass of all the plant components (stem, leaf and panicle) was reduced by salinity (Fig. 1). But the stem biomass was the most reduced, accounting for 80% and 58% of the total biomass loss in 2008 and 2009, respectively (Fig. 1A). Leaf biomass (Fig. 1B) and the panicle biomass (Fig. 1C) were the least reduced.

There was a large range of variation among the accessions for the panicle emergence and maturity. These durations changed minimally across the salinity environments and years (Table 3). The accessions ranged in shoot biomass production from 12 to 78 g under control, which changed to 9 to 81 g under salinity in 2008. Similarly a grain yield range of 5 to 19 g of the accessions under control was changed to

3 to 32 g under salinity in 2008, indicating that the genotypic range of yield was high under salinity in 2008. This extended range of yield under salinity is more due to a 'determinate' type of growth and synchrony in panicle emergence and development achieved only in selective accessions. However this accession range of 11 to 37 g in control was substantially higher than the range of 2 to 28 g pot<sup>-1</sup> in 2009. The mean harvest index and its range were increased by salinity in 2009. The heritability of the phenological traits was the highest under control and reduced marginally under salinity. Based on 35 common accessions, randomly picked across the whole range of salinity response from 2008, the heritability of shoot biomass and grain yield were high in 2009 but were moderate in 2008 (data not shown). The heritability derived out of two years pooled data was also high for the phenology (>0.92) and moderate for shoot biomass (0.73) and grain yield (0.51).

From the data of 2008, initially, ten top grain yielders; five bottom ones and four common checks were separated. The performance of these accessions was largely comparable to the 2009 reaction except for a few genotypes such as ISe 254 and ISe 1888 that yielded low (Table 4). A close association of days to maturity with the loss of shoot biomass (%) under salinity as that of control or the loss of grain yield (%) as that of control was noticed in 2009 (Fig. 2), however, with some accessions deviating from this pattern. This had shown that the early maturing accessions suffered greater grain yield loss than the late maturing ones that took close to 80 days to maturity with an option to select for contrasts within the later maturing group for grain yield. But this trend was not followed by accessions that matured beyond 90 days after sowing. The negative grain yield loss observed in one long duration accession was more due to a suppression of growth in control (denominator component) (Fig. 2A). This accession offered a poor aerial competition to the relatively vigorous growing neighbors resulting in slow growth, less tillers and poor biomass.

There are sufficient indications that a poor stem biomass accumulation leads to a poor panicle size under salinity but this effect was minimal under non-saline control. Accessions with greater stem biomass also produced heavier panicles in a curvilinear response with some exceptions for selection with the heavier plants (Fig. 3). Similar response of panicles to the stem weight can also be noticed under control condition, but this relationship was sparse with minimum gains in panicle weight for large increases in stem weight.

A pooled analysis using the common 82 genotypes in both the years showed the accessions  $\times$  year effect to be significant for all the traits that were measured both under saline and control conditions (data not shown). These interaction variance components were about half of the genotypic variance for both grain yield and shoot biomass. Therefore the individual accession means of grain yield in each year were used for hierarchical clustering.

The accessions were grouped into representative groups using the BLUPs of accessions under salinity observed in two years by a hierarchical cluster analysis using the Ward (1963) method. For the convenience of splitting these 82 accessions (part of the core) into groups of highly tolerant, moderately tolerant, tolerant only in 2009, moderately sensitive and sensitive accessions, a 5% dissimilarity level that yielded five clusters with significantly different group means were chosen (Fig 4). Based on the extent of cluster group means highly tolerant (n=13) (consistently high yielding ones under salinity both in 2008 and 2009 with mean BLUPs for 2008; 2009 as 21.8 and 23.0 g pot<sup>-1</sup>), moderately tolerant (n=16) (with mean BLUPs 13.1; 20.4 g pot<sup>-1</sup>), tolerant only in 2009 (n=12) (with mean BLUPs 6.0; 21.7 g pot<sup>-1</sup>), moderately sensitive (n=27) (with mean BLUPs 5.4; 13.8 g pot<sup>-1</sup>) and sensitive (n=14) (with mean BLUPs 4.1; 7.8 g pot<sup>-1</sup>) were identified and these means for days to panicle appearance, days to maturity, shoot biomass (g pot<sup>-1</sup>) and grain yield (g pot<sup>-1</sup>) are presented in Table 5. The data for individual accessions can be obtained from the senior author or Dr H.D. Upadhyaya. The highly tolerant group (ten presented as salinity resistant in table 4 with ISe 963, ISe 1269 and ISe 1354) can be used as tolerant parents in breeding for salinity tolerance, while the sensitive ones as parents in developing populations that can be used in various genetic and genomic studies. Overall, ISe 869 that produced panicle at 52 and 58 days after sowing, matured 80 and 86 days, produced a shoot biomass of 71.2 and 69.4 g pot<sup>-1</sup> and a grain yield 26.5 and 27.4 g pot<sup>-1</sup> in 2008 and 2009 respectively was the best and the consistent salt tolerant accession (Table 4).

## **Discussion**

The purpose of this study was to screen the germplasm under an agronomic productive level of salinity, get the best discrimination among the germplasm accessions and use these contrasting sources for further genetic improvement. Therefore, the level of salinity used in this study was moderate. Previous studies on related cereals (meadow foxtail)

have indicated that foxtail millet is moderately sensitive to salinity (Maas 1993). The salt concentration (100 mM NaCl resulting in a soil ECe of  $11.2 \pm 0.28 \text{ dS m}^{-1}$  in an Alfisol) chosen for screening, was similar to the ones used in some previous studies for sorghum (ECe 10–11  $\text{dS m}^{-1}$ ; Maas 1985; Francois *et al.* 1984; El-Haddad and O’Leary 1994; Igartua *et al.* 1995). Later works (Islam *et al.* 2011) also confirmed that saturating with 100 mM salt solution would be the most suitable for good genetic discrimination. However the top tolerant accessions need to be tested for performance under higher salt concentrations. The overall grain yield loss observed was greater in the 2009 experiment. In this year, the overall loss in shoot biomass was 41% and the grain yield 30% and this biomass reduction was almost similar to the loss reported by earlier works (Thimmaiah *et al.* 1989; Islam *et al.* 2011). As of now, no other study, except Thimmaiah *et al.* (1989) and Islam *et al.* (2011), had been known to evaluate the grain yield performance of foxtail millet for salinity tolerance. In a closely related earlier work, dealing with saline water irrigation, it was shown that grain yield and dry matter production of foxtail millet did increase at all growth stages when irrigated with saline water up to  $6 \text{ dS m}^{-1}$  but did decrease at higher salinity levels, tested up to  $21 \text{ dS m}^{-1}$  (Kubsad *et al.* 1995).

There was a large range in the shoot biomass or grain yield loss of individual accessions that varied from -17 to 79% for shoot biomass and -15 to 88% for the grain yield. This variation in loss was to a large extent explained by the variation in crop duration. Generally, the accessions that were short in duration suffered the most shoot biomass/grain yield loss compared to the long duration accessions likely due to the non-availability of enough growing time for compensation. Delay in phenology, observed under salinity, might have further reduced the active reproductive duration in the short duration accessions. In comparison to the performance under control, both the shoot biomass (a mean 60% across years) and grain yield (a mean 50% across years) suffered the maximum in the ten early duration accessions. Salinity treatment rather marginally increased the harvest index. The mean improvement in harvest index across all the accessions was 7% and 80 accessions had exhibited increases in harvest index. The overall indication is that the salinity losses can be largely overcome by a careful choice of the best adapted duration, i.e., by using 80 to 85 day duration genotypes in this location. Accessions ISe 375, ISe 376, ISe 1468 and ISe 1541 were used as checks in these trials based on their superior yield performance in the non-saline field (Upadhyaya *et al.* 2011). Based on their yield under salinity, three of these check entries were rated moderately

tolerant, whereas the accession ISe 1468 was rated only moderately sensitive. Overall, the mean shoot biomass loss in the checks due to salinity compared to control was 79% in 2008 and 35% in 2009. Similarly, the mean grain yield loss was 73 and 18%. The mean shoot biomass of the ten tolerant accessions under salinity compared to control were increased by 63% in 2008 and decreased by 30% in 2009 while the mean grain yield were increased by 157% in 2008 and decreased by 14% in 2009 demonstrating the superior value of the currently identified highly tolerant germplasm (data not shown). However, the salinity response of the newly identified highly tolerant accessions needs to be verified in field trials where the spatial variation in soil salinity is a major limitation.

Yield potential is expected to reflect on the yield performance under stress (Araus *et al.* 2002). In this case, 20 accessions that were included in this evaluation for their high yields under normal field conditions performed relatively moderate under salinity with a grain yield loss of 30% in 2008 and 37% in 2009. Therefore, the usual salinity screening procedures can not be overlooked while establishing the salinity response of foxtail millet genotypes.

Salinity reduced the accumulation of biomass in the shoots but the reduction was maximum in stem biomass followed by the panicle. The leaf biomass was the least affected component, and as a consequence the crop under salinity was relatively short statured and leafy. This trend of selective plant component reduction was observed both in foxtail and proso millets after exposing the plants to 100 mM concentrations of salinity and alkalinity stresses for 16 days at early vegetative crop growth stage (Islam *et al.* 2011). However, salinity was also found to reduce the shoot length, number and size of spikes and the grain yield (Hendawy *et al.* 2012). Saline water irrigations with salinity increasing from 1 to 16 dS m<sup>-1</sup> had been noticed to linearly decrease seed and straw yield, harvest index and 1000 grain weights in foxtail millet and the harvested seeds from these treatments were found to germinate into normal seedlings (Thimmaiah *et al.* 1989). Overall, at about 10-12 dS m<sup>-1</sup> soil EC levels, salinity would reduce the stover yield more than the grain yield and therefore accessions would show a higher harvest index. There are sufficient indications that the stem accumulation in the control plants is supra-optimal for the best panicle size. However, under salinity, the extra early accessions tend to produce suboptimal stem weights and the panicle weight linearly increased only up to a certain level. Also the cluster group means, based only on the performance under salinity, had clearly shown that the changes in grain yield followed proportionately the changes in

shoot biomass. These observations indicate that under non-saline conditions the best yields can be possible with accessions of a range of plant heights, but under salinity relatively taller accessions with a good level of shoot productivity need to be selected when stable grain yields are intended.

### **Conclusions**

This study has shown the availability of a wide genotypic variation for salinity response in the foxtail millet core collection. Salinity reduced both the shoot biomass and the grain yield but not the harvest index. Reduction in grain yield in the saline treatment was primarily associated with the reduction in total plant biomass and particularly the stem. Yield potential of the accessions provided a poor proxy for salinity tolerance in foxtail millet. Groups of highly tolerant, moderately tolerant and sensitive accessions in terms of grain yield in the saline treatment have been identified, and can be used in breeding for salinity tolerance as parents after verifying their salinity response in the field.

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## References

- Ahanchede A, Hamon SP, Darmency H (2004) Why no tetraploid cultivar of foxtail millet? *Genetic Resources and Crop Evolution* **51**, 227–230.
- Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C<sub>3</sub> cereals: What should we breed for? *Annals of Botany* **89**, 925-940.
- Austin DF (2006) Foxtail millets (Setaria: Poaceae) - abandoned food in two hemispheres. *Economic Botany* **60**, 143–158.
- Bartlett MS (1937) Properties of Sufficiency and Statistical Tests. Proceedings of the Royal Society, London, Series A, **160**, 268–282.
- Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, Estep M, Feng L, Vaughn JN, Grimwood J, Jenkins J, Barry K, Lindquist E, Hellsten U, Deshpande S, Wang X, Wu X, Mitros T, Triplett J, Yang X, Chu-Yu Ye, Mauro-Herrera M, Wang L, Li P, Sharma M, Sharma R, Ronald PC, Panaud O, Kellogg EA, Brutnell TP, Doust AN, Tuskan GA, Rokhsar D, Devos KM (2012) Reference genome sequence of the model plant Setaria. *Nature Biotechnology* doi:101038/nbt2196.
- Dwivedi S, Upadhyaya HD, Senthilvel S, Hash C, Fukunaga K, Diao X, Santra D, Baltensperger D, Prasad M (2012) Millets: Genetic and Genomic Resources. *Plant Breeding Reviews* **35**, 246–375.
- El-Haddad EHM, O’Leary JW (1994) Effect of salinity and K/Na ratio of irrigation water on growth and solute content of *Atriplex amnicola* and *Sorghum bicolor*. *Irrigation Science* **14**, 127–133.
- El-Swaify SA, Walker TS, Virmani SM (1984) Dryland management alternatives and research needs for Alfisols in the semi-arid tropics. In: An interpretive summary of the consultants’ workshop on the state of the art and management alternatives for optimizing the productivity of SAT Alfisols and related soils, pp.1-44. ICRISAT, Patancheru, A.P., India, 1-3 Dec 1983; ICRISAT Center, India, ISBN 92-9066- 079-1.9.
- Francois LE, Donovan T, Maas EV (1984) Salinity effects on seed yield, growth and germination of grain sorghum. *Agronomy Journal* **76**, 741–744.
- Hendawy SF, El-Sherbeny SE, Hussein MS, Youssef AA (2012) Evaluation of some cultivars of foxtail plants under salinity conditions. *Journal of Applied Sciences Research* **8**, 620–627.
- Igartua E, Gracia MP, Lasa JM (1995) Field responses of grain sorghum to a salinity gradient. *Field Crops Research* **42**, 15–25.

Islam MS, Akhter MM, El Sabagh A, Liu LY, Nguyen NT, Ueda A, Masaoka Y, Saneoka H (2011) Comparative studies on growth and physiological responses to saline and alkaline stresses of foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.). *Australian Journal of Crop Science* **5**, 1269–1277.

Krishnamurthy L, Turner NC, Gaur PM, Upadhyaya HD, Varshney RK, Siddique KHM, Vadez V (2011) Consistent variation across soil types in salinity resistance of a diverse range of chickpea (*Cicer arietinum* L.) genotypes. *Journal of Agronomy and Crop Science* **197**, 214–227.

Kubsad VS, Hunshal CS, Vishwanath DP, Patil SL, Gowda DSM (1995) Dry matter accumulation in *Setaria* as influence by saline water irrigation. *Journal of Maharashtra Agricultural University* **20**, 3–5.

Lu H, Zhang J, Liu KB, Wu N, Li Y, Zhou K, Ye M, Zhang T, Zhang H, Yang X, Shen L, Xu D, Li Q (2009) Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 7367–7372.

Maas EV (1985) Crop tolerance to saline sprinkling water. *Plant Soil* **89**, 273–284.

Liu Z, Bai G, Zhang D, Znu C, Xia X, Cheng Z, Shi Z (2011) Genetic diversity and population structure of elite foxtail millet (*Setaria italica* (L.) P. Beauv.) germplasm in China. *Crop Science* **51**, 1655–1663.

Maas EV (1993) Testing crops for salinity tolerance. In: *Proceedings of Workshop on Adaptation of Plants to Soil stresses*. INTSORMIL (Eds JW Maranville, BV Baligar, RR Duncan, JM Yohe) pp. 234–247. Publication No. 94-2, University of Nebraska, Lincoln, August 1–4.

Srivastava N, Vadez V, Upadhyaya HD, Saxena KB (2006) Screening for inter and intra specific variability for salinity tolerance in pigeonpea (*Cajanus Cajan*) and its related wild species. *SAT e-journal* **2**, 1–1.

Srivastava N, Vadez V, Lakshmi Narasu M, Upadhyaya HD, Nigam SN, Rupkala A, (2008) Large genotypic variation for salinity tolerance in groundnut (*Arachis hypogaea*) particular to reproductive stage. (Abstract) International dryland development commission (IDDC): Ninth International Dryland Development Conference - Sustainable development in the drylands - Meeting the challenge of global climatic change, Alexandria, Egypt. 7–10 Nov 2008, pp. 111.

Tanji KK (1990) Nature and extent of agricultural salinity. In ‘Agricultural Salinity

Assessment and Management'. (Eds KK Tanji) pp. 1-18. American Society of Civil Engineers, New York.

Thimmaiah SK, Viswanath DP, Vyakarnahal BS, Hunshal CS (1989) Effect of salinity on yield, seed quality and biochemical characteristics in *Setaria italica* L. *Cereal Chemistry* **66**, 525-527.

Upadhyaya HD, Pundir RPS, Gowda CLL, Reddy VG, Singh S (2008) Establishing a core collection of foxtail millet to enhance utilization of germplasm of an underutilized crop. *Plant Genetic Resources* **7**, 177–184.

Upadhyaya HD, Ravishankar CR, Narasimhudu Y, Sarma NDRK, Singh SK, Varshney RK, Reddy VG, Singh S, Parzies HK, Dwivedi SL, Nadaf HL, Sahrawat KL, Gowda CLL (2011) Identification of trait-specific germplasm and developing a mini core collection for efficient use of foxtail millet genetic resources in crop improvement. *Field Crops Research* **124**, 459–467. doi:10.1016/j.fcr.2011.08.004

Vetriventhan M, Upadhyaya HD, Anandakumar CR, Senthilvel S, Parzies HK, Bharathi A, Varshney RK, Gowda CLL (2012) Assessing genetic diversity, allelic richness and genetic relationship among races in ICRISAT foxtail millet core collection. *Plant Genetic Resources* **10**, 214–223.

VSN International (2011) GenStat software for windows. Release 14.1. VSN International Ltd., Hemel Hempstead, UK: <http://www.vsni.co.uk>.

Wang C, Chen J, Zhi H, Yang L, Li W, Wang Y, Li H, Zhao B, Chen M, Diao X (2010) Population genetics of foxtail millet and its wild ancestor. *BMC Genetics* **11**, 90.

Ward JH, 1963. Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association* **58**, 236–244.

## Captions for figures

**Fig. 1.** The effect of soil salinity on individual plant components at final maturity assessed as means of a part of the foxtail millet germplasm core collection (n=84) in 2009. The biomass of (A) stem, (B) leaf and (C) panicle at maturity recorded both under non-saline control and salinity in 2008 and 2009 seasons.

**Fig. 2.** Relationship between (A) the days taken to maturity and the proportion of shoot biomass under saline conditions compared to the control ( $\text{g pot}^{-1}$ ) [(Shoot biomass under control – shoot biomass under salinity)  $\times 100$ ] and (B) days taken to maturity and the proportion of grain yield ( $\text{g pot}^{-1}$ ) [(grain yield under control – grain yield under salinity)  $\times 100$ ] in the germplasm accessions of foxtail millet grown in 2009 (n=84). The accessions that took >100 days to maturity were not considered for the linear regression curve and are shown in grey on A and B.

**Fig. 3.** The relationship between the stem weight and panicle weight observed under both saline (open) and non-saline control (closed) conditions in the germplasm accessions of foxtail millet grown in 2009 (n=84).

**Fig. 4.** Dendrogram showing the distribution of various salinity response groups of the subset (n=82) of the core foxtail millet germplasm tested using the grain yield under salinity stress in two years.

**Table 1. Standard week-wise (StdWk) sums of rainfall, mm (RF) and open pan evaporation, mm (PE) and weekly means of maximum temperature, °C (MaxT), minimum temperature, °C (MinT), morning relative humidity, % (RHM) and evening relative humidity, % (RHE) during the crop growing season of the foxtail millet germplasm evaluation for salinity tolerance both during 2008 and 2009 rainy seasons.**

StdWk	2008						2009					
	RF	PE	MaxT	MinT	RHM	RHE	RF	PE	MaxT	MinT	RHM	RHE
19	0.0	81.3	38.5	23.0	43.3	21.6						
20	5.0	92.5	39.4	24.6	68.3	43.7						
21	5.8	59.6	37.4	23.5	72.7	38.6						
22	6.2	79.0	39.0	25.9	63.7	27.3						
23	3.4	64.8	35.1	24.0	75.4	44.9	63.2	82.8	37.0	24.6	76.3	38.3
24	37.2	64.3	32.2	22.8	82.1	49.4	8.2	68.1	37.1	24.8	79.6	45.9
25	9.0	71.8	34.6	23.9	76.7	43.4	24.8	59.3	35.7	23.3	88.1	58.4
26	20.2	38.5	31.6	22.4	86.6	61.1	0.8	59.3	34.5	25.0	76.9	46.3
27	1.0	56.9	33.6	23.3	80.7	56.7	22.8	50.5	32.8	23.5	83.7	51.6
28	2.0	60.6	33.8	23.5	77.3	48.9	16.8	39.8	30.6	23.1	86.1	60.6
29	14.4	45.0	34.9	23.8	81.3	56.7	19.2	50.5	31.3	23.2	86.6	59.3
30	87.5	21.5	28.6	21.4	93.4	78.0	0.4	54.2	31.9	23.3	81.9	52.7
31	84.6	29.7	29.2	21.5	93.7	72.9	0.0	52.7	33.4	24.1	77.0	47.4
32	234.1	16.9	26.4	21.5	91.0	81.4	16.0	50.0	31.7	23.7	83.7	51.7
33	12.3	25.0	29.5	21.8	92.4	72.1	46.8	37.7	31.3	23.7	87.4	60.0
34	31.6	28.2	30.1	22.0	91.3	63.6	235.3	17.9	29.3	22.4	95.9	76.9
35	29.2	26.2	31.1	22.3	94.0	65.4	247.6	27.5	28.8	21.7	92.9	73.7
36	141.7	28.4	30.8	21.8	94.1	63.6	40.9	22.0	28.3	22.4	91.0	77.0
37							4.8	30.1	31.8	22.8	91.6	57.7
38							19.0	31.8	32.4	22.4	92.6	54.7
39							74.4	29.2	31.5	22.4	91.3	61.6
40							60.1	22.1	28.7	23.0	92.9	77.0
41							0.0	26.1	30.8	21.2	93.7	53.0

**Table 2. Analysis of variance for various characters measured on the core collection of foxtail millet germplasm (n = 144) with four check accessions in the 2008 and a selected subset of core collection (n = 80) with four check accessions in the 2009 rainy seasons grown under both salinity stressed and control conditions. \*\*\* and \*\* denote significance at P =<0.001 and P=<0.01 levels, respectively.**

	<u>Salt treatment</u>		<u>Salt treatment × Genotype</u>	
	Wald statistic	Significance level	$\sigma^2_g$ (SE)	Significance level
<b>2008</b>				
Days to panicle emergence	48.4	<0.001	5.06 (1.03)	***
Days to maturity	120.5	<0.001	5.41 (1.32)	***
Shoot biomass (g pot <sup>-1</sup> )	27.3	<0.001	125.8 (30.9)	***
Grain yield (g pot <sup>-1</sup> )	0.78	0.379	28.7 (5.67)	***
Harvest index (%)	0.01	0.915	74.9 (13.14)	***
Panicle harvest index (%)	42.9	<0.001	64.6 (16.3)	**
Stem dry weight (g pot <sup>-1</sup> )	129.9	<0.001	17.6 (4.42)	**
Leaf dry weight (g pot <sup>-1</sup> )	7.18	0.008	3.95 (1.04)	**
Panicle dry weight (g pot <sup>-1</sup> )	41.8	0.418	41.8 (8.66)	***
<b>2009</b>				
Days to panicle emergence	78.5	<0.001	6.16 (1.16)	***
Days to maturity	61.3	<0.001	12.46 (2.22)	***
Shoot biomass (g pot <sup>-1</sup> )	629.0	<0.001	12.2 (3.33)	**
Grain yield (g pot <sup>-1</sup> )	300.5	<0.001	2.51 (0.64)	**
Harvest index (%)	98.7	<0.001	20.2 (4.39)	***
Panicle harvest index (%)	103.7	<0.001	13.7 (3.09)	***
Stem dry weight (g pot <sup>-1</sup> )	392.3	<0.001	66.7 (16.4)	**
Leaf dry weight (g pot <sup>-1</sup> )	136.5	<0.001	10.8 (2.44)	***
Panicle dry weight (g pot <sup>-1</sup> )	7.7	0.007	27.9 (5.48)	***

**Table 3. Mean days to panicle emergence, maturity, total shoot biomass (g pot<sup>-1</sup>), grain yield (g pot<sup>-1</sup>), harvest index (%) and panicle harvest index (%) for the core collection of foxtail millet germplasm (n = 155) with five check accessions in the 2008 and a selected subset of core collection (n = 84) in the 2009 rainy seasons grown under both salinity stressed and control conditions. In 2008, 12 accessions that had one plant per pot was removed from the analysis**

Season/Environment	Trial mean	Range of predicted means	S.Ed	$\sigma^2_g$ (SE)	Heritability (h <sup>2</sup> )
<b>2008, Salinity stressed</b>					
Days to panicle appearance	50.8	29.8 – 88.9	3.29	113.6 (14.1)	0.963
Days to maturity	74.3	55.1 – 107.3	4.13	93.8 (12.4)	0.925
Shoot biomass (g pot <sup>-1</sup> )	30.9	9.3 – 81.1	16.50	452.0 (77.4)	0.698
Grain yield (g pot <sup>-1</sup> )	10.0	3.4 – 32.3	6.19	56.8 (10.3)	0.662
Harvest index (%)	27.7	21.1 – 35.5	6.26	28.4 (13.1)	-
Panicle harvest index (%)	65.0	53.7 – 72.4	9.34	67.2 (26.8)	-
<b>2008, Control</b>					
Days to panicle appearance	48.4	26.0 – 89.0	1.87	116.4 (13.9)	0.985
Days to maturity	69.9	48.9 – 117.8	2.26	116.0 (12.8)	0.976
Shoot biomass (g pot <sup>-1</sup> )	40.6	12.0 – 77.9	9.78	163.0 (27.6)	0.706
Grain yield (g pot <sup>-1</sup> )	10.7	5.4 – 18.9	3.02	9.11 (2.26)	0.499
Harvest index (%)	27.3	11.2 – 51.9	5.05	68.5 (9.99)	-
Panicle harvest index (%)	73.8	60.6 – 85.6	5.76	37.2 (8.32)	-
<b>2009, Salinity stressed</b>					
Days to panicle appearance	49.7	24.8 – 97.9	2.13	206.0 (32.4)	0.964
Days to maturity	76.4	50.5 – 115.9	2.30	150.8 (23.9)	0.901
Shoot biomass (g pot <sup>-1</sup> )	45.9	10.4 – 102.6	7.04	424.9 (70.0)	0.929
Grain yield (g pot <sup>-1</sup> )	16.6	2.3 – 27.9	3.11	40.4 (7.08)	0.844
Harvest index (%)	38.2	4.4 – 58.5	3.32	71.9 (12.1)	-
Panicle harvest index (%)	79.0	19.3 – 87.5	3.97	70.5 (12.2)	-
<b>2009, Control</b>					
Days to panicle appearance	45.9	25.5 – 104.2	1.85	216.5 (33.9)	0.994
Days to maturity	71.9	51.1 – 130.3	2.03	230.6 (36.2)	0.997
Shoot biomass (g pot <sup>-1</sup> )	78.3	42.9 – 120.2	9.63	392.0 (69.6)	0.817
Grain yield (g pot <sup>-1</sup> )	23.7	11.3 – 36.5	3.43	34.1 (6.46)	0.743
Harvest index (%)	31.0	12.3 – 43.5	2.71	34.6 (6.07)	-
Panicle harvest index (%)	81.6	64.8 – 87.7	3.24	22.2 (4.58)	-

**Table 4. Mean days to panicle emergence, maturity, total shoot biomass (g pot<sup>-1</sup>), grain yield (g pot<sup>-1</sup>), harvest index (%) and panicle harvest index (%) under salinity for the accessions that were rated as top 10 resistant, bottom 5 sensitive and 4 check entries of foxtail millet germplasm in the 2008 with their corresponding reaction in 2009.**

Reaction group/ Accessions	Days to panicle appearance	Days to maturity	Shoot biomass (g pot <sup>-1</sup> )	Grain yield (g pot <sup>-1</sup> )	Harvest index (%)	Panicle harvest index (%)
<b>2008, Salinity resistant</b>						
ISe 254	49.7	75.6	80.3	32.3	33.4	72.4
ISe 869	52.3	79.6	73.4	27.3	31.8	72.4
ISe 1851	47.1	74.0	58.9	23.0	32.5	72.4
ISe 96	51.0	75.0	61.2	23.0	31.8	70.7
ISe 388	53.6	78.3	62.4	22.2	31.3	71.1
ISe 480	55.8	77.7	69.3	21.3	28.8	67.6
ISe 995	47.1	71.9	60.1	20.4	30.4	68.5
ISe 1629	52.9	75.9	56.4	20.1	31.5	68.3
ISe 969	51.6	75.9	57.6	20.1	31.1	70.1
ISe 1888	54.8	76.2	76.0	26.8	31.2	70.2
<b>2008, Salinity sensitive</b>						
ISe 735	-	-	-	-	-	-
ISe 1118	50.8	74.3	9.3	3.4	27.7	65.0
ISe 1597	74.0	74.3	11.3	3.4	27.7	65.0
ISe 769	-	-	-	-	-	-
ISe 1059	50.8	74.3	9.3	3.4	27.7	65.0
<b>2008, Checks</b>						
ISe 375	56.3	76.2	31.4	9.7	29.0	65.7
ISe 376	57.1	80.5	39.7	13.9	28.6	65.9
ISe 1468	49.9	71.6	18.3	6.8	30.2	67.9
ISe 1541	62.2	83.3	53.3	14.8	26.7	61.5
<b>2009, Salinity resistant</b>						
ISe 254	51.2	76.6	43.5	15.4	34.7	82.4
ISe 869	57.9	86.3	69.4	27.4	40.5	87.4
ISe 1851	54.2	80.9	72.6	26.1	36.6	81.3
ISe 96	54.0	79.0	53.4	19.0	35.9	82.6
ISe 388	52.8	82.3	71.9	27.5	39.3	85.0
ISe 480	52.6	82.1	66.6	26.4	40.4	85.8
ISe 995	53.4	79.0	62.4	20.6	33.6	77.2
ISe 1629	56.3	80.7	55.8	21.6	39.3	81.6
ISe 969	51.2	76.0	63.4	24.8	39.9	84.9
ISe 1888	49.6	76.4	51.9	17.7	34.6	80.5
<b>2009, Salinity sensitive</b>						
ISe 735	49.7	76.5	45.9	16.6	38.2	79.0
ISe 1118	32.5	59.4	31.4	14.8	46.2	78.0

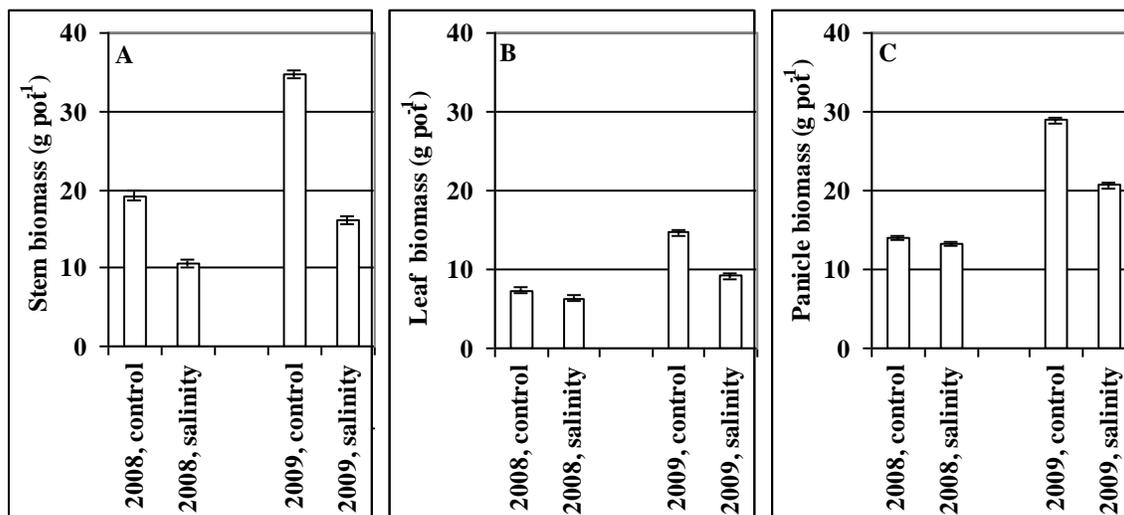
ISe 1597	77.6	102.4	58.1	15.5	26.9	80.0
ISe 769	89.3	101.3	26.0	2.3	4.4	22.3
ISe 1059	64.1	83.5	35.0	10.0	27.1	76.5
<b>2009, Checks</b>						
ISe 375	59.5	81.3	54.0	19.7	36.4	78.8
ISe 376	61.9	80.3	48.3	17.6	37.2	80.2
ISe 1468	46.4	71.1	45.6	17.2	37.3	80.8
ISe 1541	64.9	92.0	57.9	18.3	32.5	77.7

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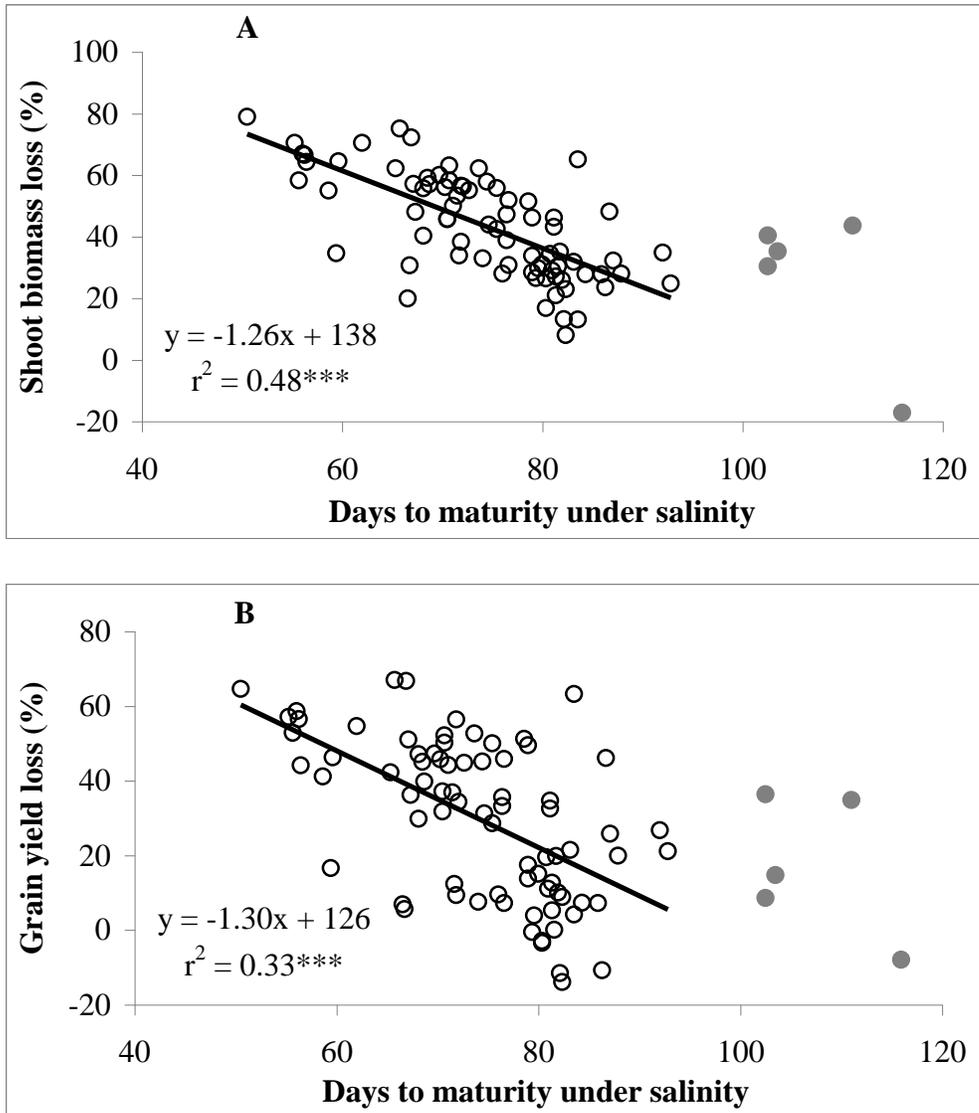
- = Data not available

**Table 5. Means of individual cluster groups with their standard errors for days to panicle appearance, days to maturity, total shoot biomass (g pot<sup>-1</sup>) and grain yield (g pot<sup>-1</sup>) of the five different groups of foxtail millet germplasm out of 82 common accessions tested in two years. The grain yield under salinity stress across two years was used for this clustering.**

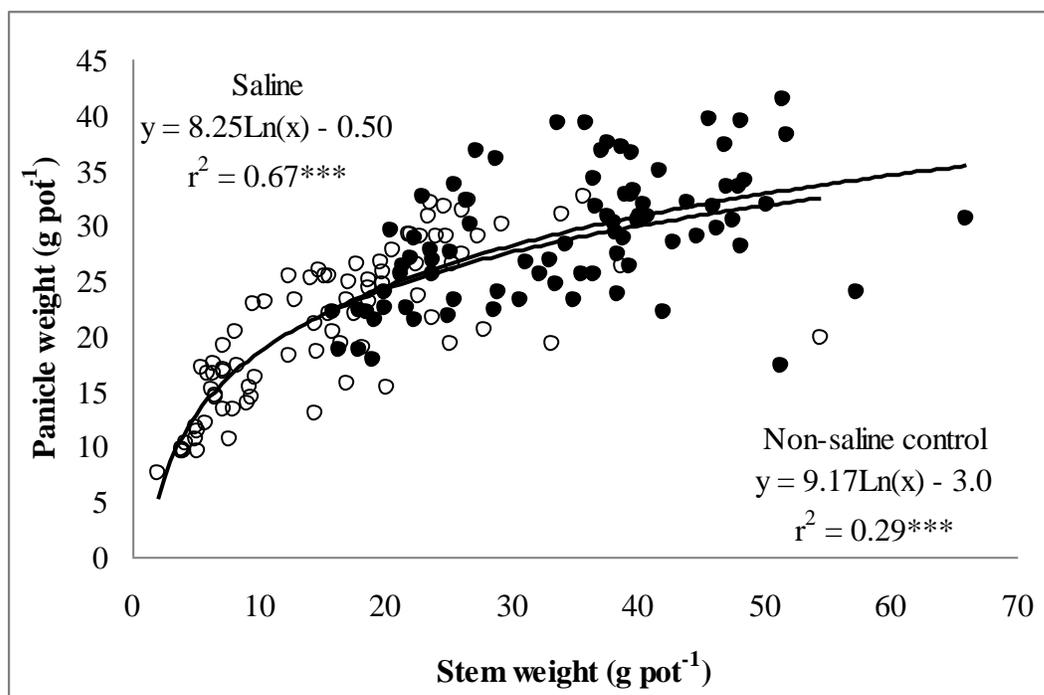
Salinity response groups	Days to panicle appearance		Days to maturity		Shoot biomass (g pot <sup>-1</sup> )		Grain yield (g pot <sup>-1</sup> )	
	2008	2009	2008	2009	2008	2009	2008	2009
Highly tolerant	52±0.82	53±0.69	77±0.72	80±0.85	60.9±2.47	63.0±3.14	21.7±1.13	23.0±1.14
Moderately tolerant	57±2.50	56±2.48	79±2.23	82±2.01	43.5±2.80	58.6±2.11	13.1±0.43	20.4±0.72
Tolerant in one season	52±3.03	54±2.94	75±2.70	80±2.64	20.9±1.81	59.5±4.42	6.0±0.54	21.7±0.79
Moderately sensitive	49±2.34	48±3.06	72±1.96	76±2.55	17.7±1.42	38.4±3.07	5.4±0.37	13.8±0.41
Sensitive	39±1.62	38±4.54	65±1.74	65±3.50	12.1±0.57	19.7±1.60	4.1±0.23	7.8±0.49



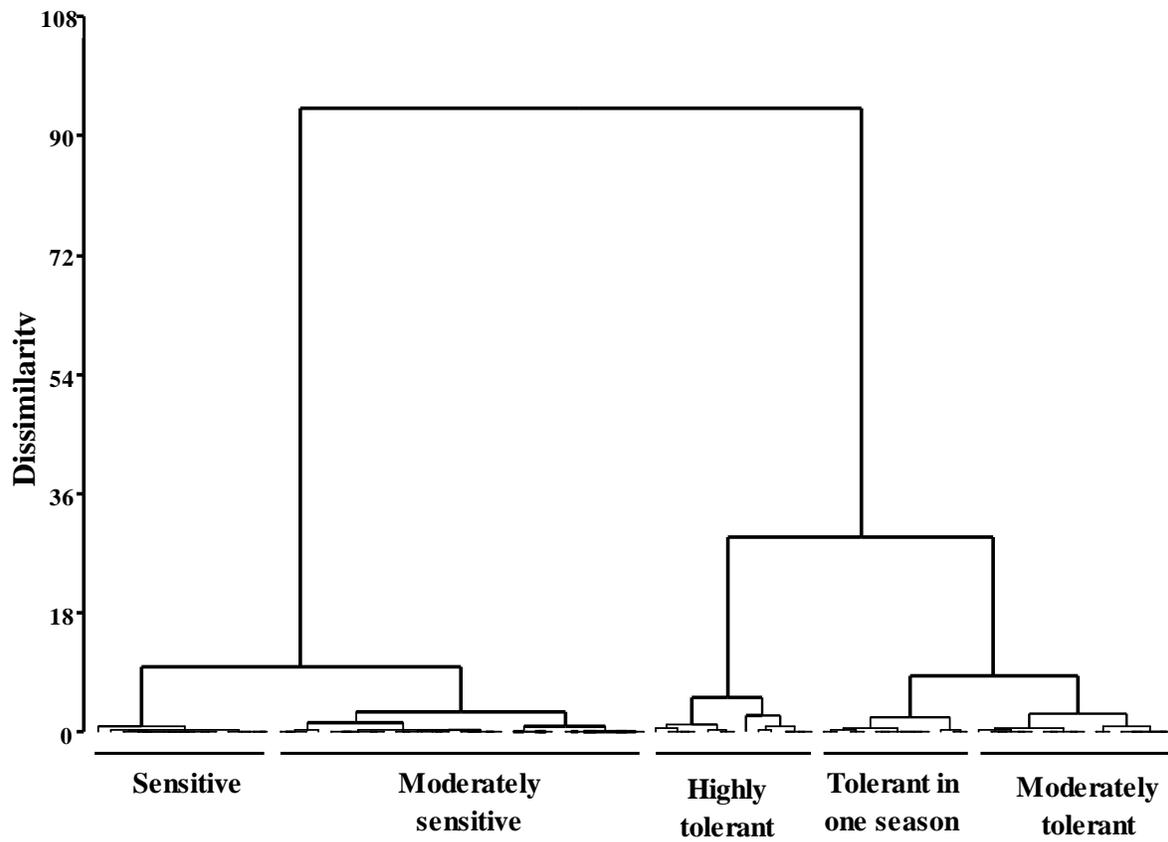
**Fig. 1.** The effect of soil salinity on individual plant components at final maturity assessed as means of a part of the foxtail millet germplasm core collection (n=84) in 2009. The biomass of (A) stem, (B) leaf and (C) panicle at maturity recorded both under non-saline control and salinity in 2008 and 2009 seasons.



**Fig. 2.** Relationship between (A) the days taken to maturity and the proportion of shoot biomass under saline conditions compared to the control ( $\text{g pot}^{-1}$ ) [(Shoot biomass under control – shoot biomass under salinity)  $\times 100$ ] and (B) days taken to maturity and the proportion of grain yield ( $\text{g pot}^{-1}$ ) [(grain yield under control – grain yield under salinity)  $\times 100$ ] in the germplasm accessions of foxtail millet grown in 2009 ( $n=84$ ). The accessions that took  $>100$  days to maturity were not considered for the linear regression curve and are shown in grey on A and B.



**Fig. 3.** The relationship between the stem weight and panicle weight observed under both saline (open) and non-saline control (closed) conditions in the germplasm accessions of foxtail millet grown in 2009 (n=84).



**Fig. 4.** Dendrogram showing the distribution of various salinity response groups of the subset (n=82) of the core foxtail millet germplasm tested using the grain yield under salinity stress in two years.