



## The extent of variation in salinity tolerance of the minicore collection of finger millet (*Eleusine coracana* L. Gaertn.) germplasm



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### ABSTRACT

Finger millet (*Eleusine coracana* L. Gaertn.) ranks third in production among the dry land cereals. It is widely cultivated in Africa and South Asia where soil salinization is a major production constraint. It is a potential crop for salt affected soils. To identify salt tolerant germplasm, the minicore finger millet germplasm ( $n=80$ ) was screened for grain yield performance in a soil saturated with NaCl solution of 100 or 125 mM. Genotype effect was significant for most traits, while salinity  $\times$  genotype interaction was significant only in one year. Salinity delayed phenology, marginally reduced shoot biomass and grain yield. There was a large range of genotypic variation in grain yield under salinity and other traits. The yield loss was higher in accessions with prolific growth and yield potential was associated with saline yields. Based on saline yields, accessions were grouped in to four groups and the top tolerant group had 22 accessions with IE 4797 remaining at the top. Salinity had no adverse impact on grain yield of five accessions. Root anatomy in selected genotype of pearl and finger millet showed presence of porous cortex and well fortified endodermis in finger millet that can exclude  $\text{Na}^+$  and enhance N absorption.

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### 1. Introduction

Finger millet (*Eleusine coracana* L. Gaertn.) is one of the most important minor millet of the tropics accounting for 12% of the global millet area. It is a potential and nutritious crop for the increasing world population, particularly in arid and semi-arid regions where it is usually ranked third in cereal production, after sorghum and pearl millet [1]. Major producers are Uganda, India, Nepal and China [2]. It is ancient with its domestication dating back to 5000 years [3]. It is widely cultivated in Africa and South Asia and is a rich source of seed protein, fiber and minerals such as iron, calcium and manganese [4]. The nutritional quality of finger millet grains makes it an ideal food for expectant women, breast-feeding mothers, children, the sick and diabetics [5]. It is a major component of the food prepared for the HIV patients in Eastern Africa. In some parts of Africa and Asia, the

grains of finger millet are used for producing beer or liquor [3]. Finger millet has also been used as a folk remedy for many diseases [6]. The finger millet straw is a highly nutritious fodder. Finger millet is considered to be an ideal crop for changing food habits of people due to its nutritional richness, high photosynthetic efficiency, and good resistance to biotic and abiotic stresses [7].

Global estimates, dating back to two decades, indicate a constant increase in salt affected soils to an extent of 10% of the arable lands [8]. It was further estimated that about 23% of the cultivated area had already been affected by salinity and 37% by sodicity. Another report estimates that approximately 10% of the Earth's total land surface may be salt-affected [9]. Crop plants were shown to differ greatly in their tolerance to salinity, as exhibited by their difference in growth responses [10]. Soil salinity drastically reduces the productivity of most crops although to a varying extent across species [11,12]. Generally, legumes are very sensitive to salinity than the cereals [13]. Among cereals, the response of rice was ranked as the most sensitive while barley the most tolerant [10] and the dryland crop species such as sorghum and pearl millet ranked moderately tolerant [14,15].

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Constant increase in salinity, both in the intensely irrigated and the dry land cropping systems, threaten crop production necessitating identification of crop species that can better tolerate the soil salinity/alkalinity and the genetic variation within each species for response to salinity. In spite of the importance of this constraint and the crop, very little work had been done on assessing the salinity response of finger millets. Currently available literature on finger millet salinity response only deal with either seedling level tolerance [16] or on biochemical differences [17] with no effort on understanding the crop yield response. During this experimentation, the plants were noticed to display nitrogen abundance in the shoot system which was contrary to the usual finding on plant growth under salinity in general. It is usual for crop plants to grow poor and to display nitrogen deficiency symptoms [14] consequent to salinity-impaired N uptake [18]. Root system, the inter-phase between the soil and the plant, and the status of its anatomy are expected to throw some understanding on the mechanism of salinity tolerance in this crop species. Root anatomy and xylem vessel characteristics were shown to help in understanding the drought tolerance strategies of various grain legumes [19]. The objectives of this study was to characterize the finger millet minicore collection [20] for the extent of natural genetic variation, to identify salinity tolerant accessions for use in crop improvement and to understand the difference in root anatomy in comparison to pearl millet, another salt tolerant crop.

## 2. Materials and methods

### 2.1. 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 from rain by a movable rain-out shelter. Experiments were undertaken, in two years at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (17°32' N, 78°16' E, altitude: 546 m above sea level) India. The crops were sown on 10 July 2008 and 2 June 2009, and harvested when matured. Maximum temperature range in the growing season was 23–35.8 °C in 2008 and 25.9–38.8 °C in 2009, and minimum temperature range was from 11.4–25.9 °C in 2008 to 14.0–27.6 °C in 2009.

The pots (27 cm diameter), containing 11 kg of Alfisol, were buried in plots spaced 0.45 × 0.35 M such that the pot rim was at the same level of the outside soil surface to avoid direct solar radiation incidence on the pots. The Alfisol (pH=6.9, CEC: clay ratio=0.29, EC=0.1 mM [21] taken from the top 10 cm of soil at the ICRISAT farm, were fertilized with di-ammonium phosphate (DAP) and muriate of potash at a rate of 200 mg kg<sup>-1</sup> soil each. Half of the pots were artificially salinized by applying a salt quantity of 1.08 g NaCl kg<sup>-1</sup> soil, equivalent to applying a 100 mM solution of NaCl in sufficient volume of water (2.035 L) to wet the Alfisol to field capacity (19.7% w/w) in 2008. The salt dose was increased to 1.35 g NaCl kg<sup>-1</sup>, equivalent to 125 mM solution of NaCl in 2009 as the 2008 dose was considered suboptimal for the best genetic discrimination. The remaining half number of 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. After 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) to avoid an increase in the salt concentration in the soil solution with the soil drying. This was on alternate days initially

and on every day after 35 days of crop age. The base of the pots of the saline treatment was sealed to avoid salt leakage, while the pots of the non-saline treatment had holes to allow drainage. Over-watering of all pots was avoided. This method has had consistently good results both in pulses and cereals [22–24].

In both the years, about 6 to >10 seeds were planted in each pot and before the seedling age of 12 days thinned to two plants per pot. The experiments were planted in a 25 × 4 alpha lattice (incomplete block design) with three replications in 2008 and in a 42 × 2 alpha lattice with five replications in 2009 under two salinity levels (saline and non-saline). In 2008, 100 entries of the finger millet accessions, that included 66 minicore and 34 agronomically superior accessions were evaluated. In 2009 the whole minicore with four agronomically superior checks ( $n=80+4$ ) was evaluated as the germplasm accessions had a good range of variation.

### 2.2. Measurements

Days to panicle emergence, days to maturity, shoot biomass (g pot<sup>-1</sup>) including grains and grain yield (g pot<sup>-1</sup>) was measured in each year. Grain yield or shoot biomass productivity under salinity was used to rate the salt tolerance of the accessions. The relative ratio of grain yield under salinity to that of control was used as index of salinity tolerance.

### 2.3. Root sampling and sectioning

Finger millet under salinity was noted, during the whole growth phase, to exhibit low senescence rate and dark green leaves relative to control plants. Therefore the root anatomy was compared with pearl millet, another salinity-tolerant crop [25], to find a clue on the possible alternate mechanisms of salt tolerance in finger millet. One of the finger millet checks used in this study [accession IE 4673 (VL 149)] and a pearl millet variety with similar duration [ICMB 88004] were harvested from plants that were at milky stage of grain filling, after digging a trench in a way to expose the roots up to 0.3 m of soil depth, from an unrelated nonsaline Alfisol field. The roots of average diameter were selected for sectioning. Freehand sections of about 50 μm thick were cut and the selected sections were stained with 50% toluidine blue, a polychromatic stain that gives different colors with different tissues, and mounted in distilled water. For each crop, three uniform sections were selected at random for observation. Pictures were taken using an optical microscope (Olympus BX43F, Tokyo, Japan) connected to a digital camera using a 10 × 10 magnification.

### 2.4. 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 ( $\sigma_g^2$ ) and error ( $\sigma_e^2$ ) and their standard errors were determined. Environment wise best linear unbiased predictors (BLUPs) for the tested accessions were calculated. Heritability in broad sense was estimated as  $h^2 = \sigma_g^2 / (\sigma_g^2 + (\sigma_e^2/r))$ . The significance of genotypic variance was assessed from the standard error of the estimate of genetic variance  $\sigma_g^2$ , assuming the ratio  $\sigma_g^2/SE(\sigma_g^2)$  to follow normal distribution asymptotically.

For the pooled analysis, homogeneity of variance was tested using Bartlett's test [26]. Here, the year (environment) was treated as a fixed effect and the genotype (G) × environment (E) interaction as random. The variance due to (G) ( $\sigma_g^2$ ) and (G) × (E) interaction ( $\sigma_{GE}^2$ ) and their standard error 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 grain 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 [27]. Principal coordinate analysis (PCoA) was also performed based on distance matrix to further assess the salinity response groups made [28]. All statistical analyses were carried out using Genstat, Release 10.1 [29].

### 3. Results

#### 3.1. Salinity effects

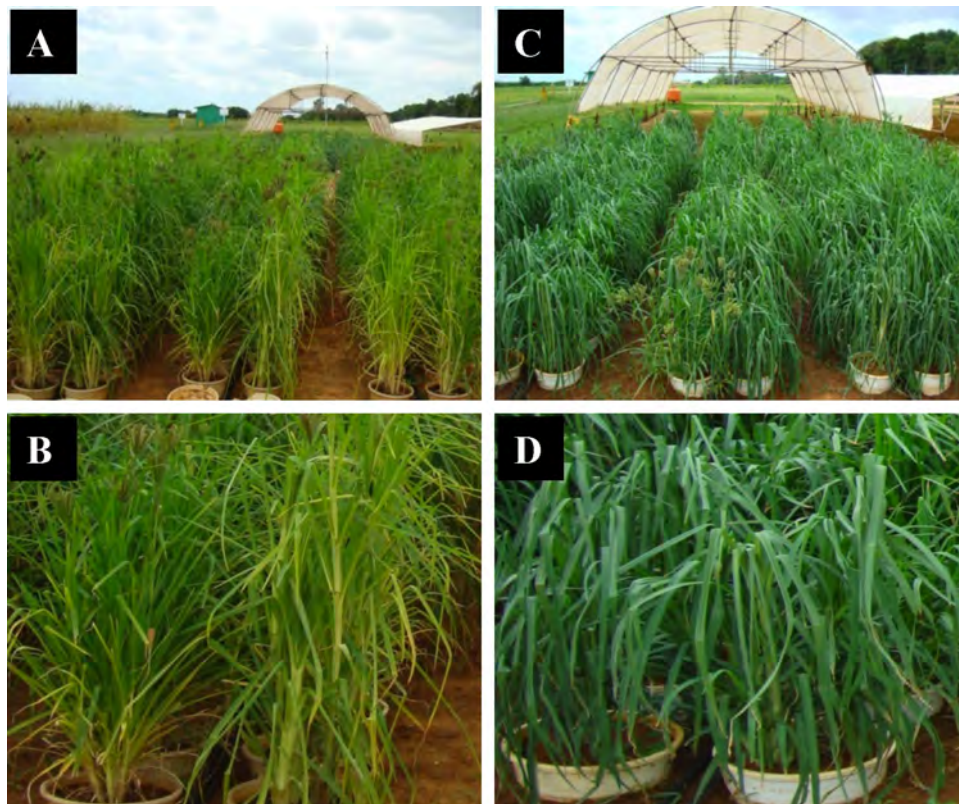
Seedling emergence was delayed by two days under salinity (data not shown). It was visually observed that salinity also delayed the development of nodal roots and the inability of seminal root to anchor the shoot led to the lodging of the plant. Large number of the lodged plants died but the required two plants per pot were achieved by sowing more seeds and careful earthing up of the seedlings.

Salt treatment significantly influenced all the traits except the stover weight in 2008 and the panicle harvest index in 2009. Genotype  $\times$  Salinity interaction was not significant for any of the traits in 2008 but this interaction was significant for all the traits in 2009 (Table 1). Salinity delayed senescence, delayed phenology and intensified the leaf greenness, extensively (Fig. 1). The delay was about 10 days in panicle emergence and maturity in both the seasons (Table 2). Salinity extended the vegetative duration by 10 days but the reproductive duration remained unaltered (26 days in 2008 and 28 days in 2009). Total shoot biomass was reduced, on average, from  $194 \pm 31.8$  g to  $182 \pm 17.3$  g in 2008 and from  $336 \pm 41.6$  g to  $236 \pm 27.8$  g during 2009 that amounted to a

reduction of 6 and 30% of the biomass in 2008 and 2009, respectively. Similarly the mean grain yield was reduced from  $50.5 \pm 10.5$  g to  $38.7 \pm 7.8$  g in 2008 and  $48.3 \pm 8.4$  to  $35.4 \pm 6.3$  g in 2009 which was a 23 and 27% reduction. Salinity reduced the harvest index by 5% in 2008 but remained unchanged in 2009. Salinity reduced panicle harvest index only in 2008. The biomass of all the plant components (stover and panicle) was reduced by salinity. During 2008, the stover was not reduced but the panicle weight was reduced by 18% whereas during 2009 both the stover and the panicle were reduced by 31 and 28%. The soil and weather variations and a higher concentration (125 mM) of salt application in 2009 could be the two reasons for these differences across years and the variation in quantum reduction. The soil and weather variation also could be a reason for the increased mean biomass productivity in the control in 2009 compared with 2008. However the panicle weight and the grain yield under control were comparable between the years.

#### 3.2. Germplasm effects

The germplasm variability for salinity tolerance was measured primarily by the grain yield and the total shoot biomass production including the grains. On the common accessions across years, the time to panicle emergence had varied by 53 days (from 51 to 104) under control and by 56 days (from 58 to 114) under salinity in 2008. It had varied by 79 days (from 49 to 128) under control and 86 days from (57 to 143) under salinity in 2009 (Table 2). Similarly in the common accessions, the days to maturity had varied by 64 days (from 73 to 137) under control and 62 days (from 83 to 145) under salinity in 2008. This was 94 days (from 67 to 161) under control and 89 days (from 80 to 169) under salinity in 2009.



**Fig. 1.** Differences in growth and canopy color of the minicore finger millet germplasm between the (A) nonsalinized control, (B) its close up and (C) salinized once with 100 mM saturation of NaCl, (D) its close up during 2008 rainy season at Patancheru. Note the delay in phenology and senescence, excessive leaf drooping and darker green canopy in salt treated plants (C, D) compared to the control (A, B).

**Table 1**  
Analysis of variance for various characters measured on the minicore collection of finger millet germplasm grown under both salinity-stressed and control conditions in the 2008 and 2009 rainy seasons, ICRISAT Center, Patancheru, India.

	Salt treatment		Salt treatment × genotype $\sigma_{tg}^2$ (SE)
	Wald statistic	Significance level	
<b>2008</b>			
Days to panicle emergence	550.6	<0.001	2.86 (1.64)
Days to maturity	365.8	<0.001	3.62 (2.46)
Shoot biomass (g pot <sup>-1</sup> )	8.3	0.005	250 (134)
Stover biomass (g pot <sup>-1</sup> )	0.02	0.884	138 (70)
Panicle biomass (g pot <sup>-1</sup> )	54.9	<0.001	16.6 (19.4)
Grain yield (g pot <sup>-1</sup> )	71.8	<0.001	18.7 (14.9)
Harvest index (%)	91.0	<0.001	2.49 (1.89)
Panicle harvest index (%)	45.1	<0.001	11.8 (5.8)
<b>2009</b>			
Days to panicle emergence	269.7	<0.001	11.4 (2.59)
Days to maturity	182.4	<0.001	14.52 (3.22)
Shoot biomass (g pot <sup>-1</sup> )	182.6	<0.001	1585 (377)
Stover biomass (g pot <sup>-1</sup> )	166.2	<0.001	825 (187)
Panicle biomass (g pot <sup>-1</sup> )	115.8	<0.001	78.4 (20.7)
Grain yield (g pot <sup>-1</sup> )	94.2	<0.001	45.8 (12.6)
Harvest index (%)	4.17	0.044	2.26 (0.55)
Panicle harvest index (%)	0.08	0.774	23.0 (5.15)

The common accessions ranged in shoot biomass production and grain yield by two-folds both under control and salinity in both the seasons. However the mean shoot biomass production and the grain yield in 2009 was substantially higher than in 2008 closely similar to Table 2. The performance of the 34 accessions, which were tested only in 2008, for all the traits was close to the means in Table 2 but with a narrow range. The harvest index range of the common accessions was 34% under control that got reduced to 30% under salinity in 2008. However this range was 12% under control and 11% under salinity in 2009. A 40% range of panicle harvest index variation of accessions was increased to 51% under salinity in 2008 but this range was 15% under control and 14% under salinity in 2009 (Table 2). The heritability of the phenological traits was the highest and salinity did not bring any consistent change (Table 2). The heritability of shoot biomass, yield, stover weight and panicle weights showed moderate heritability in 2008 but these values were high in 2009. The heritability of harvest index and the panicle harvest index were high both in 2008 and 2009 (Table 2).

**Table 2**  
Mean days to panicle emergence, maturity, total shoot biomass (g pot<sup>-1</sup>), stover (stem+leaf) biomass (g pot<sup>-1</sup>), panicle biomass (g pot<sup>-1</sup>), grain yield (g pot<sup>-1</sup>), harvest index (%) and panicle harvest index (%) for the finger millet germplasm in the 2008 and 2009 rainy seasons under salinity stressed and control conditions, ICRISAT Center, Patancheru, India.

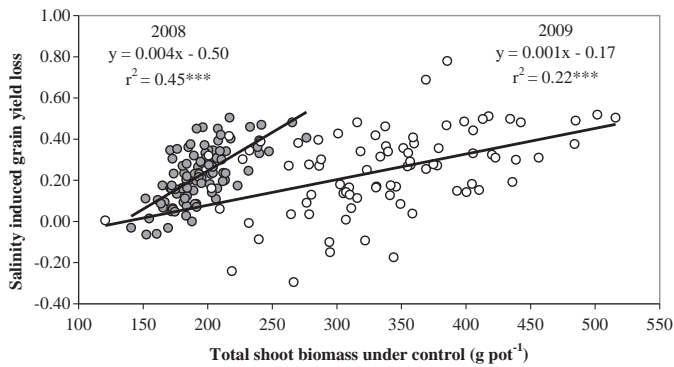
Season/traits	Salinity stressed					Control				
	Trial mean	Range of predicted means	S. Ed	$\sigma_g^2$ (SE)	Heritability (h <sup>2</sup> )	Trial mean	Range of predicted means	S. Ed	$\sigma_g^2$ (SE)	Heritability (h <sup>2</sup> )
<b>2008 (n = 100)</b>										
Days to panicle appearance	84.3	57.9–113.8	3.57	81.1 (12.53)	0.921	73.4	50.8–104.1	3.96	91.1 (14.20)	0.914
Days to maturity	110.5	82.5–144.7	4.41	119.6 (18.53)	0.919	99.5	73.3–136.8	4.91	119.2 (18.89)	0.799
Shoot biomass (g pot <sup>-1</sup> )	181.9	131.3–209.1	17.31	343.1 (90.6)	0.721	193.7	140.6–276.4	31.76	1054 (303)	0.687
Stover biomass (g pot <sup>-1</sup> )	130.2	65.9–170.0	15.90	451.8 (90.7)	0.501	130.3	72.4–207.0	24.87	987 (209)	0.460
Panicle biomass (g pot <sup>-1</sup> )	51.7	36.5–67.2	7.96	63.6 (19.1)	0.563	63.4	49.2–85.0	11.7	126.5 (41.9)	0.522
Grain yield (g pot <sup>-1</sup> )	38.7	21.0–55.4	7.77	71.8 (18.4)	0.579	50.5	30.8–69.5	10.5	120.5 (33.2)	0.542
Harvest index (%)	21.7	8.8–39.0	4.28	37.65 (7.17)	0.757	26.4	9.8–43.7	3.49	44.3 (7.32)	0.863
Panicle harvest index (%)	72.9	34.8–84.0	7.67	108.9 (21.6)	0.731	78.8	46.9–87.0	3.87	32.4 (6.07)	0.769
<b>2009 (n = 84)</b>										
Days to panicle appearance	98.9	56.7–143.1	3.46	246.8 (39.4)	0.973	88.6	48.5–127.7	2.81	338.9 (53.2)	0.981
Days to maturity	126.5	80.0–169.1	3.65	320.4 (50.8)	0.969	117.0	66.8–161.2	3.28	423.7 (66.6)	0.979
Shoot biomass (g pot <sup>-1</sup> )	236.3	148.0–335.7	27.8	1730 (369.2)	0.876	336.4	216.5–515.8	41.6	6458 (1185)	0.880
Stover biomass (g pot <sup>-1</sup> )	153.1	67.2–241.4	19.07	1256 (235.2)	0.895	221.7	91.4–363.3	29.2	4123 (722)	0.883
Panicle biomass (g pot <sup>-1</sup> )	47.8	39.8–79.2	7.60	165.4 (32.6)	0.905	66.3	65.3–112.2	10.38	334 (64.0)	0.879
Grain yield (g pot <sup>-1</sup> )	35.4	31.7–64.2	6.30	142.0 (26.5)	0.920	48.3	51.5–82.0	8.37	261.5 (48.1)	0.896
Harvest index (%)	15.0	14.7–25.7	1.45	24.0 (3.91)	0.949	14.4	14.9–26.7	1.51	19.2 (3.20)	0.939
Panicle harvest index (%)	71.6	72.7–86.6	4.23	203.2 (33.0)	0.931	71.4	68.9–83.9	4.34	106.5 (18.31)	0.901

### 3.3. Association of salt tolerance with other yield components

The association between the shoot biomass productivity under control and the grain yield loss due to salinity was close and positive in finger millet germplasm (Fig. 2). This indicated that higher is the shoot biomass under control, greater is the grain yield loss under salinity. The proportion of yield reduction under salinity compared to non-saline control can be a good measure of salinity tolerance. But in this study the grain yield of accessions under salinity was closely and positively related to their yield under non-saline control (Fig. 3) indicating that a preliminary assessment of salt tolerance can be possible through their yield potential in any region.

### 3.4. Salinity response groups

A pooled analysis using the common 68 genotypes in both the years showed significant accessions × year effect for all the traits except for the panicle harvest index (data not shown). These



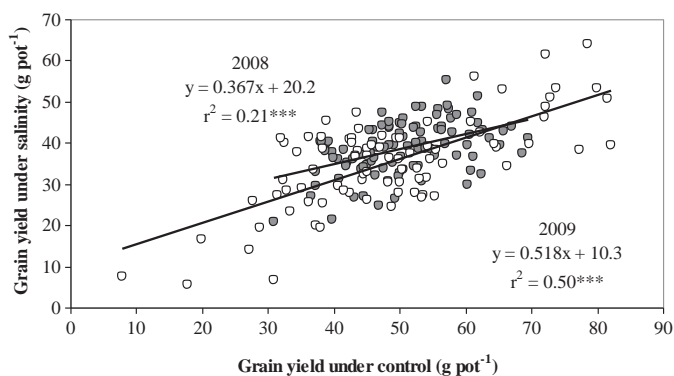
**Fig. 2.** Relationship between the shoot biomass productivity under control and the grain yield loss due to salinity in finger millet germplasm.

interaction variance components were about one fifth for the grain yield and half of the genotypic variance for shoot biomass. Therefore the individual accession means of grain yield in each year were used for selections. The accessions were grouped into representative groups using the BLUPs of accessions under salinity observed in two years by a hierarchical cluster analysis using Ward's method [24]. For the convenience of splitting these 68 accessions (part of the minicore) into distinct groups of response a 15% dissimilarity level was chosen which yielded 4 clusters with significantly different means named as tolerant, moderately tolerant, sensitive and sensitive and late accessions. Tolerant accessions ( $n=22$ ) were consistently high yielding under salinity with a mean grain yield of  $45.5 \text{ g pot}^{-1}$  in 2008 and  $43.1 \text{ g pot}^{-1}$  in 2009, moderately tolerant ( $n=20$ ) were next in order with a mean grain yield of  $36.7$  and  $38.6 \text{ g pot}^{-1}$ , sensitive ones ( $n=21$ ) with a mean grain yield of  $37.1$  and  $25.2 \text{ g pot}^{-1}$  and the sensitive and late ones ( $n=5$ ) with a mean grain yield of  $24.6$  and  $18.6 \text{ g pot}^{-1}$  were the least adapted ones. The accession IE 4797 produced the highest shoot biomass and grain yield with above average harvest index under salinity in both the years (Table 3).

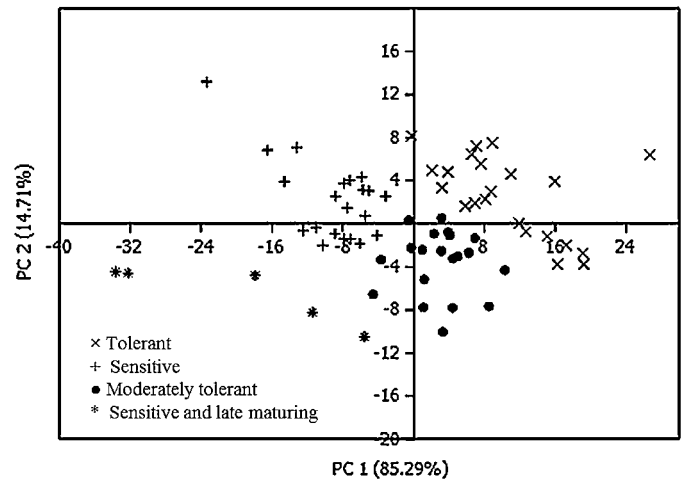
PCoA was performed to further assess the grouping of accessions done on the basis cluster analysis. PC1 and PC 2 explained 85.3% and 14.7% of the variation in grain yield under salinity. Majority of the salinity tolerant accessions grouped by cluster analysis fell in the top right coordinate of the biplot and the sensitive ones on the top left (Fig. 4). The sensitive and late accessions group fell on the bottom left. Distribution of this PCoA indices also confirmed the clustering results.

### 3.5. Root anatomy

The transverse section of the root of finger millet that was extracted at about 0.3 m soil depth from a normal (nonsaline)



**Fig. 3.** Relationship of the grain yields under non-saline control with saline ones of 100 accessions in 2008 and 84 accessions in 2009.



**Fig. 4.** Biplot of principal coordinate indices of finger millet minicore collection accessions calculated using the BLUPs of accessions under salinity observed in two years based on distance estimates. The accessions of various response groups assessed by the clustering approach (listed in Table 3) are marked differently by symbols.

Alfisol field showed two major variations in comparison to another dryland cereal, the pearl millet (Fig. 5). The cortex of the finger millet contained about two layers of collenchyma in the hypodermis below the epidermis followed by single layer of large aerenchymatous cells interspersed radially with small parenchymatous cell. All these cells had thin cell walls. In comparison, pearl millet had well suberized epidermis followed by three layers of small sclerenchymatous cortex followed by about 8–9 layers of thin walled parenchyma. In brief, pearl millet cortex possessed layers of cells that provided mechanical strength. The cortex of finger millet was collapsible. The stele offered a major variation. The stele of the finger millet also presented large differences from that of the pearl millet. The endodermis cells were relatively small, tightly packed, individual cells rectangular in shape on the outside and oval in the inside. The endodermis is followed by two definite well defined thick walled layers of pericycle. The xylem vessels were large and relatively several in finger millet. Every individual xylem vessel was surrounded by a clear layer of highly suberized companion cells. Also large number of companion cells could be noticed in between the xylem vessels with high level of suberization.

## 4. Discussion

The aim of this study was to screen the minicore collection of finger millet germplasm under salinity stress and use the resultant contrasting accessions as donors for further genetic improvement. Therefore the level of salinity used in this study was realistic and moderate. Though there have been reports on the salinity response of finger millets, most of them concentrated on seedling response [16,30] or on genetics of this response. None of these work studied the crop response in terms of total shoot or grain biomass at maturity. For some of the cereals like sorghum the salt concentration chosen for screening is saturation with 100 mM NaCl that resulted in a soil ECE of  $10\text{--}11 \text{ dS m}^{-1}$  [31–34]. Saturating the Alfisol once with 100 mM NaCl resulted in an ECE of  $11.2 \pm 0.28 \text{ dS m}^{-1}$  in this study. Recent works [35] also confirmed that this level of salinity (saturating with 100 mM salt solution) is appropriate for good genetic discrimination. The overall loss observed was the highest in the 2009 experiment when the screening was done by saturating with 125 mM salt solution. In that year, the overall loss was 30% in shoot biomass and 27% in grain yield and this biomass reduction was closely similar to the loss reported in foxtail millet [35].

**Table 3**  
Days to panicle appearance and maturity, total shoot biomass (g pot<sup>-1</sup>) and grain yield (g pot<sup>-1</sup>) of the tolerant and sensitive cluster group accessions of finger millet germplasm out of 68 common minicore accessions tested in the 2008 and 2009 rainy season, ICRISAT Center, Patancheru, India.

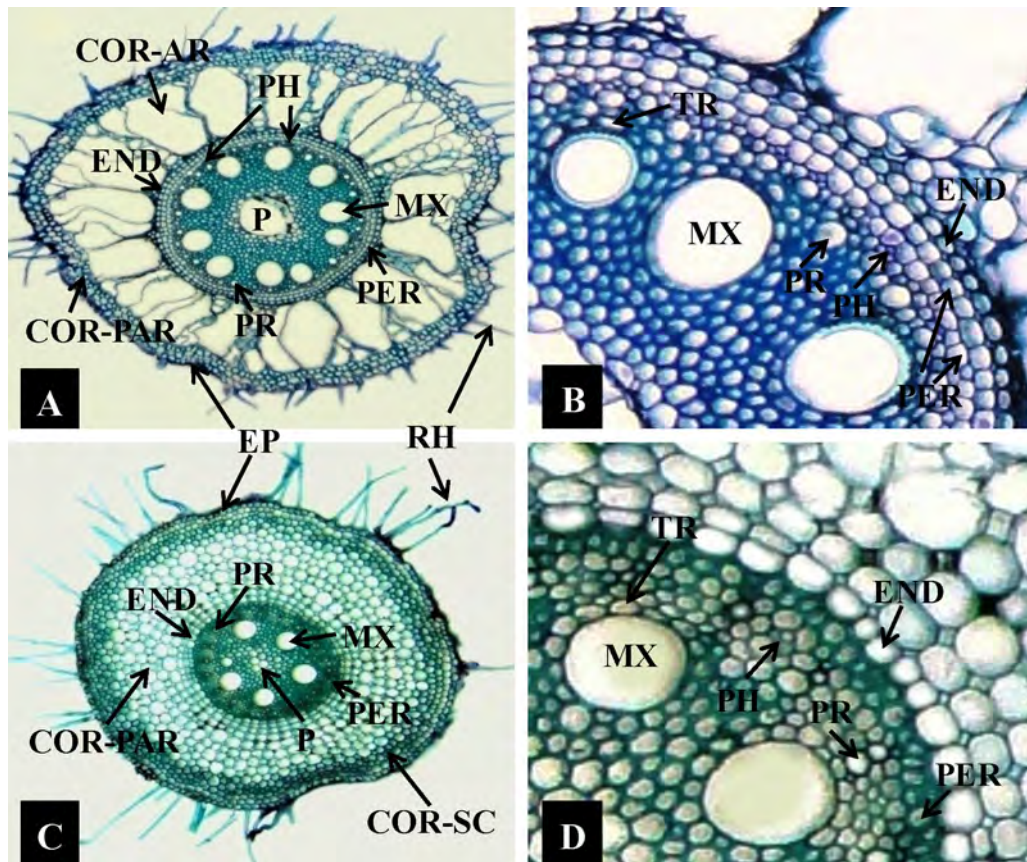
S. No.	Accessions	Days to panicle appearance		Days to maturity		Shoot biomass (g pot <sup>-1</sup> )		Grain yield		
		2008	2009	2008	2009	2008	2009	2008	2009	
<b>Tolerant</b>										
1	IE 518	78	87	101	113	185.4	253.3	43.8	48.7	
2	IE 2034	94	108	119	142	200.6	335.7	44.0	53.0	
3	IE 2217	76	86	99	116	177.3	246.5	47.4	42.6	
4	IE 2790	93	101	125	129	184.9	277.1	41.9	50.8	
5	IE 2872	93	95	122	119	192.2	263.8	43.9	51.0	
6	IE 3045	86	103	110	127	196.2	225.7	44.1	34.3	
7	IE 3077	79	93	101	116	179.9	280.7	43.6	45.3	
8	IE 3391	83	108	112	139	182.6	234.5	46.1	30.9	
9	IE 3470	79	86	101	109	178.6	245.9	43.2	46.3	
10	IE 3973	83	95	111	123	191.2	235.3	42.6	39.1	
11	IE 4073	87	106	116	140	189.4	235.6	47.3	37.8	
12	IE 4329	83	93	114	118	177.9	215.6	44.1	40.9	
13	IE 4671	70	106	94	138	161.0	244.0	48.2	38.0	
14	IE 4673 (C)	64	116	90	147	160.4	291.2	48.8	47.4	
15	IE 4757	76	62	100	85	176.2	171.8	46.9	39.1	
16	IE 4789	84	86	108	114	184.5	255.0	43.1	53.5	
17	IE 4795	81	90	106	118	190.8	244.1	43.1	36.0	
18	IE 4797	82	98	106	122	197.3	302.5	55.4	56.2	
19	IE 5066	79	97	108	124	195.6	243.2	49.2	39.5	
20	IE 6154	88	104	112	133	178.9	244.3	45.0	41.2	
21	IE 6165	82	98	105	121	178.8	228.6	44.7	36.0	
22	IE 6326	83	93	107	119	177.7	233.4	43.3	40.0	
	Group mean	82	96	108	123	183.5	250.3	45.5	43.1	
<b>Sensitive</b>										
1	IE 2312	90	110	118	142	197.8	259.4	40.3	27.3	
2	IE 2430	83	98	108	128	151.9	219.0	34.3	27.8	
3	IE 2437	88	103	116	135	172.5	198.8	32.7	25.9	
4	IE 2457	93	103	118	129	192.9	220.5	34.3	26.7	
5	IE 2589	88	106	114	133	182.4	233.8	37.2	29.1	
6	IE 2619	92	127	118	154	202.2	310.6	37.0	27.0	
7	IE 2821	78	89	105	114	169.3	220.1	34.6	29.6	
8	IE 2957	58	119	82	146	131.3	257.8	39.9	19.5	
9	IE 3392	82	98	110	127	184.6	223.8	39.8	30.5	
10	IE 3721	86	108	114	137	188.8	237.7	36.1	31.1	
11	IE 3945	81	100	104	128	209.1	229.2	39.5	28.6	
12	IE 3952	91	108	118	141	206.7	251.6	33.9	24.5	
13	IE 4028	75	99	101	122	172.8	222.1	39.3	27.9	
14	IE 4545	84	102	109	129	197.0	207.7	39.5	26.2	
15	IE 4734	60	55	88	73	132.6	149.5	41.3	7.7	
16	IE 4816	94	124	121	153	199.8	205.7	38.3	16.6	
17	IE 5201	78	111	107	143	191.5	256.8	37.5	25.3	
18	IE 6082	69	86	96	111	152.0	161.6	36.4	19.5	
19	IE 6337	81	96	110	125	169.9	200.9	34.5	28.5	
20	IE 6350	90	105	117	139	177.9	188.6	33.1	23.3	
21	IE 6473	87	101	113	132	196.2	218.6	39.0	25.7	
	Group mean	82	102	109	131	180.0	222.6	37.1	25.2	
<b>Sensitive and late maturing</b>										
1	IE 2710	99	130	140	160	179.0	237.8	27.1	20.0	
2	IE 2871	102	121	129	153	171.0	263.2	26.6	27.3	
3	IE 5106	95	103	123	132	190.8	235.7	26.9	33.6	
4	IE 2572	106	143	137	167	191.0	253.4	21.5	6.8	
5	IE 6537	114	136	145	169	169.8	216.9	21.0	5.5	
	Group mean	103	127	135	156	180.3	241.4	24.6	18.6	

(C)=Checks used in this study.

However, the most useful observation was the presence of a large range of variation among accessions for yield loss due to salinity. This varied from -20 to 32% in 2008 and -24 to 52% in 2009 for shoot biomass and -10 to 50% in 2008 and -29 to 78% in 2009 for the grain yield providing good perspectives for selection. The accessions that were consistently greater in tolerance and ranged from marginal gain to only a 10% loss in grain yield were IE-4073, -4797, -5870, -6326 and IE 6154 and accessions that lost >40% in both the years were IE-2572, -2710 and IE 4545. In the first season with 100 mM saline saturation, the biomass lost was minimal but the grain yield was affected through a reduced partitioning to grains making this crop species as one of the potential choice for

salt-affected areas. Generally, the prolific shoot biomass producing accessions under non-saline environments were the more sensitive to salinity or lost more yield under salinity. This has indicated that a greater reproductive success under control can be an indirect trait in selecting for good performance under salinity. Also grain yield under non-saline control explained about 50% of the variation in saline grain yields in 2009 when the range of saline grain yields were largest. This observation would also help in narrowing down the size of the screening material on the basis of their grain yield potential in any region.

Some clear morphological differences that were noticed under salinity were dark green canopy, drooping leaves and delayed



**Fig. 5.** Photomicrographs of transverse freehand root sections of finger millet [A ( $\times 100$ ) and B (a portion of the stele enlarged, not to scale)] and pearl millet [C ( $\times 100$ ) and D (a portion of the stele enlarged, not to scale)] stained with 50% toluidine blue. Roots were extracted from 0.3 m soil depth in an Alfisol. EP = Epidermis, END = Endodermis, RH = Root hair, PR = Proto xylem, PH = Phloem, P = Pith, MX = Meta xylem, TR = Tracheid, COR-PAR = Cortical parenchyma, COR-SC = Cortical sclerenchyma, COR-AR = Cortical aerenchyma, PER = Pericycle.

senescence compared to the pale green and largely erect leaves under control (Fig. 1). The permanent drooping can be explained by less turgid and water deficient leaves. However, the intense green color and the delayed senescence of the leaves were indicative of a better N status in the leaves. Impaired nitrogen uptake was one of the primary consequences of salinity. Under salinity, better growing or salinity-tolerant genotypes were found to contain poor shoot N concentrations than the poor growing genotypes of the dryland cereals such as sorghum and pearl millet [14,15]. This was explained as a consequence of tissue N dilution (from 0.9% to 0.5% in sorghum and from 1.3 to 0.3% in pearl millet) that was needed to promote plant growth when N uptake was impaired in salt-affected soils. Salt-affected plants tend to reduce water uptake and result in lowered transpiration and stomatal conductance. This is expected to reduce the N uptake. Finger millet in general seems to have mechanisms to overcome this hurdle and to ensure N uptake that needs detailed further studies.

The presence of salt in the soil is known to affect the plant growth in two ways, namely osmotic tolerance or ionic tolerance or both [10]. In cereals, the reduction in total leaf area of the plant is achieved through reductions in tillers and the size of individual leaves. This modification was to achieve a reduced leaf area development relative to root growth and as a consequence reduce the water use by plants. Subsequent measures of ionic tolerance are through either exclusion  $\text{Na}^+$  and  $\text{Cl}^-$  ions or compartmentalization of the  $\text{Na}^+$  and  $\text{Cl}^-$ . In this study, the loss in shoot biomass or grain yield of finger millet due to salinity was about 30%. A constant wilted appearance of the crop under salinity, generalized across genotypes, was indicative of the osmotic stress the plants were

subjected to but dark green leaves, the absence of the characteristic early senescence of the old leaves as a symptom of accumulation of toxic  $\text{Na}^+$  ions were indicative of a largely successful  $\text{Na}^+$  and  $\text{Cl}^-$  ion exclusion or compartmentalization [10]. This crop species seems to possess some important salt-tolerant genes [30] and further work is needed to confirm the mechanism of its salt tolerance.

The seedling stage seems to be the most sensitive stage of crop ontogeny at which the plants tend to lodge and never recover. At this stage the formation of secondary roots seem to be delayed as a consequence of avoidance of the stressful environment. The more the plants delay such nodal root development the more plant mortality is experienced leading to a characteristic patchy crop. The relative salt tolerance as grain yield and shoot biomass only comes after successful plant establishment. Therefore it is necessary to look for accessions that reduce the lag phase of nodal roots as well agronomic options that would help normal nodal root development.

Three major differences were seen in the anatomy of roots of finger millet compared with pearl millet; a crop species also known to be tolerant to salinity and drought [36]. Extensive increase in the root porosity with the presence of large aerenchymatous cells in the hypodermal cortex is an indication of requirements for greater levels of aerobic respiration. Presence of aerenchyma in the roots increase the longitudinal oxygen flow from the above ground part to the root [37] and also decrease the metabolic cost and cell respiration [38]. Besides, these are responsible for the ventilation (root-atmosphere) of excess gas such as ethylene, methane, and carbon dioxide which can have growth retarding effect at higher concentrations. As these roots in the current study were harvested from well-drained Alfisol, this aerenchyma presence can

be considered as a constitutive trait of this crop. Under salinity stress, when the energy demand for many altered plant functions is expected to increase, a greater elaboration these aerenchymatous cells can be expected. The presence of a mechanism for high energy turnover can only be explained for active discrimination in ion uptake and in overcoming resistances to water and minerals uptake. The additional resistances to the Na<sup>+</sup> uptake are the well equipped endodermis, double-layered pericycle and a xylem vessel uniformly surrounded by companion cells. These additional fortifications explain the slow and delayed senescence, low requirement for N remobilization from basal leaves and the presence of dark green leaves in plants under saline soils. These tolerance symptoms are possible by Na-exclusion and enhanced N absorption. The formation of aerenchyma under waterlogged conditions was also reported for other crops, such as soybean [39], wheat, barley, and oat [40]. An intensive production of aerenchyma was observed in maize cultivars, such as cv. Seneca Horizon [41]. Finger millet is claimed to be capable of performing better under adverse soil and weather conditions compared to other crops [42].

## 5. Conclusion

This study has shown that finger millet minicore collection is a good resource for identifying sources of salinity tolerance. The overall reduction caused by salinity in shoot biomass or grain yield varied from minimal to moderate. The finger millet minicore collection had exhibited the existence of a wide genotypic variation for salinity response. Salinity reduced the grain yield through the reductions both in the shoot biomass and the harvest index. Groups of accessions with variation in salinity tolerance have been identified which may be used in cultivar development. A well fortified endodermis and pericycle and a well-protected individual xylem vessels in the roots suggested potential for an effective ionic exclusion in finger millet.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.plantsci.2014.07.001>.

## References

- [1] M.S. Bisht, Y. Mukai, Genome organization and polyploid evolution in the genus *Eleusine* (Poaceae), *Plant Syst. Evol.* 233 (2002) 243–258.
- [2] ICRISAT, ICRISAT Website, International Crops Research Institute for the Semi-Arid Tropics, 2008, <http://www.icrisat.org/index.htm> (Accessed 21.10.08).
- [3] K.W. Hilu, J.M.J. Dewet, Domestication of *Eleusine coracana*, *Econ. Bot.* 30 (1976) 199–208.
- [4] H.D. Upadhyaya, N.D.R.K. Sarma, C.R. Ravishankar, T. Albrecht, Y. Narasimhudu, S.K. Singh, S.K. Varshney, V.G. Reddy, S. Singh, S.L. Dwivedi, N. Wanyera, C.O.A. Oduori, M.A. Mgonja, D.B. Kisandu, H.K. Parzies, C.L.L. Gowda, Developing a mini-core collection in finger millet using multi location data, *Crop Sci.* 50 (2010) 1924–1931.
- [5] National Research Council, Finger millet, in: F.R. Ruskin (Ed.), *Lost crop of Africa, Grains*, vol. 1, National Academy Press, Washington, DC, 1996, pp. 39–58.
- [6] J.M. Watt, M.G. Breyer-Brandwijk, *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, Second edition, E. and S. Livingstone Ltd, Edinburgh and London, 1962, pp. 1457.
- [7] T.K. Babu, R. Sharma, H.D. Upadhyaya, P.N. Reddy, S.P. Deshpande, S. Senthilvel, N.D.R.K. Sarma, R.P. Thakur, Evaluation of genetic diversity in *Magnaporthe grisea* populations adapted to finger millet using simple sequence repeats (SSRs) markers, *Physiol. Mol. Plant Pathol.* 84 (2013) 10–18.
- [8] K.K. Tanji, Nature and extent of agricultural salinity, in: K.K. Tanji (Ed.), *Agricultural Salinity Assessment and Management*, American Society of Civil Engineers, New York, 1990, pp. 1–18.
- [9] R.V. Schofield, M.J. Kirkby, Application of salinization indicators and initial development of global soil salinization scenario under climatic change, *Glob. Biogeochem. Cycles* 17 (2003) 1078.
- [10] R. Munns, M. Tester, Mechanisms of salinity tolerance, *Annu. Rev. Plant Biol.* 59 (2008) 651–681.
- [11] L.E. Francois, E.V. Maas, Crop response and management on salt-affected soils, in: M. Pessarakli (Ed.), *Handbook of Plant and Crop Stress*, Marcel Dekker, New York, 1994, pp. 149–181.
- [12] R. Munns, Comparative physiology of salt and water stress, *Plant Cell Environ.* 25 (2002) 239–250.
- [13] A. Lauchli, Salt exclusion: an adaptation of legumes for crops and pastures under saline conditions, in: R.C. Staples, G.H. Toenniessen (Eds.), *Salinity Tolerance in Plants, Strategies for Crop Improvement*, Wiley, New York, 1984, pp. 171–187.
- [14] L. Krishnamurthy, R. Serraj, C.T. Hash, J.D. Abdullah, B.V.S. Reddy, Screening sorghum genotypes for salinity tolerant biomass production, *Euphytica* 156 (2007) 15–24.
- [15] L. Krishnamurthy, R. Serraj, K.N. Rai, C.T. Hash, J.D. Abdullah, Identification of pearl millet [*Pennisetum glaucum* (L.) R. Br.] lines tolerant to soil salinity, *Euphytica* 158 (2007) 179–188.
- [16] H.B. Shailaja, S. Tirumeni, Evaluation of salt tolerance in finger millet (*Eleusine coracana*) genotypes at seedling stage, *Indian J. Agric. Sci.* 77 (2007) 672–674.
- [17] A. Ediga, S. Hemalatha, B. Meriga, Effect of salinity stress on antioxidative defense system of two finger millet cultivars (*Eleusine coracana* (L.) Gaertn.) differing in their sensitivity, *Adv. Biol. Res.* 7 (2013) 180–187.
- [18] D.C. Bowman, G.R. Cramer, D.A. Devitt, Effect of salinity and nitrogen status on nitrogen uptake by tall fescue turf, *J. Plant Nutr.* 29 (2006) 1481–1490.
- [19] R. Purushothaman, M. Zaman-Allah, N. Mallikarjuna, R. Pannirselvam, L. Krishnamurthy, C.L.L. Gowda, Root anatomical traits and their possible contribution to drought tolerance in grain legumes, *Plant Prod. Sci.* 16 (2013) 1–8.
- [20] H.D. Upadhyaya, R. Ortiz, A mini core subset for capturing diversity a promoting utilization of chickpea genetic resources, *Theor. Appl. Genet.* 102 (2001) 1292–1298.
- [21] S.A. El Swaify, P. Pathak, T.J. Rego, S. Singh, Soil measurement for optimized productivity under rainfed conditions in the semi-arid tropics, in: *Advances in Soil Science*, Springer-Verlag New York Inc., New York, 1985, pp. 1–64.
- [22] L. Krishnamurthy, N.C. Turner, P.M. Gaur, H.D. Upadhyaya, R.K. Varshney, K.H.M. Siddique, V. Vadez, Consistent variation across soil types in salinity resistance of a diverse range of chickpea (*Cicer arietinum* L.) genotypes, *J. Agron. Crop Sci.* 197 (2011) 214–227.
- [23] N. Srivastava, V. Vadez, H.D. Upadhyaya, K.B. Saxena, Screening for inter and intra specific variability for salinity tolerance in pigeonpea (*Cajanus cajan*) and its related wild species, *SAT eJournal* 2 (2006) 1.
- [24] N. Srivastava, V. Vadez, M. Lakshmi Narasu, H.D. Upadhyaya, S.N. Nigam, A. Rupkala, 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 November, 2008, p. 111.
- [25] Biosalinity Awareness Project, Salt Tolerant Plants, 2005, Available from: <http://www.biosalinity.org/salt-tolerant-plants.htm>
- [26] M.S. Bartlett, Properties of sufficiency and statistical tests, *Proc. R. Soc. London A* 160 (1937) 268–282.
- [27] J.H. Ward, Hierarchical grouping to optimize an objective function, *J. Am. Stat. Assoc.* 58 (1963) 236–244.
- [28] M. Nei, Analysis of gene diversity in subdivided populations, *PNAS* 70 (1973) 3321–3323.
- [29] R.W. Payne, The Guide to GenStat® Release 6.1. Part: 2 Statistics, VSN International Ltd., Oxford, UK, 2002.
- [30] P. Aarati, B.T. Krishnaprasad, Ganeshkumar, M. Savitha, R. Gopalakrishna, G. Ramamohan, M. Udayakumar, Expression of an ABA responsive 21 kDa protein in finger millet (*Eleusine coracana* Gaertn.) under stress and its relevance in stress tolerance, *Plant Sci.* 164 (2003) 25–34.
- [31] E.V. Maas, Crop tolerance to saline sprinkling water, *Plant Soil* 89 (1985) 273–284.
- [32] L.E. Francois, T. Donovan, E.V. Maas, Salinity effects on seed yield, growth and germination of grain sorghum, *Agron J.* 76 (1984) 741–744.
- [33] E.H.M. El-Haddad, J.W. O'Leary, Effect of salinity and K/Na ratio of irrigation water on growth and solute content of *Atriplex amnicola* and *Sorghum bicolor*, *Irrig. Sci.* 14 (1994) 127–133.
- [34] E. Igartua, M.P. Gracia, J.M. Lasa, Field responses of grain sorghum to a salinity gradient, *Field Crop. Res.* 42 (1995) 15–25.
- [35] M.S. Islam, M.M. Akhter, A. El Sabagh, L.Y. Liu, N.T. Nguyen, A. Ueda, Y. Masaoka, H. Saneoka, Comparative studies on growth and physiological responses to



- saline and alkaline stresses of foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.), *AJCS* 5 (2011) 1269–1277.
- [36] P.C. Sharma, D. Sehgal, D. Singh, G. Singh, R.S. Yadav, A major terminal drought tolerance QTL of pearl millet is also associated with reduced salt uptake and enhanced growth under salt stress, *Mol. Breed.* 27 (2011) 207–222.
- [37] T.D. Colmer, Long-distance transport of gases in plants a perspective on internal aeration and radial oxygen loss from roots, *Plant Cell Environ.* 26 (2003) 17–36.
- [38] J.P. Lynch, M.D. Ho, Rhizoeconomics: carbon costs of phosphorus acquisition, *Plant Soil* 269 (2005) 45–56.
- [39] A.L. Thomas, S.M.C. Guerreiro, L. Sodek, Aerenchyma formation and recovery from hypoxia of the flooded root system of nodulated soybean, *Ann. Bot.* 96 (2005) 1191–1198.
- [40] T.L. Setter, I. Waters, Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats, *Plant Soil* 253 (2003) 1–34.
- [41] D.E. Enstone, C.A. Peterson, Suberin lamella development in maize seedling root grown in aerated and stagnant conditions, *Plant Cell Environ.* 28 (2005) 444–455.
- [42] H.D. Upadhyaya, R.P.S. Pundir, C.L.L. Gowda, V.G. Reddy, S. Singh, Establishing a core collection of foxtail millet to enhance utilization of germplasm of an underutilized crop, *Plant Genet. Resour. Characterization Util.* 7 (2008) 177–184.