

## 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 climates and stressed environments due to its short duration, high photosynthetic efficiency and good level of resistance to pest and diseases. Soil salinisation is an increasing problem, with 23% of the global cultivated land already affected. Foxtail millet has potential as a crop for salt-affected soils, with its high tolerance to salinity. The foxtail millet core collection ( $n = 155$ ) was screened in a soil saturated once with 100 mM NaCl and in a non-saline control in 2008 and a subset ( $n = 84$ ) in 2009 in a partly controlled environment using Alfisol to identify the best salt-tolerant germplasm. Plants were grown in pots and protected from rain. 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 accessions. Salinity delayed panicle emergence and maturity, and reduced shoot biomass by 24–41% and grain yield by 7–30%. Salinity did not reduce the harvest index. Among the plant components, stem biomass was reduced most by salinity. There was a large 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 accessions were grouped into five sets based on grain yield under saline conditions, and the 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, panicle harvest index, salinity, shoot biomass.

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### 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, the need for hand-weeding, inadequate breeding efforts for improvement (Ahanchede *et al.* 2004) and limited utilisation of genetic variability for the development of improved cultivars are the reasons for its low yield (Vettrivethan *et al.* 2012). Foxtail millet has attracted international research attention because of its high drought tolerance, photosynthetic efficiency, nutritional value 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 growing 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 an ideal crop for the changing climate due to its short duration, high photosynthetic

efficiency, nutritional richness and low incidence of pest and diseases (Vettrivethan *et al.* 2012). Changes foreseen under climate change scenarios are changes in the pattern of rainfall, rather than the quantum, leading to long periods of drought and spells of waterlogging of 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 20 years indicate a constant increase in salt-affected soils. It was estimated that ~23% of the cultivated area was already affected by salinity and 37% by sodicity (Tanji 1990). In addition, the usual course of salinity increase in intensely irrigated cropping systems, as well as transient dryland salinity, threaten crop production, making it necessary to identify crop species that can tolerate soil salinity–alkalinity and to understand the genetic variation within each species for tolerance to saline conditions. Foxtail millet is a potential crop for salt-affected soils because of its high tolerance to salinity (Maas 1985) and its salt 'escape' potential arising from its short growing duration.

Core collections were considered ideal to understand the extent of variation in the germplasm, as these are subsets representing the diversity of the entire collection and comprising ~10% of the entire collection. A core collection of foxtail millet with 155 accessions representing the entire collection of 1474 accessions, based on data on taxonomic and qualitative traits, was developed by Upadhyaya *et al.* (2008).

The objectives of this study were to characterise 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 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 2 years at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) 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 mature.

The pots (27 cm diameter), containing 11 kg of Alfisol, were buried in plots spaced 0.45 by 0.35 m apart such that the pot rim was level with the outside soil surface to avoid direct solar heating of the pots. These Alfisols were a clayey-skeletal, mixed, iso-hyperthermic family of Udic Rhodustalfs (Soil Survey Staff 2010) of sandy clay loam to clay type, neutral soils with a cation exchange capacity (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 dS m<sup>-1</sup> (El-Swaify *et al.* 1984). The soil was taken from the top 10 cm at the ICRISAT farm and was fertilised with di-ammonium phosphate (DAP) and muriate of potash, each at a rate of 200 mg kg<sup>-1</sup> soil. In both 2008 and 2009, half of the pots were artificially salinised 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 it was similar to that successfully used in previous studies with sorghum (Francois *et al.* 1984; Maas 1985; 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 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, as a more realistic representation of 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 at 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 bases of the saline treatment pots were sealed to avoid salt leakage, whereas the pots of the non-saline treatment had holes to allow drainage. The

availability of initial pot plus soil weight and the water it held helped to crosscheck, if required, and thus over-watering of all pots was avoided. This method had yielded consistently good results in pulses and cereals (Srivastava *et al.* 2006, 2008; Krishnamurthy *et al.* 2011).

Seeds were planted in each pot (~12 in 2008 and ~20 in 2009) 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 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. In 2009, only 80 accessions and four checks that were tested in 2008 were included. The selection of 80 accessions was based on extreme contrasts in salinity yields in the top ( $n = 10$ ) and bottom ( $n = 5$ ) of the range and a whole spectrum of reaction ( $n = 35$ ) as well as for 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 showed a similar pattern of decrease. The first 4 weeks of 2008 were relatively warm with less precipitation, higher pan evaporation and lower relative humidity than 2009, as sowing was 20 days earlier 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 19.8–27.4°C in 2008 and 20.4–27.6°C in 2009.

### *Measurements*

Days to panicle emergence, days to maturity, and 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 per cent ratio of grain yield to total shoot biomass, and the panicle harvest index as the per cent ratio of grain biomass to panicle biomass. The relative ratio of grain yield under salinity to that of the control was used for assessing the salinity tolerance.

### *Statistical analyses*

The replication-wise values of various traits in each salt environment were used for statistical analysis via REML considering genotypes as random. Variance components due to genotypes ( $\sigma_g^2$ ) and error ( $\sigma_e^2$ ), and their standard errors (s.e.), were determined. Environment-wise best linear unbiased predictors (BLUPs) for the mini core accessions and controls were calculated. Broad-sense heritability was estimated as  $h^2 = \sigma_g^2 / (\sigma_g^2 + (\sigma_e^2/r))$ . The significance of genetic variability among accessions was assessed from the standard error of the estimate of genetic variance  $\sigma_g^2$ , assuming the ratio  $\sigma_g^2/\text{s.e.}(\sigma_g^2)$  to follow a normal distribution asymptotically.

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

Std Wk	2008						2009					
	Rainfall	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

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 ( $\sigma_g^2$ ) and G × E interaction ( $\sigma_{GE}^2$ ), 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 yields 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 2 days under salinity (data not shown). Salinity was observed to delay the development of nodal roots and led to the inability of the seminal root to anchor the shoot, leading to lodging and death of the seedlings. In pilot trials, the majority of the lodged plants never recovered and died, leading to a single plant per pot or none in many cases. Despite this, in the 2008 trial, there were only a few cases of single plants ( $n = 12$ ) that were not considered for the ANOVA, and the means are presented for 148 accessions. During 2009, the target of two plants was fully achieved by using ~20 seeds per pot and later thinning to two.

Salt treatment significantly influenced all traits except the harvest index and the panicle harvest index during 2008. The salinity × genotype interaction was significant for all traits in both years (Table 2). Salinity delayed phenology, by 2 days for

panicle emergence and 4 days for maturity during 2008 and by 4 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, grain yield was reduced by 7% in 2008 and by 30% in 2009. Salinity increased the harvest index by 7% during 2009 only. Panicle harvest index was reduced substantially by salinity in 2008.

The biomass of all plant components (stem, leaf and panicle) was reduced by salinity (Fig. 1), with the stem biomass 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 panicle biomass (Fig. 1c) were the least reduced.

There was a large range of variation among the accessions for panicle emergence and maturity. These durations changed minimally across the salinity environments and years (Table 3). The accessions had a range in shoot biomass production of 12–78 g under control conditions, which changed to 9–81 g under salinity in 2008. Similarly, a grain yield range of 5–19 g of the accessions under control conditions was changed to 3–32 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 due to a 'determinate' type of growth and synchrony in panicle emergence and development achieved only in selected accessions. However, this accession range of 11–37 g in the control was substantially higher than the range of 2–28 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 highest under control conditions and reduced marginally under salinity. Based on 35 common accessions randomly picked across the whole range of salinity response from 2008, the heritabilities of shoot biomass and grain

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

	Salt treatment		Salt treatment $\times$ genotype	
	Wald statistic	Significance level	$\sigma_g^2$ (s.e.)	Significance level
<i>2008</i>				
Days to panicle emergence	48.43	<0.001	5.06 (1.03)	***
Days to maturity	120.51	<0.001	5.41 (1.32)	***
Shoot biomass (g pot <sup>-1</sup> )	27.30	<0.001	125.80 (30.9)	***
Grain yield (g pot <sup>-1</sup> )	0.78	0.379	28.73 (5.67)	***
Harvest index (%)	0.01	0.915	74.92 (13.14)	***
Panicle harvest index (%)	42.92	<0.001	64.56 (16.3)	**
Stem dry weight (g pot <sup>-1</sup> )	129.90	<0.001	17.58 (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> )	41.84	0.418	41.81 (8.66)	***
<i>2009</i>				
Days to panicle emergence	78.52	<0.001	6.16 (1.16)	***
Days to maturity	61.29	<0.001	12.46 (2.22)	***
Shoot biomass (g pot <sup>-1</sup> )	629.00	<0.001	12.20 (3.33)	**
Grain yield (g pot <sup>-1</sup> )	300.53	<0.001	2.51 (0.64)	**
Harvest index (%)	98.71	<0.001	20.24 (4.39)	***
Panicle harvest index (%)	103.68	<0.001	13.73 (3.09)	***
Stem dry weight (g pot <sup>-1</sup> )	392.31	<0.001	66.69 (16.4)	**
Leaf dry weight (g pot <sup>-1</sup> )	136.54	<0.001	10.82 (2.44)	***
Panicle dry weight (g pot <sup>-1</sup> )	7.73	0.007	27.88 (5.48)	***

yield were high in 2009 but moderate in 2008 (data not shown). The heritability derived from 2 years of pooled data was also high for the phenology (>0.92) and moderate for shoot biomass (0.73) and grain yield (0.51).

From the 2008 data, 10 top grain yielders, five bottom ones and four common checks were initially 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, with low yields (Table 4). A close association of days to maturity with loss of shoot biomass (%) under salinity compared with the control or the loss of grain yield (%) compared with the control was observed in 2009 (Fig. 2); however, some accessions deviated from this pattern. The early-maturing accessions suffered greater grain yield loss than the late-maturing ones that took ~80 days to maturity, with an option to select for contrasts within the later maturing group for grain yield. This trend was not followed in accessions that matured beyond 90 days after sowing. The negative grain-yield loss observed in one long-duration accession was due to a suppression of growth in the control (denominator component) (Fig. 2a). This accession offered a poor aerial competition to its relatively vigorously growing neighbours, resulting in slow growth, fewer tillers and poor biomass.

There are indications that poor stem-biomass accumulation led to poor panicle size under salinity, but this effect was minimal under non-saline control conditions. Accessions with greater stem biomass also produced heavier panicles, in a curvilinear response, with some exceptions for selection with the heavier plants (Fig. 3). A similar response of panicles to stem weight was noticed under control conditions, but this relationship was sparse,

with minimum gains in panicle weight for large increases in stem weight.

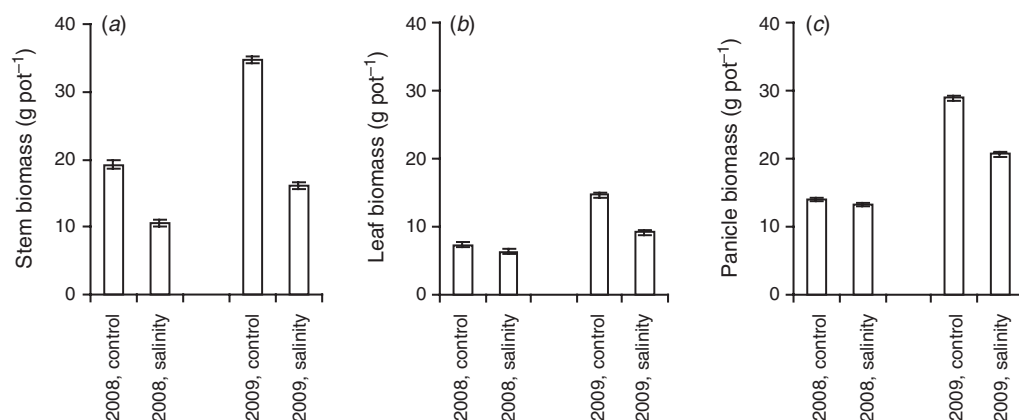
A pooled analysis using the 82 genotypes common to both the years showed the accession  $\times$  year effect to be significant for all traits measured both under saline and control conditions (data not shown). These interaction variance components were about half of the genotypic variance for both grain 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 2 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 was chosen (Fig. 4). Based on the extent of cluster group means, highly tolerant ( $n = 13$ ) (consistently high-yielding under salinity in both 2008 and 2009 with mean BLUPs for 2008 and 2009 as 21.8 and 23.0 g pot<sup>-1</sup>), moderately tolerant ( $n = 16$ ) (with mean BLUPs 13.1 and 20.4 g pot<sup>-1</sup>), tolerant (only in 2009;  $n = 12$ ) (with mean BLUPs 6.0 and 21.7 g pot<sup>-1</sup>), moderately sensitive ( $n = 27$ ) (with mean BLUPs 5.4 and 13.8 g pot<sup>-1</sup>), and sensitive ( $n = 14$ ) (with mean BLUPs 4.1 and 7.8 g pot<sup>-1</sup>) groups were identified. 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 (10 presented as salinity-resistant in Table 4 with ISe 963, ISe 1269 and ISe 1354)

**Table 3.** Mean days to panicle emergence and maturity, total shoot biomass, grain yield, harvest index and panicle harvest index for the core collection of foxtail millet germplasm ( $n = 155$ ) with five check accessions in 2008 and a selected subset of the core collection ( $n = 84$ ) in 2009, in the rainy seasons grown under salinity stressed and control conditions

In 2008, 12 accessions that had one plant per pot were removed from the analysis

	Trial mean	Range of predicted means	s.e.d.	$\sigma_g^2$ (s.e.)	Heritability ( $h^2$ )
<i>2008, salinity stressed</i>					
Days to panicle appearance	50.8	29.8–88.9	3.3	113.6 (14.1)	0.963
Days to maturity	74.3	55.1–107.3	4.1	93.8 (12.4)	0.925
Shoot biomass (g pot <sup>-1</sup> )	30.9	9.3–81.1	16.5	452.0 (77.4)	0.698
Grain yield (g pot <sup>-1</sup> )	10.0	3.4–32.3	6.2	56.8 (10.3)	0.662
Harvest index (%)	27.7	21.1–35.5	6.3	28.4 (13.1)	–
Panicle harvest index (%)	65.0	53.7–72.4	9.3	67.2 (26.8)	–
<i>2008, control</i>					
Days to panicle appearance	48.4	26.0–89.0	1.9	116.4 (13.9)	0.985
Days to maturity	69.9	48.9–117.8	2.3	116.0 (12.8)	0.976
Shoot biomass (g pot <sup>-1</sup> )	40.6	12.0–77.9	9.8	163.0 (27.6)	0.706
Grain yield (g pot <sup>-1</sup> )	10.7	5.4–18.9	3.0	9.1 (2.26)	0.499
Harvest index (%)	27.3	11.2–51.9	5.1	68.5 (9.99)	–
Panicle harvest index (%)	73.8	60.6–85.6	5.8	37.2 (8.32)	–
<i>2009, salinity stressed</i>					
Days to panicle appearance	49.7	24.8–97.9	2.1	206.0 (32.4)	0.964
Days to maturity	76.4	50.5–115.9	2.3	150.8 (23.9)	0.901
Shoot biomass (g pot <sup>-1</sup> )	45.9	10.4–102.6	7.0	424.9 (70.0)	0.929
Grain yield (g pot <sup>-1</sup> )	16.6	2.3–27.9	3.1	40.4 (7.08)	0.844
Harvest index (%)	38.2	4.4–58.5	3.3	71.9 (12.1)	–
Panicle harvest index (%)	79.0	19.3–87.5	4.0	70.5 (12.2)	–
<i>2009, control</i>					
Days to panicle appearance	45.9	25.5–104.2	1.9	216.5 (33.9)	0.994
Days to maturity	71.9	51.1–130.3	2.0	230.6 (36.2)	0.997
Shoot biomass (g pot <sup>-1</sup> )	78.3	42.9–120.2	9.6	392.0 (69.6)	0.817
Grain yield (g pot <sup>-1</sup> )	23.7	11.3–36.5	3.4	34.1 (6.46)	0.743
Harvest index (%)	31.0	12.3–43.5	2.7	34.6 (6.07)	–
Panicle harvest index (%)	81.6	64.8–87.7	3.2	22.2 (4.58)	–



**Fig. 1.** 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. Biomass of (a) stem, (b) leaf and (c) panicle at maturity recorded under non-saline control and salinity conditions in 2008 and 2009 seasons. Capped lines are  $\pm$  s.e.

can be used as tolerant parents in breeding for salinity tolerance, whereas the sensitive group can be used as parents for populations that can be used in various genetic and genomic studies. Overall,

ISe 869, which produced panicles at 52 and 58 days after sowing, matured 80 and 86 days, and 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

**Table 4.** Mean days to panicle emergence and maturity, total shoot biomass, grain yield, harvest index and panicle harvest index under salinity for the accessions rated as top 10 resistant and bottom five sensitive, and four check entries of foxtail millet germplasm in 2008, with their corresponding reaction in 2009

–, Data not available

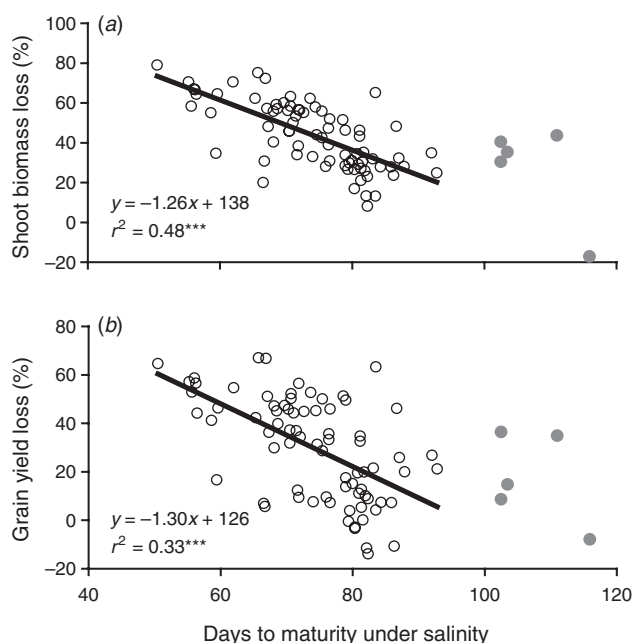
Accessions	Days to panicle appearance	Days to maturity	Shoot biomass (g pot <sup>-1</sup> )	Grain yield	Harvest index	Panicle harvest index (%)
<i>2008, salinity resistant</i>						
ISe 254	49.7	75.6	80.3	32.3	33.4	72.4
ISe 869	52.3	79.6	73.4	27.3	31.8	72.4
ISe 1851	47.1	74.0	58.9	23.0	32.5	72.4
ISe 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
<i>2008, salinity sensitive</i>						
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
<i>2008, checks</i>						
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
<i>2009, salinity resistant</i>						
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
<i>2009, salinity sensitive</i>						
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
<i>2009, checks</i>						
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

and 2009, respectively, was the best and most consistent salt-tolerant accession (Table 4).

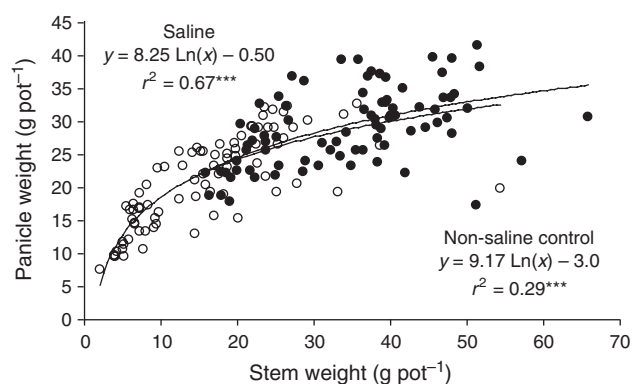
## Discussion

The purpose of this study was to screen the germplasm under an agronomically realistic level of salinity, to achieve 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  $11.2 \pm 0.28$  dS m<sup>-1</sup> in an Alfisol) chosen for screening was

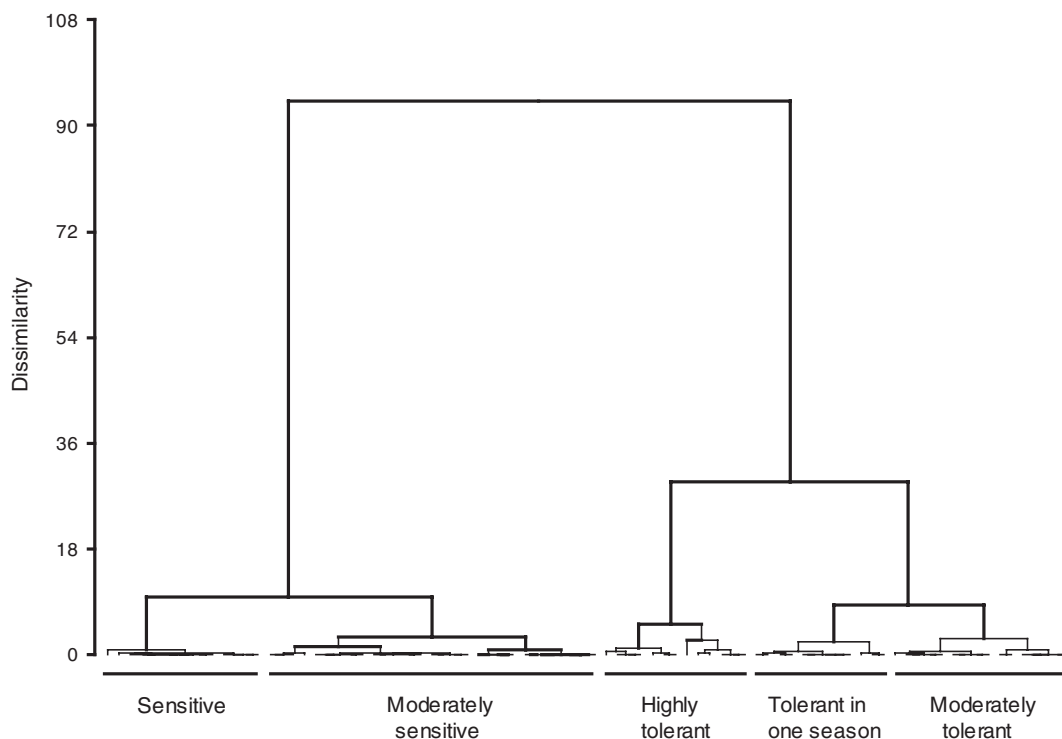


**Fig. 2.** Relationship between days to maturity and (a) proportion of shoot biomass ( $\text{g pot}^{-1}$ ) ((shoot biomass under control – shoot biomass under salinity)  $\times$  100), and (b) proportion of grain yield ( $\text{g pot}^{-1}$ ) ((grain yield under control – grain yield under salinity)  $\times$  100) under saline conditions compared with the control in the germplasm accessions of foxtail millet grown in 2009 ( $n=84$ ). Accessions that took  $>100$  days mature were not considered for the linear regression curve and are shown as grey circles.



**Fig. 3.** Relationship between the stem weight and panicle weight under both saline ( $\circ$ ) and non-saline control ( $\bullet$ ) conditions in the germplasm accessions of foxtail millet grown in 2009 ( $n=84$ ).

similar to those used in previous studies with sorghum ( $\text{ECe } 10\text{--}11 \text{ dS m}^{-1}$ ; Francois *et al.* 1984; Maas 1985; El-Haddad and O’Leary 1994; Igartua *et al.* 1995). Later works (Islam *et al.* 2011) confirmed that saturating with  $100 \text{ mM}$  salt solution would be most suitable for genetic discrimination. However, the most 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 loss in grain yield 30%, and this biomass reduction was similar to the loss reported by earlier works (Thimmaiah *et al.* 1989; Islam *et al.* 2011). No other



**Fig. 4.** Dendrogram showing the distribution of various salinity response groups of the subset ( $n=82$ ) of the core collection of foxtail millet germplasm tested using the grain yield under salinity stress in 2 years.

**Table 5. Means of individual cluster groups with their standard errors for days to panicle appearance, days to maturity, total shoot biomass and grain yield of the five different groups of foxtail millet germplasm out of 82 common accessions tested in 2 years**  
Grain yield under salinity stress across 2 years was used for this clustering

Salinity response group	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	52.3 ± 0.8	53.2 ± 0.7	76.7 ± 0.7	79.9 ± 0.9	60.9 ± 2.5	63.0 ± 3.1	21.7 ± 1.1	23.0 ± 1.1
Moderately tolerant	56.6 ± 2.5	55.8 ± 2.5	79.4 ± 2.2	82.0 ± 2.0	43.5 ± 2.8	58.6 ± 2.1	13.1 ± 0.4	20.4 ± 0.7
Tolerant in one season	52.5 ± 3.0	53.8 ± 2.9	75.5 ± 2.7	80.1 ± 2.64	20.9 ± 1.8	59.5 ± 4.4	6.0 ± 0.5	21.7 ± 0.8
Moderately sensitive	48.9 ± 2.3	48.5 ± 3.1	72.4 ± 2.0	75.9 ± 2.6	17.7 ± 1.4	38.4 ± 3.1	5.4 ± 0.4	13.8 ± 0.4
Sensitive	38.6 ± 1.6	38.5 ± 4.5	64.5 ± 1.7	64.9 ± 3.5	12.1 ± 0.6	19.7 ± 1.6	4.1 ± 0.2	7.8 ± 0.5

studies, except Thimmaiah *et al.* (1989) and Islam *et al.* (2011), have 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, grain yield and dry matter production of foxtail millet did increase at all growth stages when irrigated with saline water up to 6 dS m<sup>-1</sup> but decreased at higher salinity levels, tested up to 21 dS m<sup>-1</sup> (Kubsad *et al.* 1995).

There was a large range in the shoot biomass or grain yield loss of individual accessions, varying from -17 to 79% for shoot biomass and -15 to 88% for the grain yield. This variation in loss was largely explained by the variation in crop duration. Generally, the short-duration accessions suffered the greatest shoot biomass-grain yield loss compared with the long-duration accessions, likely due to insufficient growing time for compensation. The delay in phenology observed under salinity might have further reduced the active reproductive duration in the short-duration accessions. Compared with the performance under control conditions, both shoot biomass (mean 60% across years) and grain yield (mean 50% across years) suffered most in the 10 early-duration accessions. Salinity treatment marginally increased the harvest index. The mean improvement in harvest index across all the accessions was 7%, and 80 accessions exhibited increases in harvest index. The indication is that salinity losses can be largely overcome by a careful choice of the best adapted duration, i.e. by using genotypes of 80–85-day duration in this location. Accessions ISe 375, ISe 1468 and ISe 1541 were used as checks in these trials, because of 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 accession ISe 1468 was rated only moderately sensitive. Overall, the mean shoot biomass loss in the checks due to salinity, compared with 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 10 tolerant accessions under salinity was increased by 63% in 2008 and decreased by 30% in 2009 compared with control, whereas, the mean grain yield was 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 for their high yields under normal field conditions

performed moderately well under salinity with a grain yield loss of 30% in 2008 and 37% in 2009. Therefore, the usual salinity screening procedures cannot be overlooked while establishing the salinity response of foxtail millet genotypes.

Salinity reduced the accumulation of biomass in the shoots, but the reduction was greatest in stem biomass, followed by the panicle. Leaf biomass was the least affected component; consequently, the crop under salinity was relatively short-statured and leafy. This trend of selective reduction of plant components was observed in foxtail and proso millets after exposing the plants to 100 mM concentrations of salinity and alkalinity stresses for 16 days at the early vegetative crop growth stage (Islam *et al.* 2011). However, salinity also reduced 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> was observed 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 soil EC levels ~10–12 dS m<sup>-1</sup>, salinity would reduce the stover yield more than the grain yield and accessions would therefore 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, clearly showed that the changes in grain yield followed proportionally 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, 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 core collection of foxtail millet. 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, 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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