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RESEARCH ARTICLE

Salinity tolerance at seed germination and early seedling stage in mini core collection of pearl millet [*Pennisetum glaucum* (L.) R. Br.]

Anju U. Lathika · Shivanagouda R. Doddagoudar · Santosh K. Pattanashetti · Thupakula Harish Vikram

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Abstract Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is grown for food and forage in arid and semi-arid tropical regions of south Asia and sub-Saharan Africa. Salinity stress has become a serious threat to pearl millet cultivation in these marginal environments. Seed germination and early seedling stage are most prone to salinity stress and critical for crop establishment. To identify diverse germplasm sources of salinity tolerance, the mini core collection of pearl millet (238 accessions) along with eight accessions used as controls including mini core controls (IP3616, IP3757, IP6098, IP6105), salinity tolerant (IP10876, IP10878, IP18406) and susceptible controls (IP17862) were subjected to preliminary evaluation for salinity tolerance at seed germination and early seedling stage using factorial completely randomized design under control and moderate level of salinity stress (100 mM NaCl) conditions in a laboratory experiment. Enormous genetic variability and significant reduction under salinity stress were noted for salinity related traits; i.e., germination, root length, shoot length, seedling dry weight, and seedling vigour indices (SVI–1, SVI–2). Principal component analysis for six-salinity related traits grouped mini core (238) and controls (8) into six distinct clusters. The highly salinity tolerant germplasm were grouped in cluster 1 (33 accessions; controls IP3616, IP10876, IP18406) and from this cluster, based on lesser percent reduction under salinity stress across six traits, the best 10 promising sources from the mini core were chosen, and subjected to detailed evaluation at different salinity levels (0, 100, 150, 200, 250 mM NaCl) to confirm their tolerance at higher levels of salinity and also assess critical and maximum level of salinity tolerance in pearl millet as a species. These promising sources could tolerate 100 mM comfortably, but drastic reduction for salinity related traits was noted at 150 mM and onwards with significant genotypic differences. Only two highly salinity tolerant

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A. U. Lathika · S. R. Doddagoudar
Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur 584104, India
e-mail: anjuul.95@gmail.com

S. R. Doddagoudar
e-mail: srdoddagoudar@uasraichur.edu.in; srdsst@gmail.com

S. K. Pattanashetti (✉) · T. H. Vikram
Department of Genetics and Plant Breeding, College of Agriculture, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga 577204, India
e-mail: santoshpattanashetti@uahs.edu.in; santosh.pattanashetti@gmail.com

T. H. Vikram
e-mail: thupakulaharishvikram@uahs.edu.in

S. K. Pattanashetti
ICRISAT, Genebank, Patancheru 502324, India

accessions; i.e., IP14294 and IP21312 recorded more than 80% seed germination at 150 mM, suggesting it to be the critical limit of salinity that pearl millet as a species can tolerate at seed germination and early seedling stage and any higher salinity levels would not be tolerable.

Keywords Genetic variability · Mini core · Pearl millet · Salinity tolerance · Seed germination · Seedling stage

Abbreviations

ANOVA	Analysis of variance
DF	Degrees of freedom
GAM	Genetic advance as per cent of mean (%)
GCV	Genotypic coefficient of variation (%)
GLM	Generalized linear model
GP	Germination percentage
H	Heritability broad sense
HSD	Tukey's studentized range test
ISTA	International Seed Testing Association
M.ha	Million hectare
$M.km^2$	Million-kilometer square
mM	Milli molar
MS	Mean squares
M.t	Million tonnes
NaCl	Sodium chloride
PCV	Phenotypic coefficient of variation (%)
PR	Percent reduction
RL	Root length (cm)
SAS	Statistical analysis system
SDW	Seedling dry weight (mg)
SL	Shoot length (cm)
SVI–1	Seedling vigour index–1
SVI–2	Seedling vigour index–2

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br., Syn. *Cenchrus americanus* (L.) (Morrone)] is the sixth most important cereal worldwide and mainly grown for food and forage in India and Africa, while as a forage crop in the Americas. Its grain is a staple food for around 90 million people in the Sahelian region of Africa and north-western India. Worldwide, millets are cultivated on 31.33 M.ha with a production of 31.59 M.t with majority of the area (~98%) being in Africa (19.68 M.ha) and Asia (11 M.ha) wherein, India (13.50 M.t) is the largest producer followed by Niger (3.34 M.t) (Faostat 2022). Pearl millet is a hardy crop grown mostly in marginal environments in the arid and semi-arid tropical regions of south Asia and sub-Saharan Africa. This crop has high degree of tolerance to heat and drought and also adapted to saline, acidic, and aluminium toxic soils (Yadav and Rai 2013).

Abiotic stresses such as drought, salinity, water logging, extreme temperatures and heavy metals pose serious constraint to plant growth and development causing more than 50% yield reduction for major crop plants (Bray et al. 2000). Salinity stress is one of the most dangerous threats for crop production and limits the agricultural productivity globally (Morton et al. 2019). In the arid and semi-arid regions of the world, salinity stress has become a serious threat to crop production due to limited rainfall, high evapotranspiration, poor soil and water management practices (Munns et al. 2002). Salinity in the soil or water source used for irrigation, adversely affects the crop growth at all developmental stages and ultimately reduces yield and quality of seeds. Crop tolerance to salinity is of enormous significance due to constant increase in salt-affected areas in arid and semi-arid regions. In a recent study, soil salinization caused by unreasonable water resource utilization was assessed for Shiyang River basin of Northwest China's arid region for two decades. Although salinization area remained stable, the degree of salinization was intensifying, which is caused by groundwater evaporation near reservoirs, agricultural irrigation evaporation, and downstream ecological water input evaporation (Meng et al. 2025).

Soil salinity can be overcome by cultivation of salt tolerant plant species (halophytes) or using available technology for reclamation of these soils. The halophytes can withstand extraordinary intensities of salt amounts; i.e., beyond 300 to 1020 mmol L^{-1} (Mushtaq et al. 2021). In a recent study on reclamation, the addition of straw and gypsum treatments could mitigate soil salinization and enhance soil fertility by increasing soil organic carbon levels and microbial community structure within the aggregates (Zhao et al. 2025). The glycophytic (salt sensitive) crop plants under saline conditions often suffer from decline in growth and productivity (Nyagah and Musyimi 2009; Jha et al. 2022). Pearl millet being fairly-tolerant to salinity could be an alternative crop

option for salt affected areas (Krishnamurthy et al. 2007). It is a suitable crop to grow while leaching is occurring and a quick-growing summer forage or grain crop (Oleiwi et al. 2015). Although pearl millet has capacity to withstand soil salinity to some extent, salinity acts as a significant abiotic constraint for its cultivation in several areas of Africa and India, with more intense effects in the West Asia and North Asia (WANA) zones of Central Asia.

Plants exhibit great variability in their capacity to tolerate salinity, they evolve adaptive mechanisms, which enable them to continue the various metabolic and physiological growth process. The negative effect of salinity prevails at every stage of crop growth but seed germination and early seedling growth are most prone. Salinity affects seed germination by creating osmotic stress that prevents water uptake or ionic stress allowing entry of ions that may be toxic to embryo or developing seedlings (Almodares et al. 2007; Sawamery and Mojaddam 2014). For efficient production under saline conditions, seeds must germinate, and seedlings must vigorously pass through the salty layer of the soil and survive (Aliakbar and Kobra 2008). Seed germination is an important stage in the life cycle of crop plants particularly in saline soils and determines the degree of crop establishment. Rapid and uniform seed germination and seedling vigour under saline condition not only increases early seedling establishment but also provides higher drought tolerance (Bradford 1995).

Compared to other cereal crops, limited information is available on response of pearl millet germplasm to soil salinity at seed germination and early seedling stage. Most investigations in pearl millet either used limited number of germplasm, cultivars or breeding material with narrow genetic base for assessing salinity tolerance (Ali et al. 2004; Mukhopadhyay et al. 2005; Kulkarni et al. 2006; Krishnamurthy et al. 2007; Yakubu et al. 2010; Venkata et al. 2012). Diverse germplasm representing global diversity of pearl millet was not assessed earlier for salinity response. The world's largest collection of pearl millet germplasm (25,794 accessions) representing 52 countries is conserved in Genebank of International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, India (https://genebank.icrisat.org). The mini core collection of pearl millet comprising 238 accessions that represents 1% of entire collection representing 45 countries was developed earlier at ICRISAT (Upadhyaya et al. 2011). The passport information including country of origin of the mini core accessions is provided (Supplementary Table 1). The detailed passport information can also be found at ICRISAT Genebank website (https://genebank.icrisat.org/IND/Passport?Crop=Pearl+millet). The pearl millet mini core collection developed at ICRISAT was assessed in the present study for their response to different levels of salinity at seed germination and early seedling stage through preliminary screening followed by confirmation of selected germplasm sources to identify diverse promising tolerant sources for their subsequent use in pearl millet breeding program.

Millets are not resistant to high salt concentrations, hence, grouped into glycophytes and can tolerate average salt threshold of about 6 dSm^{-1} with variation among species. Glycophytes are critically repressed or destroyed at 100–200 mmol L^{-1} NaCl (Mushtaq et al. 2021). Earlier investigations in pearl millet have indicated 17 dSm^{-1} as critical level to distinguish salinity tolerant and sensitive genotypes (Venkata et al. 2012); soil salinized with 200 mM concentration in a lysimeter experiment was found to decrease ~ 25% of shoot, grain and total biomass weight (Oleiwi et al. 2015), and; significant reduction of grain yield on using high salinity levels (8, 12, or 15 dSm^{-1}) (Ribadia et al. 2018; Heidari and Jamshid 2010; Yakubu et al. 2010; Yadav et al. 2012). In the light of these investigations in pearl millet and also a large set of germplasm to be evaluated in the present study, a moderate level of salinity; i.e., 100 mM NaCl (measured EC 12.4 dSm^{-1}) which is slightly higher than the field level of salinity in pearl millet growing areas (6 to 8 dSm^{-1}) was chosen for preliminary screening of pearl millet mini core collection. Further, identified promising accessions of mini core were subjected to higher salinity levels (100, 150, 200, 250 mM NaCl) along with control to confirm their tolerance and also identify critical level of tolerance in this diverse sub-set of global pearl millet germplasm. The major objectives of the present study were (i) assess the genetic variability for salinity tolerance at seed germination and early seedling stage in the mini core collection of pearl millet, (ii) detailed evaluation of identified tolerant sources at much higher salinity levels for confirmation, and (iii) assess critical level of tolerance in pearl millet as a species. These would be helpful in identification of genetically diverse promising sources of germplasm that could be

utilized in breeding programs towards development of salinity tolerant pearl millet cultivars.

Materials and methods

Preliminary evaluation

Plant material

The experimental material was obtained from ICRISAT Genebank that comprised of pearl millet mini core collection (238 accessions) and eight germplasm accessions used as controls including mini core controls (IP3616, IP3757 from India; IP6098, IP6105 from Niger), salinity tolerant (IP10876, IP10878, IP18406) and susceptible control (IP17862). The germplasm accessions IP10876, IP10878 (landraces from Sudan), and IP18406 (landrace from Namibia) were earlier reported to be salinity tolerant at germination and early seedling stage (Ali et al. 2004, 2006), hence, used as tolerant controls. As reported earlier, IP17862 an advanced/improved cultivar (ICTP8203) was used as a susceptible control (Kulkarni et al. 2006). Earlier, mini core controls IP3757 (Krishnamurthy et al. 2007), IP6098, IP3616, IP6105 (Kulkarni et al. 2006) were reported as highly tolerant to salinity and also give better grain and fodder yield. The mini core collection is frequently regenerated at ICRISAT Genebank, Patancheru, India, to maintain optimum germination (> 90%) and also stored in air tight containers under medium term storage at 4 °C to maintain viability and supplied to the end users. The age of the seed samples was generally of few years old and properly conserved to maintain the viability. From ICRISAT Genebank, the seed samples were sent in air tight seed packets to the concerned scientist at UAS, Raichur, Karnataka, India for research purpose and were properly stored at low temperature and immediately used. The viability of the seed lots used in the study was optimum (> 90%).

Experiment details

The laboratory experiments to identify sources of salinity tolerance at seed germination and early seedling stages from mini core collection of pearl millet were conducted at the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences Raichur, India. Towards this, 246 germplasm accessions as noted above were preliminarily evaluated in a factorial completely randomized design comprising of two factors: salinity levels and genotypes. The first factor comprised of two salinity levels; i.e., control (0 mM NaCl or distilled water) and salinity stress (100 mM NaCl). This salinity stress level (100 mM NaCl, ~ 12 dSm^{-1}) was used as it is slightly higher than the practical salinity levels (6 to 8 dSm^{-1}) found in most of the pearl millet growing areas in the arid and semi-arid regions and will allow to cull-out salinity sensitive genotypes in the preliminary evaluation. The second factor had 238 genotypes and eight controls. To obtain 100 mM NaCl solution, 5.84 g of sodium chloride (NaCl) was dissolved and made up one litre using distilled water. The measured electrical conductivity (EC) of control (0 mM NaCl) and 100 mM NaCl solutions were 0.01 dSm^{-1} and 12.4 dSm^{-1}; pH was 6.83 and 6.96, respectively, and closer to neutral pH. However, the osmotic potential was not measured. Germination papers were soaked in distilled water for control (0 mM NaCl), while in saline solution for salt stress (100 mM NaCl) for evaluation. Each of the accession was received as a single seed sample of same seed lot from ICRISAT, Patancheru, India. Four random samples were drawn from this lot and used as two replicates each for testing under control (0 mM NaCl) and salinity stress (100 mM NaCl) conditions. Each of the treatments were randomized and incubated in walk in germination room under appropriate conditions.

The environmental conditions in the seed germination chamber were monitored on daily basis with the set temperature of 25 ± 2 ℃ and relative humidity of 90 ± 5% and there were no fluctuations or breakdown during the testing period as a backup generator facility was available during the testing period to the seed germination chamber. The regular weight seed germination papers of grade LAX-90 (38 lbs) creeped to hold small and medium sized seeds were purchased from M/s Laxmi Industries, Hyderabad, India. This paper has excellent water holding capacity and its special structure prevents the seed roots from growing through. It also has high absorbent capacity and has 6.6 to 7.0 pH range with good bursting strength. The dry thickness of the paper was approximately 1.4 mm. The seed germination papers were soaked in the prepared salt solution and then the seeds were placed in

these papers. Any crystal formation or drying of germination papers was not observed during the testing period due to the maintenance of seed germination chamber at 90 ± 5% relative humidity. Before placing the seeds for seed germination testing, the seeds were sterilized with 0.5% sodium hypochlorite solution. According to ISTA Rules, the normal seedlings were counted based on the characters such as, (i) Well developed primary and secondary roots showing good elongation, (ii) Healthy plumule (embryonic shoot) with an emerging coleoptile (protective sheath), (iii) Balanced growth indicating strength and potential for normal seedling development, and (iv) No significant damage, disease or malformation that would hinder future seedling growth.

Data collection

(i) Germination percentage (GP): The standard germination test was done by using between-paper method (ISTA 2013). Each accession was tested by placing 100 seeds per replicate uniformly on the germination paper. The roll towels were kept in a germination chamber maintained at 25 ± 2 °C temperature and 90 ± 5% relative humidity. The final count of germination was taken on seventh-day. Germination percentage (GP) was calculated according to the International Seed Testing Association (ISTA) method as below.

$$GP = (\text{Number of normally germinated seeds} / \text{Number of total seeds sown}) \times 100$$

(ii) Root length (cm) (RL): From the germination test, ten normal seedlings were selected randomly from each treatment replication-wise on the day of final count; i.e., seventh-day. The root length was measured from base of the hypocotyl to primary root tip with a precision of ± 0.1 cm. The mean root length was expressed in centimetre (cm).

(iii) Shoot length (cm) (SL): The same ten normal seedlings used for measuring of root length were used for measuring the shoot length. The shoot length was measured from the base of the primary leaf to the base of hypocotyl with a precision of ± 0.1 cm. The mean shoot length was expressed in centimetre (cm).

(iv) Seedling dry weight (mg) (SDW): The same ten normal seedlings used for measuring root and shoot length were placed in a butter paper packet and dried in a hot air oven maintained at 70 ± 2 °C for 24 h. The dried seedlings were removed, weighed in an electronic balance with a precision of ± 1.0 mg. The mean seedling dry weight was expressed in milligram (mg).

(v) Seedling Vigour Index–1 (SVI–1): It was computed as suggested by Abdul-Baki and Anderson (1973) as below. Seedling length include root length and shoot length.

$$\text{SVI}-1 = \text{Germination (\%)} \times \text{Mean seedling length (cm)}$$

(vi) Seedling Vigour Index–2 (SVI–2): It was computed as below.

$$\text{SVI}-2 = \text{Germination (\%)} \times \text{Mean seedling dry weight (mg)}$$

Data analyses

The analysis of variance (ANOVA) for data generated using factorial experiment excluding controls (n = 238) and including controls (n = 246) were analysed by using dplyr, openxlsx, and tidyr packages in R (version 4.4.1). The mean squares (MS) due to salinity, genotypes and salinity x genotypes interaction and their significance were tested by F test. The genetic components of variability were estimated for data under salinity stress using traitstats package in R (version 4.4.1). The genetic components such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) (Burton and Devane 1953), Heritability in broad sense (H) (Hanson et al. 1956) and genetic advance as per cent of mean (GAM) (Johnson et al. 1955) were estimated. The GCV and PCV were categorized as low (0–10%), moderate (10–20%) and high (> 20%) as suggested by Sivasubramanian and Menon (1973). The heritability (H) was classified as low (0–30%), moderate

(30–60%) and high (> 60%) as suggested by Robinson et al. (1949). The GAM was categorized as low (0–10%), moderate (10–20%) and high (> 20%) as suggested by Johnson et al. (1955). The mean comparison between control and salinity was done using Tukey's Studentized Range (HSD) Test ($p = 0.01$). Percent Reduction (PR) between control and salinity stress was computed as below.

$$PR = \left[(\text{Control value} - \text{Salinity stress value}) / \text{Control value}\right] \times 100$$

The violin plot analysis was performed using the readxl and ggplot2 packages in R (version 4.4.1) to visualize trait distributions across treatments. Principal component analysis (PCA) and k-means clustering was done using the FactoMineR and factoextra packages in R (version 4.4.1) to explore multi-trait variation.

Evaluation of promising accessions

Plant material

Preliminarily evaluation of 246 accessions under salinity stress (100 mM) helped in identification of accessions from mini core collection of pearl millet that are highly salinity tolerant at germination and early seedling stage. Among them, top 10 best performing accessions viz., IP14294, IP21312, IP11811, IP3626, IP7422, IP11247, IP8205, IP11930, IP12650, and IP16402 with least reduction for salinity tolerance traits were chosen for further evaluation under control and different salinity levels. This aims to understand the critical and maximum level of salinity that pearl millet as a species can tolerate and also to confirm the level of tolerance among these selected accessions.

Experiment details

To confirm the salinity tolerance and understand the critical level of salinity tolerance in pearl millet among diverse germplasm representing global collection, selected 10 accessions from mini core collection were tested in a factorial completely randomized design comprising two factors. The first factor had five salinity levels; i.e., control (0 mM NaCl or distilled water) and different salinity levels (100, 150, 200, and 250 mM NaCl), while second factor had 10 genotypes. To obtain 100, 150, 200, and 250 mM NaCl solutions, 5.84, 8.76, 11.68, 14.6 g of sodium chloride (NaCl), respectively were dissolved and made up to one litre using distilled water. The measured electrical conductivity (EC) of control (0 mM), 100, 150, 200, and 250 mM NaCl solutions were 0.01, 12.4, 18.1, 24, and 29 dSm^{-1}, respectively; pH of these solutions was 6.82, 6.97, 6.78, 6.66, and 6.76, respectively. Germination papers were soaked in distilled water for control (0 mM NaCl), while in saline solution for different salinity levels (100, 150, 200, and 250 mM NaCl) for evaluation. Each accession was kept for germination as four replicates with 100 seeds per replicate under control and different salinity levels.

Data collection

Data on salinity tolerance related traits such as germination (%), root length (cm), shoot length (cm), seedling dry weight (mg), SVI–1, and SVI–2 were recorded as noted earlier under preliminary evaluation.

Data analyses

The data generated using factorial experiment was analysed by generalized linear model using SAS program version 9.1 (SAS 2004). The ANOVA revealed mean squares due to salinity, genotypes and salinity × genotypes interaction and their significance were tested by F test. Mean comparison between genotypes, control and different salinity levels was tested using Tukey's studentized range (HSD) test ($p = 0.01$). Percent reduction between control and different salinity levels was computed as noted in preliminary evaluation under data analyses section. To explain the genotype x salinity interaction, the multivariate stability analysis was performed based on Additive Main effect Multiplicative Interaction (AMMI) analysis using the 'metan' package in R (version 4.4.1). AMMI biplot exemplify genotype × salinity levels

Table 1 Mean squares for salinity tolerance traits in mini core collection of pearl millet [$] Data analyses excluding controls (n = 238) and including controls (n = 246) presented separately ***, **, * Significant at $p < 0.0001$, $p = 0.01$, $p = 0.05$, respectively.

Source of variation	df	GP	RL	SL	SDW	SVI–1	SVI–2
Excluding controls[$]							
Salinity	1	45,895***	2487.9***	645.5***	41,347.8***	137,641,975***	815,152,660***
Genotype	237	993.93***	99.40***	60.95***	1490.67**	5,551,312***	25,964,838***
Salinity × Genotype	237	40.036	0.00	0.062	7.926	4205	26,017
Error	476	41.991	7.58	3.344	151.693	253,982	1,698,134
Including controls[$]							
Salinity	1	46,399***	2562***	668.4***	42,765***	141,352,098***	840,087,864***
Genotype	245	467.65**	47.24*	33.17**	588.9*	2,773,064**	11,156,986*
Salinity × Genotype	245	4.093	0.019	0.077	6.662	8.064	51,395
Error	492	43.109	7.61	3.363	152.63	257,403	1,717,892

GP germination percentage, *RL* root length (cm), *SL* shoot length (cm), *SDW* seedling dry weight (mg), *SVI-1* seedling vigour index–1, *SVI-2* seedling vigour index–2, *df* degrees of freedom

interaction. The graph generated is based on multi environment evaluation (which-won-where pattern), and tested salinity levels raking (discriminative versus representative).

Results

Preliminary evaluation

Variance components

The ANOVA of factorial experiment for analysis excluding controls (n = 238), revealed highly significant differences ($p < 0.0001$ to $p = 0.01$) for mean squares due to salinity and genotypes for all salinity tolerance related traits such as germination percentage, root length, shoot length, seedling dry weight, SVI–1, and SVI–2, but non-significant interaction for salinity × genotypes interaction (Table 1). This indicated large variation between the genotypes of mini core collection for all six traits related to salinity tolerance at seed germination and early seedling stage. However, the ANOVA for analysis including controls (n = 246), revealed highly significant differences ($p < 0.0001$) for mean squares due to salinity for all six traits, but significant differences ($p = 0.01$ to $p = 0.05$) among genotypes for six-traits and non-significant differences for salinity x genotypes interaction for all traits.

Genetic variability

Enormous genotypic differences were evident among mini core accessions (n = 238) under control (0 mM NaCl) and salinity stress (100 mM NaCl) conditions for all six salinity response traits (Table 2). Wide variation was evident based on range under control (0 mM) and salinity stress (100 mM) conditions for germination % (82.5–97%, 43.5–94.5%), root length (12.1–26 cm, 6.5–21.7 cm), shoot length (7.4–15.6 cm, 5.2–13.6 cm), seedling dry weight (39–107.5 mg, 18–84 mg), SVI–1 (1661–4001, 509–3249), and SVI–2 (3302–10376, 778–7937), respectively. The range was high for all six traits under salinity stress compared to control indicating differential response of mini core accessions to salinity stress. This revealed large genotypic variation in pearl millet mini core collection for salinity stress response at seed germination and early seedling stage. Under control condition, genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were low for germination percentage (GP), moderate for root length (RL), shoot length (SL), seedling dry weight (SDW), and SVI–1, but higher for SVI–2; heritability (H) was high for all six traits, while genetic advance as per cent of mean (GAM) was high for all traits except GP which was low. Under salinity stress, GCV and PCV were moderate for GP, RL, and SL, but higher for SDW, SVI–1, and SVI–2; H and GAM were high for all six traits. Higher values of genetic components

Table 2 Genetic components of variability for salinity tolerance traits under control and salinity stress

Condition*/Trait[$]	Mean	Range	GCV (%)	PCV (%)	H (%)	GAM (%)
Control						
GP	90.5	82.5–97.0	2.86	3.64	61.73	4.63
RL	18.6	12.1–26.0	13.90	14.51	91.73	27.43
SL	11.0	7.40–15.6	15.62	16.71	87.42	30.09
SDW	64.8	39.0–107.5	18.16	19.47	87.02	34.91
SVI–1	2692	1611–4001	17.41	17.87	94.94	34.95
SVI–2	5894	3302–10376	20.97	22.34	88.11	40.55
Salinity stress						
GP	76.7	43.5–94.5	10.70	11.31	89.56	20.86
RL	15.3	6.5–21.7	17.91	18.56	93.10	35.59
SL	9.4	5.2–13.6	18.85	19.65	91.99	37.23
SDW	51.6	18–84	22.69	23.52	93.12	45.11
SVI–1	1931	509–3249	27.34	27.78	96.81	55.41
SVI–2	4044	778–7937	31.69	32.41	95.60	63.82

*Control—0 mM NaCl, Salinity stress—100 mM NaCl

[$]*GP* germination percentage, *RL* root length (cm), *SL* shoot length (cm), *SDW* seedling dry weight (mg), *SVI-1* seedling vigour index–1, *SVI-2* seedling vigour index–2. *GCV* genotypic coefficient of variation (%), *PCV* phenotypic coefficient of variation (%), *H* heritability broad sense (%), *GAM* genetic advance as per cent of mean (%)

observed under salinity stress compared to control indicate scope for selecting salinity tolerant accessions from mini core collection.

Influence of salinity stress on traits

The distribution of data of factorial experiment for all six traits is presented as violin plots (Supplementary Fig. 1). Comparison of performance of genotypes under control and salinity stress indicated drastic changes in distribution for germination percentage (GP). Under control condition less range was observed for GP, while under salinity stress range was very high indicating differential influence of salinity stress on genotypes, wherein sensitivity ranged from highly tolerant to highly sensitive among mini core accessions. For other traits such as root length (RL), shoot length (SL), seedling dry weight (SDW), SVI-1, and SVI-2, the distribution and range was slightly narrowed down under control and salinity stress. The salinity stress drastically reduced SDW, SVI-1, and SVI-2, however, this reduction was moderate for RL and SL but influence was almost similar among all genotypes.

Mean performance and Percent reduction

The mean performance of 238 accessions of pearl millet mini core collection and eight controls for six salinity tolerance related traits under control (0 mM NaCl) and salinity stress (100 mM NaCl) has been summarized in Supplementary Table 2. The percent reduction (PR) under salinity stress compared to control for six salinity tolerance related traits is presented in Table 3. These results are presented below.

(i) Germination percentage (GP)

The mean of all genotypes under control (90.5%) and salinity stress (76.7%) indicated highly significant percent reduction in GP (PR-GP) (15.2%) under salinity stress based on HSD test ($p=0.01$) (Supplementary Table 2). The PR-GP ranged from 2.6% (IP14294, IP21312, IP7422) to 51.9% (IP5964) across all genotypes (Table 3). Among controls, highest PR-GP was noted in susceptible control IP17862 (24.3%) and 24 accessions recorded much higher PR-GP (24.7–51.9%) showing higher susceptibility. Overall, 60 accessions showed less than 10% PR-GP under salinity stress compared to control. Among them, 11 genotypes from mini core collection viz., IP14294, IP21312, IP7422, IP3626, IP11811, IP11930, IP8205, IP11247, IP12650,

Table 3 Percent reduction (PR) under salinity stress (100 mM) compared to control (0 mM) for salinity tolerance traits in pearl millet mini core collection

Entry no	IP #	PR-GP	PR-RL	PR-SL	PR-SDW	PR-SVI-1	PR-SVI-2
1	196	14.3	13.0	12.6	23.0	25.2	33.9
2	277	16.4	16.2	14.8	22.3	29.4	35.3
3	446	10.9	13.8	12.2	18.7	22.8	27.6
4	869	8.1	14.9	10.7	15.4	20.4	22.3
5	952	18.2	17.0	15.8	19.3	31.9	34.0
6	1060	24.7	21.6	20.3	28.0	40.6	45.8
7	1098	11.4	14.2	13.2	21.4	23.6	30.5
8	1405	10.3	15.1	10.2	16.7	22.2	25.2
9	1536	11.1	15.7	11.6	16.5	23.7	25.8
10	1566	9.3	14.1	12.5	21.6	21.5	28.9
11	1625	20.3	14.5	10.7	17.6	30.7	34.4
12	1834	19.7	14.9	10.8	17.1	30.4	33.5
13	1917	16.8	15.7	14.9	17.5	29.6	31.4
14	2083	12.6	13.1	12.6	17.2	23.9	27.5
15	2167	12.0	14.5	10.8	14.1	23.6	24.4
16	2246	27.1	17.1	15.5	16.8	39.1	39.3
17	2322	19.5	21.9	19.9	23.8	36.6	38.8
18	2704	9.7	19.1	14.5	18.8	25.3	26.7
19	2761	8.2	13.3	11.9	21.6	20.0	28.2
20	2789	10.3	14.8	9.8	20.3	21.9	28.5
21	3110	5.3	15.5	11.8	19.1	18.6	23.4
22	3329	10.8	14.7	10.3	16.1	22.5	25.1
23	3432	8.8	13.8	11.9	22.0	20.7	29.0
24	3489	6.4	14.9	12.8	15.7	19.6	21.1
25	3525	20.2	12.2	15.5	17.4	30.8	34.1
26	3626	3.1	17.8	13.2	16.1	18.7	18.7
27	3642	8.8	14.3	13.3	17.6	21.5	24.8
28	3646	13.5	14.4	12.0	16.1	25.2	27.5
29	3706	12.6	14.0	12.4	18.8	24.2	29.1
30	3852	8.1	14.0	11.2	17.3	20.0	24.0
31	4177	8.2	13.2	14.8	17.9	20.9	24.7
32	4291	5.3	14.9	15.2	19.4	19.4	23.8
33	4363	7.6	14.9	12.5	17.1	20.6	23.5
34	4488	21.1	14.3	16.4	20.9	33.0	37.8
35	4747	9.1	16.3	15.8	17.9	23.8	25.4
36	4903	9.9	17.2	12.5	19.1	23.8	27.2
37	4979	10.1	14.7	10.8	14.1	22.0	22.8
38	5085	8.2	13.6	11.9	21.6	20.3	28.0
39	5153	12.8	15.0	11.3	22.8	24.7	32.6
40	5185	13.8	14.5	10.7	14.7	25.1	26.6
41	5261	19.3	14.7	11.1	18.4	30.0	34.0
42	5298	8.3	13.8	11.7	17.4	20.2	24.3
43	5389	7.6	15.3	10.2	16.6	20.0	22.9
44	5407	14.3	14.5	10.8	16.1	25.5	28.1
45	5438	17.1	16.9	15.1	20.2	30.5	33.7
46	5455	13.2	16.2	15.6	16.9	27.0	27.9
47	5581	7.0	13.2	11.7	16.9	18.7	22.6

Table 3 (continued)

Entry no	IP #	PR-GP	PR-RL	PR-SL	PR-SDW	PR-SVI-1	PR-SVI-2
48	5711	9.7	14.9	10.7	17.0	21.8	25.1
49	5719	20.7	17.8	17.9	48.7	34.9	59.3
50	5793	9.8	14.2	11.5	17.9	21.7	25.9
51	5869	32.4	27.8	22.5	20.0	50.0	46.1
52	5957	14.2	13.9	12.5	19.0	25.7	30.5
53	5964	51.9	45.7	30.3	53.8	71.1	77.9
54	6057	11.2	15.8	15.0	17.5	25.0	26.8
55	6113	39.8	41.2	27.6	40.2	61.6	63.9
56	6193	20.9	21.1	16.0	21.8	36.1	38.1
57	6275	11.0	14.9	15.3	17.5	24.5	26.6
58	6278	21.6	17.4	14.9	17.8	34.5	35.5
59	6324	41.2	36.4	23.3	34.9	59.7	61.8
60	6340	33.3	35.4	25.6	33.0	54.7	55.2
61	6517	18.6	15.0	14.9	19.5	30.8	34.3
62	6769	11.9	15.0	15.0	16.4	25.0	26.2
63	6798	20.9	17.8	16.4	18.3	34.4	35.3
64	6805	11.0	18.8	15.5	17.7	26.7	26.9
65	7118	10.9	15.0	14.9	16.5	24.3	25.7
66	7259	18.4	20.8	19.7	19.3	35.1	34.1
67	7358	8.2	14.0	14.0	17.3	21.1	24.1
68	7422	2.6	19.2	12.8	15.4	19.0	17.7
69	7497	17.8	18.9	14.2	16.8	32.0	31.7
70	7537	9.1	13.0	12.1	16.6	20.6	24.2
71	7675	19.4	23.0	19.5	25.0	36.9	39.4
72	7829	27.0	33.5	27.6	40.9	49.8	56.8
73	7846	11.4	13.7	11.7	17.3	22.8	26.8
74	7860	16.6	21.1	18.4	20.2	33.4	33.3
75	7886	23.3	26.2	18.9	34.7	41.5	49.9
76	7915	17.4	20.8	15.7	20.0	33.0	34.1
77	7978	11.6	17.5	21.0	17.8	28.1	27.2
78	8022	9.1	16.4	15.8	17.9	23.8	25.4
79	8051	18.5	19.3	14.9	18.6	32.9	33.5
80	8074	9.9	13.6	11.7	17.3	21.5	25.4
81	8155	14.8	15.0	12.1	16.9	26.6	29.1
82	8205	3.7	17.8	14.1	15.0	19.4	18.1
83	8220	8.1	13.9	11.1	21.3	19.9	27.8
84	8245	8.9	14.4	14.1	17.1	21.9	24.4
85	8276	26.3	22.6	19.8	27.9	42.1	46.9
86	8288	12.1	16.5	15.8	19.5	26.4	29.2
87	8350	10.4	14.0	13.3	17.4	22.8	26.1
88	8418	10.7	13.5	11.8	22.1	22.1	30.4
89	8472	8.9	14.2	13.3	16.9	21.5	24.3
90	8529	15.9	17.5	15.2	19.5	29.9	32.5
91	8540	16.6	18.4	16.8	17.7	31.5	31.4
92	8562	35.5	37.5	25.8	33.7	56.9	57.3
93	8672	9.9	17.5	15.2	18.6	24.9	26.6
94	8707	12.8	14.4	14.3	47.3	25.3	54.1

Table 3 (continued)

Entry no	IP #	PR-GP	PR-RL	PR-SL	PR-SDW	PR-SVI-1	PR-SVI-2
95	8818	9.8	17.1	15.5	16.8	24.6	24.9
96	8863	8.5	14.6	15.2	22.4	22.1	28.9
97	8913	21.1	21.3	27.0	23.8	39.5	40.0
98	9000	14.6	17.8	16.0	18.3	29.2	30.1
99	9026	47.3	44.0	29.6	45.6	67.6	71.4
100	9157	26.7	25.9	23.0	38.1	44.9	54.5
101	9198	10.9	14.2	14.7	18.0	23.6	26.9
102	9449	13.8	13.9	12.1	22.1	25.1	32.8
103	9464	11.0	20.7	15.0	18.8	27.6	27.7
104	9492	11.6	20.4	15.5	17.8	28.0	27.4
105	9527	14.9	14.8	10.7	18.5	26.1	30.7
106	9596	23.7	17.0	15.0	16.8	36.1	36.7
107	9617	12.7	13.9	12.2	38.2	24.3	46.1
108	9645	36.8	39.4	21.7	39.8	57.6	62.3
109	9692	11.8	13.5	14.0	29.4	23.9	37.8
110	9795	20.1	22.7	19.7	27.2	37.4	41.8
111	9813	7.7	13.9	14.6	19.0	20.7	25.3
112	9934	14.1	20.4	17.6	18.9	30.8	30.2
113	10,085	15.5	17.7	15.3	18.8	29.7	31.3
114	10,151	7.7	14.0	14.1	17.6	20.7	23.9
115	10,263	19.8	20.9	16.5	22.0	35.3	37.5
116	10,371	29.6	34.6	19.8	38.0	50.0	56.5
117	10,399	39.8	40.5	27.7	43.8	61.3	66.2
118	10,437	22.5	22.5	20.0	30.4	39.3	46.0
119	10,467	6.6	13.9	13.8	19.0	19.5	24.1
120	10,601	10.4	13.8	12.5	18.4	22.4	26.9
121	10,632	19.3	20.8	21.1	23.9	36.0	38.7
122	10,665	8.2	16.1	11.9	18.9	21.5	25.6
123	10,713	17.8	20.7	19.5	21.5	34.5	35.4
124	10,729	10.8	16.0	11.1	16.3	23.4	25.3
125	10,761	19.8	16.2	17.0	17.1	33.0	33.4
126	10,925	24.9	29.7	25.3	36.6	45.9	52.2
127	10,953	8.5	15.6	12.4	20.0	21.6	26.8
128	11,010	19.4	14.9	15.3	18.8	31.4	34.4
129	11,036	18.6	14.8	15.1	16.8	30.8	32.4
130	11,044	27.5	21.0	15.9	18.2	41.2	40.5
131	11,113	11.6	16.6	12.5	20.3	25.0	29.6
132	11,247	3.7	21.3	17.0	17.5	22.6	20.6
133	11,268	8.1	15.0	10.7	19.0	20.4	25.6
134	11,405	8.2	13.4	16.2	24.2	21.5	30.8
135	11,428	17.5	15.5	15.6	16.0	30.3	30.7
136	11,546	19.6	14.3	12.3	18.0	30.4	34.0
137	11,666	29.7	20.8	19.9	21.5	44.1	45.0
138	11,799	18.6	17.6	17.9	21.4	33.0	36.1
139	11,811	3.1	19.2	15.8	20.8	20.5	23.4
140	11,930	3.2	17.8	12.1	17.7	18.3	20.3
141	11,943	30.6	27.3	16.6	36.0	46.9	55.9

Table 3 (continued)

Entry no	IP #	PR-GP	PR-RL	PR-SL	PR-SDW	PR-SVI-1	PR-SVI-2
142	12,221	22.2	20.8	18.5	22.9	37.5	40.1
143	12,364	17.3	15.1	15.4	16.8	29.9	31.2
144	12,374	18.8	16.3	14.9	16.7	31.5	32.3
145	12,418	12.8	16.2	17.6	17.5	27.6	28.2
146	12,431	10.7	16.5	15.6	18.0	25.2	26.8
147	12,498	13.8	25.6	14.8	17.9	32.5	29.1
148	12,533	10.2	13.9	11.3	19.0	21.8	27.2
149	12,546	13.6	14.9	19.1	16.4	28.0	27.8
150	12,644	11.6	20.5	15.2	17.8	28.0	27.3
151	12,650	3.7	17.1	15.7	19.6	19.6	22.6
152	12,669	15.3	20.5	20.8	18.9	32.7	31.2
153	12,731	19.1	21.2	19.9	24.3	35.8	38.8
154	12,805	11.8	13.7	12.0	23.0	23.3	32.1
155	12,839	12.6	16.5	14.7	17.5	26.4	27.9
156	12,993	11.0	17.1	15.4	18.5	25.7	27.3
157	13,261	8.6	16.1	14.9	16.7	22.9	23.9
158	13,345	14.5	18.0	16.1	18.3	29.3	30.3
159	13,387	9.7	15.2	14.2	16.6	23.1	24.6
160	13,523	9.7	14.1	12.3	16.8	21.9	24.8
161	13,623	8.3	14.4	13.0	20.8	21.1	27.4
162	13,624	12.8	15.0	14.8	16.4	25.9	27.0
163	13,636	19.7	16.7	20.4	18.8	34.1	34.9
164	13,760	8.2	14.2	12.8	20.1	20.8	26.7
165	13,875	16.0	14.5	13.0	17.6	27.6	30.7
166	13,991	17.6	15.9	16.7	16.8	30.9	31.4
167	14,294	2.6	16.8	16.3	17.6	18.8	19.8
168	14,428	8.1	13.8	14.9	16.3	21.2	23.2
169	14,522	19.2	17.4	15.3	18.8	32.6	34.5
170	14,537	19.4	20.7	15.2	23.9	34.6	38.8
171	14,542	9.7	14.3	14.9	17.6	22.8	25.5
172	14,599	12.4	13.7	12.5	21.3	24.1	31.2
173	14,753	23.7	21.3	19.9	21.5	39.6	40.0
174	14,776	21.3	22.8	19.6	23.1	38.3	39.4
175	14,787	11.3	15.0	10.8	17.0	23.2	26.4
176	15,095	9.1	12.7	16.3	16.0	21.9	23.7
177	15,119	11.3	16.2	15.6	22.8	25.5	31.5
178	15,256	13.0	14.0	12.8	19.4	24.8	29.8
179	15,273	20.9	12.8	12.2	18.1	30.8	35.2
180	15,372	13.6	16.0	15.5	16.1	27.3	27.5
181	15,448	18.8	23.2	21.2	31.1	37.0	44.1
182	15,556	32.8	27.2	21.9	35.0	49.8	56.2
183	15,829	5.9	15.0	10.4	16.1	18.3	21.1
184	15,836	9.9	13.7	11.9	22.0	21.6	29.5
185	15,953	19.3	22.8	19.1	24.0	36.6	38.9
186	16,402	4.2	19.1	16.1	17.3	21.3	20.8
187	16,489	27.1	18.9	14.1	17.7	39.7	40.2
188	16,540	10.2	14.4	10.7	17.6	21.8	26.0

Table 3 (continued)

Entry no	IP #	PR-GP	PR-RL	PR-SL	PR-SDW	PR-SVI-1	PR-SVI-2
189	16,754	13.4	13.9	11.3	15.9	24.5	27.2
190	16,863	4.8	14.9	11.7	22.3	17.9	26.1
191	17,396	13.5	14.7	11.0	16.4	25.1	27.8
192	17,465	23.1	20.8	13.8	24.1	37.1	41.5
193	17,490	14.6	19.1	15.4	41.6	29.7	50.1
194	17,532	10.7	13.1	12.2	20.0	22.1	28.6
195	17,775	10.3	16.0	14.9	16.7	24.3	25.3
196	18,040	9.2	17.1	11.2	17.1	22.7	24.8
197	18,353	20.9	16.4	15.7	18.0	33.7	35.1
198	18,545	21.8	21.6	19.9	29.5	38.2	45.0
199	18,579	28.2	26.1	21.8	29.4	45.9	49.4
200	18,657	12.6	17.9	14.9	16.5	27.2	27.0
201	18,824	12.6	17.0	16.4	23.3	27.3	33.1
202	18,854	18.4	21.4	19.1	29.5	35.2	42.7
203	18,900	22.5	21.0	19.6	28.0	38.3	44.0
204	19,072	10.6	16.0	11.9	16.6	23.5	25.4
205	19,141	11.9	14.8	15.2	20.5	25.1	30.0
206	19,305	16.1	14.1	13.4	16.9	27.8	30.2
207	19,415	22.8	26.8	25.0	36.2	43.0	50.7
208	19,425	12.0	19.0	12.5	18.7	26.5	28.6
209	19,448	21.7	14.9	15.2	16.7	33.5	34.8
210	19,629	12.1	13.1	12.6	17.8	23.6	27.7
211	19,722	11.9	13.9	13.5	16.9	24.0	26.7
212	19,816	19.8	16.3	15.1	21.3	32.4	37.2
213	19,851	23.3	18.0	15.4	17.5	36.4	36.8
214	19,913	12.1	17.1	17.8	18.5	27.4	28.3
215	19,964	24.1	21.3	14.4	22.4	38.4	41.2
216	20,249	12.3	19.9	15.0	19.8	28.2	29.8
217	20,274	19.3	16.2	15.6	17.9	32.2	33.8
218	20,409	21.9	20.7	18.9	28.2	37.6	44.0
219	20,576	17.5	19.4	15.1	18.9	32.2	33.0
220	20,577	16.6	16.4	15.1	18.0	29.8	31.5
221	20,611	22.0	15.9	15.5	21.8	34.3	38.8
222	20,715	21.5	14.4	16.1	17.6	33.3	35.4
223	20,768	24.0	21.0	16.0	19.1	38.5	38.6
224	20,929	14.3	17.0	15.5	16.8	28.4	28.8
225	20,955	15.5	15.7	14.9	17.5	28.6	30.5
226	21,066	16.9	21.1	15.4	18.9	32.7	32.7
227	21,093	22.0	16.1	15.1	17.5	34.3	35.7
228	21,127	14.5	16.3	12.1	19.8	27.2	31.5
229	21,187	15.1	17.9	14.9	16.8	29.3	29.5
230	21,201	20.5	16.3	14.6	17.4	32.9	34.3
231	21,244	23.8	17.1	15.5	18.5	36.4	37.9
232	21,283	8.1	15.0	10.9	16.7	20.5	23.4
233	21,312	2.6	19.1	14.5	24.7	19.6	26.6
234	21,438	27.6	23.7	15.8	19.1	42.6	41.3
235	21,503	10.2	13.7	15.1	17.3	23.0	25.9

Table 3 (continued)

Entry no	IP #	PR-GP	PR-RL	PR-SL	PR-SDW	PR-SVI-1	PR-SVI-2
236	22,269	10.9	14.4	11.3	17.1	22.7	26.2
237	22,449	27.3	25.5	21.2	30.1	44.8	49.3
238	22,489	15.8	15.6	15.5	17.7	28.9	30.7
	Mean	15.4	17.9	15.4	21.0	29.5	32.9
	Minimum	2.6	12.2	9.8	14.1	17.9	17.7
	Maximum	51.9	45.7	30.3	53.8	71.1	77.9
	Mini core controls						
239	3616	3.2	14.0	12.3	18.7	16.1	21.3
240	3757	11.6	14.9	10.9	16.8	23.5	26.5
241	6098	14.7	16.3	16.2	19.0	28.6	30.9
242	6105	7.0	13.0	11.8	19.4	18.6	25.0
	Salinity controls						
243	10,876 (T)	4.8	14.4	13.3	17.6	18.1	21.5
244	10,878 (T)	10.3	14.9	14.8	18.3	23.6	26.6
245	17,862 (S)	24.3	20.6	19.5	21.3	39.6	40.4
246	18,406 (T)	3.8	15.0	14.7	17.0	18.1	20.1

PR-GP—percent reduction in GP, PR-RL percent reduction in RL, PR-SL—percent reduction in SL, PR-SDW—percent reduction in SDW, PR-SVI-1– percent reduction in SVI–1, PR-SVI-2—percent reduction in SVI–2

IP16402, IP16863 and three salinity tolerant controls viz., IP3616, IP18406, IP10876 recorded less than 5% PR-GP under salinity stress compared to control. Based on higher mean value under salinity stress, the best 10 promising sources from the mini core collection with higher germination (94.5–91%) were IP14294, IP21312, IP7422, IP3626, IP11811, IP11930, IP8205, IP11247, IP12650, and IP16402 compared to the best salinity tolerant control IP3616 (90.5%).

(ii) Root length (cm) (RL)

Based on mean of all genotypes under control (18.6 cm) and salinity stress (15.3 cm), highly significant percent reduction in root length (PR-RL) (17.7%) was noted under salinity stress based on HSD test ($p=0.01$) (Supplementary Table 2). The PR-RL ranged from 12.2% (IP3525) to 45.7% (IP5964) across all genotypes (Table 3). Compared to susceptible control IP17862 (20.6% reduction), 54 accessions showed much higher PR-RL (20.7–45.7%) showing their higher susceptibility than control. None of the accessions from mini core showed less than 10% PR-RL under salinity stress compared to control. However, the best performing five genotypes from mini core collection viz., IP3525, IP15095, IP15273, IP7537, IP196, and one salinity tolerant control IP6105 recorded less than 13% PR-RL under salinity stress compared to control. Based on the higher mean value under salinity stress, best 10 promising sources from mini core collection with higher root length (21.7–19.6 cm) under salinity stress were IP14294, IP21312, IP11811, IP3626, IP7422, IP8205, IP11930, IP12650, IP4903, and IP11113 compared to the best salinity tolerant control IP6098 (19.6 cm).

(iii) Shoot length (cm) (SL)

The mean of all genotypes under control (11.1 cm) and salinity stress (9.4 cm) indicated highly significant percent reduction in shoot length (PR-SL) (15.3%) under salinity stress based on HSD test ($p=0.01$) (Supplementary Table 2). The PR-SL ranged from 9.8% (IP2789) to 30.3% (IP5964) (Table 3). Compared to susceptible control IP17862 (19.5% reduction), 35 accessions recorded much more PR-SL (19.6–30.3%) showing higher susceptibility. Only one accession from mini core; i.e., IP2789 showed less than 10% PR-SL under salinity stress compared to control. However, 19 genotypes from mini core collection and one salinity tolerant control (IP3757) recorded less than 11% PR-SL under salinity stress compared to control. Based on higher

mean value under salinity stress, the best 10 promising sources from the mini core collection with higher shoot length (13.6–12.6 cm) under salinity stress were IP7422, IP11930, IP3626, IP8205, IP11247, IP11811, IP14294, IP12650, IP16402, and IP21312 compared to the best salinity tolerant control IP6098 (12 cm).

(iv) Seedling dry weight (mg) (SDW)

Based on mean of all genotypes under control (64.8 mg) and salinity stress (51.6 mg), highly significant percent reduction in seedling dry weight (PR-SDW) (20.4%) was observed under salinity stress as indicated by HSD test ($p=0.01$) (Supplementary Table 2). The PR-SDW ranged from 14.1% (IP4979, IP2167) to 53.8% (IP5964) (Table 3). Compared to susceptible control IP17862 (21.3% reduction), 68 accessions showed much higher PR-SDW (21.4–51.9%) showing their higher susceptibility. However, 10 genotypes from mini core collection viz., IP4979, IP2167, IP5185, IP8205, IP7422, IP869, IP3489, IP16754, IP11428, and IP15095 recorded less than 16% PR-SDW under salinity stress compared to control. Based on higher mean value under salinity stress, the best 11 promising sources from the mini core collection with higher seedling dry weight (84–74 mg) under salinity stress were IP14294, IP7422, IP11811, IP21312, IP11247, IP8205, IP3626, IP16402, IP11930, IP4903, and IP12650 compared to the best salinity tolerant control IP6098 (72.5 mg).

(xxii) Seedling vigour index–1 (SVI–1)

The mean of all genotypes under control (SVI–1: 2692) and salinity stress (SVI–1: 1931) indicated highly significant percent reduction in seedling vigour index–I (PR-SVI-1) (28.3%) under salinity stress based on HSD test ($p=0.01$) (Supplementary Table 2). The PR-SVI-1 ranged from 17.9% (IP16863) to 71.1% (IP5964) (Table 3). Compared to susceptible control IP17862 (39.6% reduction), 25 accessions showed much more PR-SVI-1 (39.7–71.1%) showing their higher susceptibility. Eighteen accessions of mini core collection and four tolerant controls (IP3616, IP10876, IP18406, IP6105) recorded less than 20% PR-SVI-1 under salinity stress compared to control. Based on higher mean value under salinity stress, the best 10 promising sources from the mini core collection with higher SVI–1 (3249–2915) under salinity stress were IP14294, IP3626, IP7422, IP21312, IP11811, IP11930, IP8205, IP12650, IP11247, and IP16402 compared to the best salinity tolerant control IP6105 (SVI–1: 2596).

(vi) Seedling vigour index–2 (SVI–2)

Mean of all genotypes under control (SVI–2: 5894) and salinity stress (SVI–2: 4044) showed drastic percent reduction in SVI–2 (PR-SVI-2) (31.4%) under salinity stress based on HSD test ($p=0.01$) (Supplementary Table 2). The PR-SVI-2 ranged from 17.7% (IP7422) to 77.9% (IP5964) (Table 3). Compared to susceptible control IP17862 (40.4% reduction), 37 accessions recorded much more PR-SVI-2 (40.5–77.9%) showing higher susceptibility than control. Nineteen accessions of mini core collection and three tolerant controls (IP18406, IP3616, IP10876) recorded less than 23.5% PR-SVI-2 under salinity stress compared to control. Based on higher mean value under salinity stress, the best 10 promising sources from the mini core collection with higher SVI–2 (7937–6772) under salinity stress were IP14294, IP7422, IP11811, IP21312, IP11247, IP8205, IP3626, IP11930, IP16402, and IP12650 compared to the best salinity tolerant control IP6098 (SVI–2: 5905).

Principal component analysis and clustering

The principal component analysis (PCA) revealed six principal components (PC), wherein two major PC's (PC1: 5.01 Eigen value, 83.6% variance; PC2: 0.46 Eigen value, 7.7% variance) explaining over 91% of variance. The PCA biplot and k-means clustering grouped 238 accessions of mini core into six clusters with 33, 10, 53, 64, 48 and 30 accessions, respectively (Fig. 1). The highly salinity tolerant germplasm were grouped in cluster 1 (33 accessions; controls IP3616, IP10876, IP18406), while salinity tolerant in cluster 4 (64 accessions; controls IP3757, IP6105) and cluster 5 (48 accessions; controls IP6098, IP10878), moderately tolerant in cluster 3 (53 accessions), susceptible in cluster 6 (30 accessions, control IP17862), and highly susceptible in cluster 2 (10 accessions) (Supplementary Table 3). The accessions grouped under cluster 1 (33 accessions; controls IP3616, IP10876, IP18406) showed least percent reduction in salinity stress compared to control for mean and range of PR-GP (6.4%, 2.6–10.2%), PR-RL (15.6%, 12.7–21.3%), PR-SL (14.2%, 10.4–17.0%), PR-SDW (18.1%, 15.0–24.7%), PR-SVI-1 (20.5%,

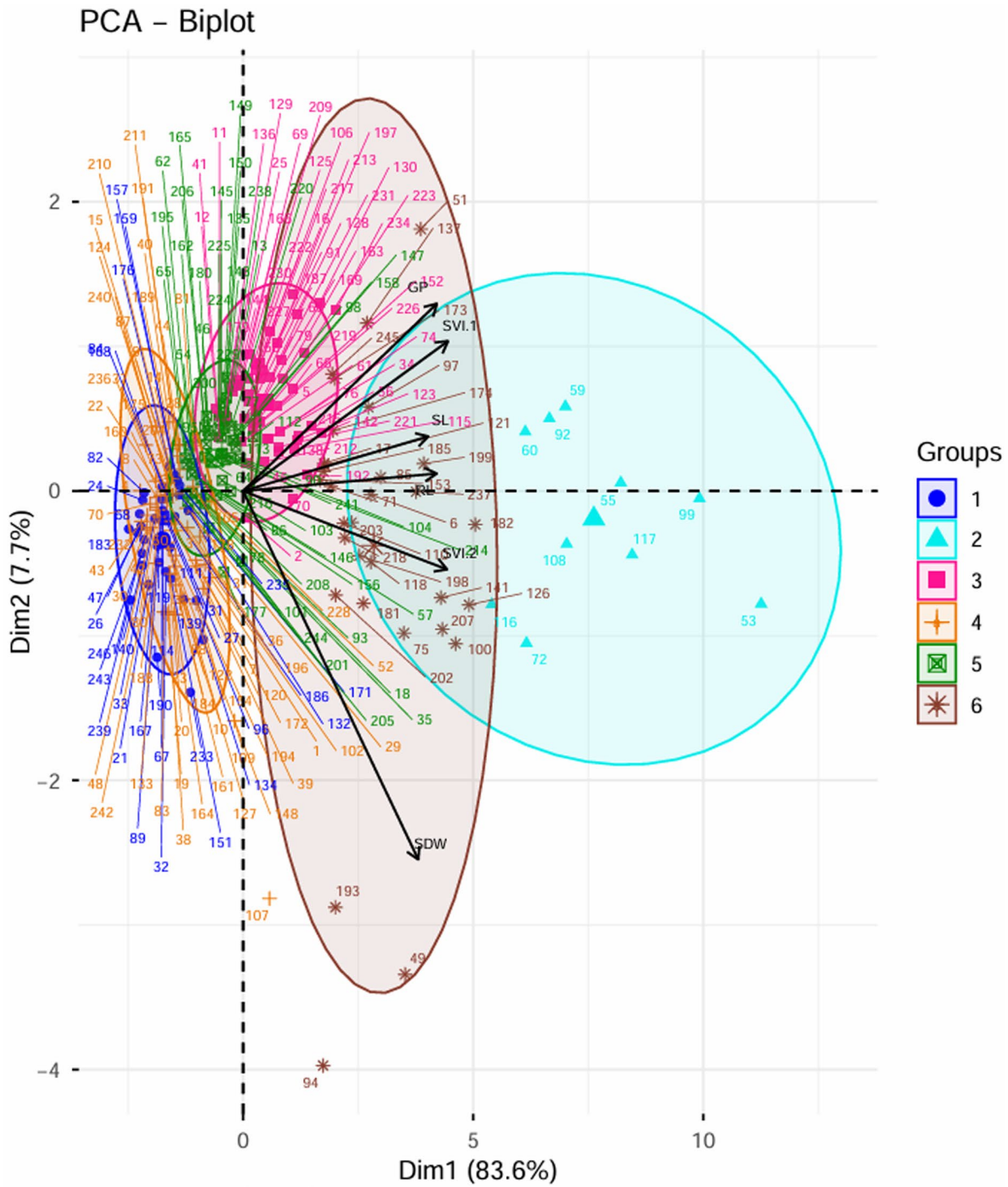

Fig. 1 Principal component analysis (PCA) biplot and k-means clustering for salinity tolerance traits in pearl millet mini core collection

17.9–23.1%), and PR-SVI-2 (23.4%, 17.7–30.8%) (Supplementary Table 3). Accessions that were separately grouped under cluster 1 and showing least reduction under salinity stress across six traits; i.e., the best 10 promising sources from the mini core collection viz., IP14294, IP21312, IP3626, IP11811, IP7422, IP11930, IP8205, IP11247, IP16402, and IP12650 were chosen for detailed evaluation. They were further tested under control and different salinity levels to confirm their salinity tolerance and also to find out the critical and maximum level of salinity they can tolerate for pearl millet as a species.

Evaluation of identified promising accessions

Preliminary evaluation of the mini core collection led to identification of best 10 promising sources viz., IP14294, IP21312, IP3626, IP11811, IP7422, IP11930, IP8205, IP11247, IP16402, and IP12650. To confirm the salinity tolerance and understand the critical level of tolerance in diverse germplasm of pearl millet, these 10 accessions were evaluated under different salinity levels [control (0 mM), 100, 150, 200, and 250 mM NaCl].

Variance components

The ANOVA of factorial experiment revealed highly significant differences ($p < 0.0001$) for mean squares due to salinity, genotypes, and salinity × genotypes interaction for all salinity tolerance related traits such as germination, root length, shoot length, seedling dry weight, SVI–1, and SVI–2 (Supplementary Table 4). This indicated large variation between the 10 selected genotypes from mini core collection for all six traits related to salinity tolerance at germination and early seedling stage.

Genetic variability

Enormous genotypic differences were evident under different salinity levels (control, 100, 150, 200, and 250 mM NaCl) for all the six-salinity tolerance related traits (Table 4). Considering range across different salinity levels, germination was highest in control (92–96%) followed by 100 mM (89.8–93%), but started declining sharply as the salinity level was increasing; i.e., 150 mM (61.5–84.5%), 200 mM (16–59.3%), and 250 mM (7.5–35%). Highest root length was observed in control (22.7–25.2 cm) followed by 100 mM (18.8–21.2 cm), and it started decreasing sharply as the salinity levels were increased viz., 150 mM (8.6–16.7 cm), 200 mM (7.1–13.2 cm), and 250 mM (5.1–10.2 cm). Similarly, shoot length was highest in control (14.3–15.5 cm) followed by 100 mM (11.7–13 cm), however, declined sharply as the salinity levels increased viz., 150 mM (5.3–8.7 cm), 200 mM (4.4–6.4 cm), and 250 mM (2.2–5.9 cm). Highest seedling dry weight was noted in control (87.5–101.8 mg) followed by 100 mM (71.5–82 mg), and this declined sharply as the salinity levels increased viz., 150 mM (47.5–64.3 mg), 200 mM (41–61.3 mg), and 250 mM (29.8–56.3 mg). The SVI–1 was highest in control (SVI–1: 3399–3900) followed by 100 mM (2728–3179), but declined sharply as the salinity level was increased; i.e., 150 mM (854–2142), 200 mM (184–1159), and 250 mM (55–562). Similarly, higher SVI–2 was noted in control (SVI–2: 8046–9768) followed by 100 mM (6419–7630), but it decreased evidently as the salinity levels increased viz., 150 mM (2919–5428), 200 mM (670–3631), and 250 mM (219–1969). These results indicated large variation among selected 10 accessions for salinity response at germination and early seedling stage under different salinity levels.

Genetic variability was assessed among the best 10 promising sources (Supplementary Table 5). The GCV and PCV were higher for SVI–2, but moderate for germination, shoot length, root length, seedling dry weight, and SVI–1. Further, difference between GCV and PCV was minimum for all six traits indicating greater influence of genotype, but less influence of environment on the expression of these traits. The heritability (H) was high for root length, shoot length, seedling dry weight, SVI–1 and SVI–2, but comparatively lesser for germination. The genetic advance as per cent of mean (GAM) was highest for SVI–2 and high for root length, shoot length, seedling dry weight, and SVI–1.

Promising sources across traits

Consideration across six traits related to salinity tolerance suggested that among different salinity levels, the promising accessions could tolerate 100 mM comfortably, but there will be drastic reduction from 150 mM onwards and their intensity would differ

Table 4 Response of selected mini core accessions under different salinity levels

Trait	Salinity levels	Genotypes (IP #)										Mean [$]
		14,294	21,312	11,811	3626	7422	11,247	8205	11,930	12,650	16,402	
GP	Control	96.0	95.5	95.0	94.5	94.0	93.8	93.5	93.5	93.0	92.0	94.1 [a]
	100 mM	93.0	92.0	92.0	91.8	91.5	91.5	91.3	90.5	90.0	89.8	91.3 [b]
	150 mM	84.5	81.3	76.3	75.0	72.3	71.5	69.5	65.5	64.0	61.5	72.1 [c]
	200 mM	59.3	57.8	43.0	38.5	37.8	32.5	29.0	26.0	19.0	16.0	35.9 [d]
	250 mM	35.0	27.5	22.0	21.5	21.3	14.8	13.5	12.5	7.8	7.5	18.3 [e]
	Mean [#]	73.6 [a]	70.8 [b]	65.7 [c]	64.3 [c]	63.4 [cd]	60.8 [de]	59.4 [ef]	57.6 [f]	54.8 [g]	53.4 [g]	–
		S	G	S×G								
	SEm ±	0.36	0.51	1.13								
	LSD	1.30	1.84	4.12								
RL	Control	25.2	24.8	24.5	24.3	24.0	23.6	23.1	23.0	22.8	22.7	23.8 [a]
	100 mM	21.2	20.8	20.3	20.0	20.0	19.4	19.1	19.0	18.9	18.8	19.7 [b]
	150 mM	16.7	14.9	14.8	14.6	14.3	13.5	13.2	13.2	12.8	8.6	13.6 [c]
	200 mM	13.2	11.6	11.6	11.4	11.3	11.3	10.8	9.8	9.2	7.1	10.7 [d]
	250 mM	10.2	10.2	10.2	10.1	9.8	8.8	8.1	7.3	6.3	5.1	8.6 [e]
	Mean [#]	17.3 [a]	16.4 [b]	16.3 [b]	16.1 [b]	15.9 [bc]	15.3 [cd]	14.8 [de]	14.5 [ef]	14.0 [f]	12.4 [g]	–
		S	G	S×G								
	SEm ±	0.09	0.13	0.29								
	LSD	0.33	0.46	1.06								
SL	Control	15.5	15.4	15.1	14.8	14.7	14.7	14.6	14.4	14.3	14.3	14.8 [a]
	100 mM	13.0	12.8	12.7	12.3	12.1	12.1	12.1	12.1	12.0	11.7	12.3 [b]
	150 mM	8.7	8.5	8.2	7.2	7.1	7.1	6.7	6.6	5.9	5.3	7.1 [c]
	200 mM	6.4	6.2	6.1	6.0	5.8	5.5	5.2	5.2	5.0	4.4	5.6 [d]
	250 mM	5.9	4.9	4.4	3.9	3.5	3.0	2.8	2.8	2.4	2.2	3.6 [e]
	Mean [#]	9.9 [a]	9.6 [ab]	9.3 [bc]	8.8 [cd]	8.6 [de]	8.5 [de]	8.3 [ef]	8.2 [ef]	7.9 [fg]	7.6 [g]	–
		S	G	S×G								
	SEm ±	0.07	0.09	0.21								
	LSD	0.23	0.33	0.77								
SDW	Control	101.8	101.3	100.5	100.0	97.3	92.5	92.3	89.0	88.0	87.5	95.0 [a]
	100 mM	82.0	81.0	77.0	76.5	74.3	74.0	73.3	73.0	72.0	71.5	75.5 [b]
	150 mM	64.3	60.0	59.0	56.3	54.8	53.5	50.5	50.5	48.8	47.5	54.5 [c]
	200 mM	61.3	54.8	52.0	50.8	48.0	46.8	45.8	43.8	41.0	41.5	48.6 [d]
	250 mM	56.3	54.3	49.0	46.3	42.3	39.8	37.5	34.5	32.8	29.8	42.2 [e]
	Mean [#]	73.1 [a]	70.3 [ab]	67.5 [bc]	66.0 [cd]	63.3 [de]	61.3 [ef]	59.9 [e–g]	58.2 [f–h]	56.5 [gh]	55.6 [h]	–
		S	G	S×G								
	SEm ±	0.52	0.73	1.64								
	LSD	1.89	2.67	5.97								
SVI–1	Control	3900	3837	3759	3695	3635	3588	3524	3494	3448	3399	3628 [a]
	100 mM	3179	3087	3036	2967	2933	2883	2844	2816	2780	2728	2925 [b]
	150 mM	2142	1895	1751	1632	1542	1466	1381	1292	1194	854	1515 [c]
	200 mM	1159	1025	759	668	643	542	463	390	270	184	610 [d]
	250 mM	562	413	320	302	283	175	146	125	67	55	245 [e]
	Mean [#]	2188 [a]	2052 [b]	1925 [c]	1853 [cd]	1807 [de]	1731 [ef]	1672 [fg]	1623 [gh]	1552 [h]	1444 [i]	–
		S	G	S×G								
	SEm ±	11.7	16.5	36.9								
	LSD	42.5	60.3	134.5								

Table 4 (continued)

Trait	Salinity levels	Genotypes (IP #)										Mean $
		14,294	21,312	11,811	3626	7422	11,247	8205	11,930	12,650	16,402	
SVI–2	Control	9768	9673	9547	9449	9144	8668	8626	8324	8183	8046	8943 [a]
	100 mM	7630	7456	7084	7016	6793	6771	6683	6604	6481	6419	6894 [b]
	150 mM	5428	4877	4499	4216	3958	3827	3513	3310	3117	2919	3966 [c]
	200 mM	3631	3170	2236	1963	1811	1519	1331	1137	782	670	1825 [d]
	250 mM	1969	1485	1078	994	892	584	503	430	251	219	840 [e]
	Mean #	5685 [a]	5332 [b]	4889 [c]	4728 [cd]	4519 [de]	4274 [ef]	4131 [fg]	3961 [gh]	3763 [hi]	3655 [i]	–
		S	G	S×G								
	SEm ±	40	56.6	126.6								
	LSD	145.8	206.3	461.4								

GP—Germination (%), RL—Root length (cm), SL—Shoot length (cm), SDW—Seedling dry weight (mg), SVI-1—Seedling vigour index–1, SVI-2—Seedling vigour index–2

Mean #—Tukey's studentized range (HSD) test ($\alpha = 0.01$) for genotypes; Mean $—Tukey's studentized range (HSD) test ($\alpha = 0.01$) for salinity levels;

SEm—Standard error of means for salinity levels (S), genotypes (G), salinity × genotype (S × G) interaction component

LSD—Least significant difference ($p = 0.01$) for salinity levels, genotypes, salinity × genotype interaction component

based on the genotype (Table 4). Among different salinity levels, only two highly salinity tolerant accessions IP14294 and IP21312 recorded more than 80% germination at 150 mM suggesting it to be the critical limit of salinity that pearl millet can tolerate. Salinity level anything beyond 150 mM would not be tolerable in pearl millet.

AMMI analysis

Additive main effects and multiplicative interaction (AMMI) analysis was done to understand interactions between promising genotypes and different salinity levels (Fig. 2). For GP, interaction between genotypes and salinity levels was least for 150 mM, but highest for 200 mM and 250 mM followed by 100 mM and control; IP14294 performed better at 250 mM, while IP21312 at 200 mM. For RL, interaction was least for 200 mM, but highest for 250 mM followed by 150 mM and 100 mM; IP14294 performed better at 150 mM, while IP16402 at 100 mM. For SL, interaction was highest for 150 mM and 250 mM followed by 100 mM, control and 200 mM; IP14294 and IP21312 performed better at 250 mM, while IP11247 at 200 mM. For SDW, interaction was least for 150 mM, but highest for 250 mM and control followed by100 mM and 200 mM; IP14294 performed better at 250 mM, IP21312 at 200 mM, IP16402 at 100 mM. For SVI-1, interaction was least for control and 250 mM, but highest for 200 mM and 150 mM followed by 100 mM; IP21312 and IP14294 performed better at 200 mM, while IP16402 at 250 mM and IP11811 at 150 mM. For SVI-2, interaction was least for 150 mM and 250 mM, but highest for control followed by 200 mM and 100 mM; IP14294 and IP21312 performed better at 200 mM, while IP16402 at 100 mM. The most promising genotypes were IP14294 and IP21312, which could tolerate highest level of salinity among tested genotypes and their critical level of salinity tolerance is 150 mM. Among salinity levels, 250 mM showed higher interaction with genotypes for traits such as GP, RL, SL, and SDW, while 200 mM showed higher interaction for GP, SVI-1, SVI-2, SL and SDW.

Discussion

Soil salinity is an important abiotic stress for crop cultivation in arid and semi-arid regions of Africa and Asia wherein high surface evaporation, low precipitation, and faulty irrigation methods cause higher levels of soluble salts which make the ground water unavailable to plants (Goyal 2004). Recent study on unreasonable water resource utilization in river basin of arid region indicated that although

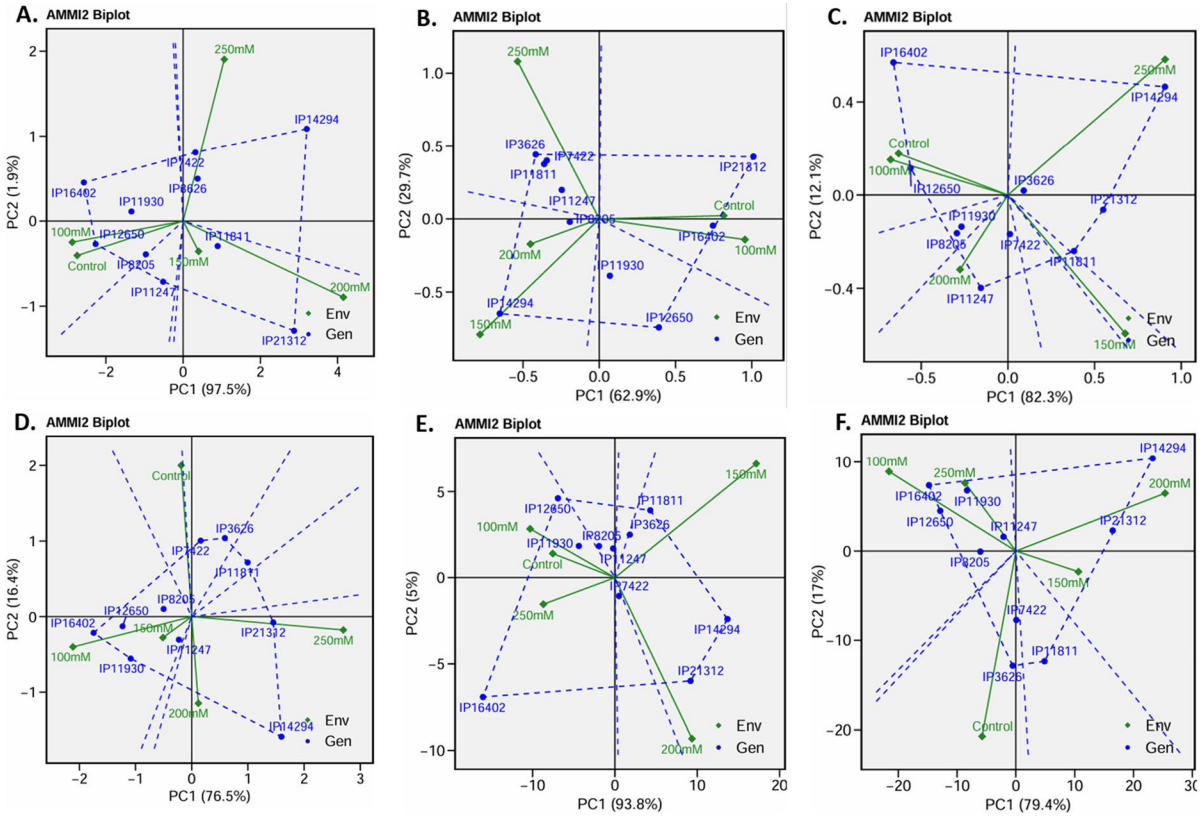

Fig.2 Addiitve main effects multiplicative interaction (AMMI) analysis biplots depicting interactions between promising genotypes and salinity levels for, **A**. Germination percentage (GP), **B**. Root length (RL), **C**. Shoot length (SL), **D**. Seedling dry weight (SDW), **E**. Seedling vigour index-1 (SVI-1), **F**. Seedling vigour index-2 (SVI-2)

salinization area remained stable, the degree of salinization was intensifying due to groundwater evaporation near reservoirs, agricultural irrigation evaporation, and downstream ecological water input evaporation (Meng et al. 2025). In areas of intense drought and high-temperature stress, salinization becomes more severe due to increased upward movement of capillary water- and water-soluble salts to the root zone of plants. Globally, 11.737 M.km^2 area was estimated to be salt-affected from 1980 to 2018, wherein 16.49 M.ha of the salt-affected areas were in croplands (Hassani et al. 2020). Further, modern agriculture management practices contribute to salinity by remobilizing salts from deep layers of the soil and salinity also occurs in non-irrigated environment. The soil salinity problem can be addressed by two approaches: (i) providing technology for reclaiming these soils, (ii) biological exploitation of such soils through cultivation of salt tolerant plant species (halophytes). Addition of straw and gypsum treatments could mitigate soil salinization and enhance soil fertility by increasing soil organic carbon levels and microbial community structure within the aggregates (Zhao et al. 2025). Compared to salt sensitive (glycophytes) crop plants, the halophytes can withstand extraordinary intensities of salt amounts; i.e., beyond 300 to 1020 mmol L^{-1} (Mushtaq et al. 2021).

Seed germination and early seedling growth are important stages for the establishment of plants especially under abiotic stress conditions that affects the initial plant stand and will have enormous effect on the ultimate productivity in such stressful growing conditions. The germination process comprises of two distinct phases; i.e., 'imbibition', and 'heterotrophic growth phase' between imbibition and emergence. Imbibition is a process of diffusion of water in plants where the net movement of water is along

a diffusion gradient. High salt concentration causes a more negative water potential, and this brings about a decrease in the rate at which water is imbibed and thus the amount of water taken up. The mechanisms involved in salt damage during germination are not fully understood (Almodares et al. 2007). The effect of salinity on plant growth and development (Jha et al. 2022) is increasingly problematic worldwide and is a complex syndrome that involves osmotic stress, ion toxicity and mineral deficiencies (Musyimi et al. 2007). Although pearl millet is fairly a salt tolerant crop, its growth is reduced with the increase of salt treatment (Krishnamurthy et al. 2007).

Availability of salinity stress tolerance within the pearl millet germplasm offers a great scope for understanding salinity tolerance related traits and to integrate these tolerant genotypes into appropriate management programs to improve the productivity of the saline soils. Identification of genetic materials contrasting in tolerance level to salinity stress is an important step in generating salt tolerant cultivars in an efficient breeding program. Compared to other cereal crops, limited information is available on response to soil salinity at seed germination and early seedling stage using diverse germplasm of pearl millet. Most of the earlier investigations in pearl millet have used either limited number of germplasm and cultivars, or they used breeding material with narrow genetic base (Ashraf and McNeilly 1992; Ali et al. 2004; Mukhopadhyay et al. 2005; Kulkarni et al. 2006; Krishnamurthy et al. 2007; Yakubu et al. 2010; Venkata et al. 2012). Hence, in the present study, we selected mini core collection developed at ICRISAT, which is a representative sub-set; i.e., 1% of global collection of pearl millet (Upadhyaya et al. 2011) and was assessed for their response to salinity stress at seed germination and early seedling stage through preliminary screening in a laboratory experiment followed by confirmation of selected germplasm sources under different salinity levels to identify promising tolerant sources for their subsequent use in pearl millet breeding program.

Earlier investigations in pearl millet have assessed germplasm or breeding lines at different levels of salinity to understand the level of tolerance. Initial screening of 15 accessions of pearl millet at different levels of salinity (EC 9, 13, 17, 21, 25 dSm^{-1}) in Hoagland's solution has identified 17 dSm^{-1} as critical salinity level to distinguish salinity tolerant and sensitive genotypes. In a lysimeter experiment, soil was salinized with different salinity levels (0, 50, 100, 150, 200 mmol L^{-1}) and 200 mM was found to decrease around 25% of shoot, grain and total biomass weight in pearl millet (Oleiwi et al. 2015). In a pot culture experiment, genotypic variability of vegetative-stage salinity tolerance was assessed among 100 pearl millet breeding lines with 250 mM NaCl solution as basal application having soil ECe of 18.1 dSm^{-1} and differentiated tolerant and sensitive genotypes (Krishnamurthy et al. 2007). The significant reduction of grain yield was observed mainly at the higher salinity level (8, 12, or 15 dSm^{-1}) compared to control in pearl millet (Ribadia et al. 2018; Heidari and Jamshid 2010; Yakubu et al. 2010). Preliminary evaluation of breeding lines and germplasm for salinity tolerance was done at 200 mM under pot conditions at ICRISAT, whereas under field conditions at 5, 10, and 15 dSm^{-1} salinity levels at ICBA Dubai. Further screening has led to identification of high degree of salinity tolerance under salinity levels up to 15 dSm^{-1} (Yadav et al. 2012). In the light of these investigations in pearl millet, a moderate level of salinity; i.e., 100 mM NaCl (EC 12.4 dSm^{-1}) which is closer to salinity levels found under field conditions where pearl millet is being grown in arid and semi-arid regions was chosen for preliminary screening of mini core collection. Further, identified promising accessions of mini core were subjected to higher salinity levels (100, 150, 200, 250 mM NaCl) along with control to confirm their tolerance and also identify critical level of tolerance in this diverse sub-set of global pearl millet germplasm.

In the present study, greater variability was observed in mini core collection under salinity stress (100 mM NaCl) for germination (43.5–94.5%) and seedling stage traits such as root length (6.5–21.7 cm), shoot length (5.2–13.6 cm), seedling dry weight (18–84 mg), SVI–1 (509–3249), and SVI–2 (778–7937) (Table 2). Similarly, considerable genotypic variation for different salinity tolerance traits was noted earlier in pearl millet (Ashraf and McNeilly 1992; Ali et al. 2004; Kulkarni et al. 2006; Krishnamurthy et al. 2007), related wild species *Pennisetum clandestinum* (Muscolo et al. 2003) and foxtail millet (Krishnamurthy et al. 2014). Salinity stress (100 mM NaCl) in comparison to control had decreased germination, root length, shoot length,

seedling dry weight, and seedling vigour indices. Sodium chloride may inhibit the activities of some enzymes that may play a critical role in seed germination and may also damage the embryo under salinity stress. Increasing level of salinity has been demonstrated to decrease germination percentage, seedling vigour index, shoot length, shoot and root weight in pearl millet (Mukhopadhyay et al. 2005; Ali et al. 2006; Yakubu et al. 2010; Krishnamurthy et al. 2007; Ali and Idris 2015) and foxtail millet (Ardie et al. 2015). Among different salinity levels tested (0, 0.5%, 1.0%, 1.5%) in pearl millet, ~ 50% reduction in germination was noted at 1.5% NaCl (Ali et al. 2014). Similarly, reduction in shoot and root growth and plant dry weight was noticed among 24 accessions at higher level of salinity level (20 dSm^{-1}) and only three accessions were found tolerant (Ashraf and McNeilly 1992). The effect on seed germination and seedling traits could be due to osmotic stress that prevents water uptake or ion toxicity that may be toxic to embryo or developing seedlings (Almodares et al. 2007; Sawamery and Mojaddam 2014). Salt stress in pearl millet has been shown to degrade seed proteins and break up these polypeptides into new smaller peptides (Rani 2011). However, the mechanisms involved in salt damage during germination are not fully understood (Almodares et al. 2007). The present study aimed to identify diverse sources of salinity tolerance at seed germination and early seedling stage. Hence, in-depth investigations on mechanisms involved were not studied considering the large number of germplasm being evaluated.

For preliminary screening of mini core, moderate level of salinity (100 mM) was used to cull out salt sensitive germplasm accessions. Similarly, decrease in relative root length has been noted as the salinity level increased up to 160 mM NaCl in pearl millet (Ali et al. 2004, 2006) and 100 to 150 mM NaCl in foxtail millet (Ardie et al. 2015). Increased Na^+ concentration by salt stress (EC 10 dSm^{-1}) under hydroponics has been also found to reduce root length and shoot length in rice (Krishnamurthy et al. 2016). In pearl millet, the genetic analysis for salinity related traits showed predominance of non-additive gene action for stem Na and dominance component for leaf Na/K ratio, while both additive and non-additive components for stem Na/K ratio (Venkata et al. 2012). Hence, for screening a large number of germplasm, moderate level of salinity can be imposed. However, for detailed evaluation higher concentration of NaCl can be imposed.

Based on preliminary evaluation, 10 promising accessions from pearl millet mini core collection were subjected to detailed evaluation for six salinity tolerance traits under control and different salinity levels. The mean values of 10 genotypes across different salinity levels (100, 150, 200, 250 mM NaCl) for germination (91.3%, 72.1%, 35.9%, 18.3%), and seedling stage traits such as root length (19.7 cm, 13.6 cm, 10.7 cm, 8.6 cm), shoot length (12.3 cm, 7.1 cm, 5.6 cm, 3.6 cm), seedling dry weight (75.5 mg, 54.5 mg, 48.6 mg, 42.2 mg), SVI–1 (2925, 1515, 610, 245), and SVI–2 (6894, 3966, 1825, 840) were highest at 100 mM, but started declining sharply as the salinity level was increased to 150, 200, and 250 mM, respectively (Table 4). The promising accessions could tolerate 100 mM NaCl comfortably for all six traits, but they showed drastic reduction from 150 mM through 250 mM NaCl, with intensity differing based on the genotype. Drastic reduction in germination and seedling parameters among pearl millet germplasm with increasing salinity concentration was noted earlier in pearl millet. Earlier, only three out of 28 inbred lines tested showed germination over 70% at 150 mM (Mukhopadhyay et al. 2005). Relative root length compared to control decreased as the salinity level increased (40, 80, 120, 160 mM) and at 160 mM NaCl, the reduction varied among parents (25.8–61.3%) and hybrids (22.5–69.5%) (Ali et al. 2006). In this study, we could identify highly tolerant 10 germplasm lines whose mean germination was much higher at 150 mM (72.1%) and five accessions IP14294, IP21312, IP11811, IP3626, IP7422, and IP11247 showing high germination (84.5–71.5%) compared to previous investigations indicating the potential of these identified lines, which could be employed in breeding for salinity tolerance (Table 4). Similarly, pearl millet was found sensitive for germination stage at ECe of 16 dS m^{-1} and beyond, and this sensitivity to some extent could be compensated by the tillering capability. Some reports suggest that salinity response estimated at germination stage does not correlate well with plant performance at later stages (Munns and James 2003), however, effect of salinity on initial plant stand as well as final population under saline soils specifically in

arid and semi-arid regions are most damaging and greatly influence in realizing the potential productivity.

As compared to other cereal crops, only limited information is available on response of soil salinity to pearl millet (Shivhare and Lata 2017). During the crop growth period, pearl millet tolerates soil salinity (ECe) of up to 6 to 8 dSm^{-1} without a significant decrease in dry matter production (Toderich et al. 2018). An average reduction of 20% in shoot biomass and ~ 40% in grain yield has been reported in pearl millet germplasm under salinity stress (Krishnamurthy et al. 2014; Toderich et al. 2018). However, under high saline stress, a significant reduction of 47–86% in grain yield and 51% in fodder yield was noted in pearl millet (Choudhary et al. 2019; Kulkarni et al. 2006; Ribadiya et al. 2018). Salinity slows germination; however, early vigour directly boosts field establishment under salinity because vigorous seeds germinate faster and produce stronger seedlings, which are better equipped to withstand negative effects (osmotic stress, ion toxicity) of salt stress by maintaining better growth (shoot/root mass) and limiting sodium accumulation, leading to better crop stands and yields. High-vigour seeds resist the initial shock, ensuring more uniform emergence, crucial for overcoming the detrimental impacts of salt stress on sensitive early growth stages, even if genotypic tolerance is key. Salinity tolerance in pearl millet has been found associated with higher shoot biomass ratio (Krishnamurthy et al. 2007), reduced shoot N content and increased K^+ and Na^+ content (Dwivedi et al. 2012), which could be used as potential selection criteria for screening of pearl millet germplasm at vegetative stage (Shivhare and Lata 2017). Yield and other yield related parameters of millets decreased by salinity stress and this reduction was more prominent only at high level of salinity (9.5 dSm^{-1}) (Kafi et al. 2009). Salinity has been shown to decrease grain yield during rainy (70%), early-summer (80%) and late-summer (86%) seasons, and high temperature can compound the effect of salinity and high vapour pressure deficit (Choudhary et al. 2019).

A generalized scheme was proposed earlier to describe the events happening in pearl millet during salinity stress that includes different metabolic pathways, transcription factors, and ion transporters that act synergistically to mediate salinity stress tolerance in pearl millet (Shinde et al. 2018). Recently investigations on nutrient recycling indicated that nodulating N_2-fixing plants may not necessarily be more effective than non-nodulating endophytic N_2-fixing plants in promoting increased available P, alkaline phosphatase activity, and abundance of microbial P transformation genes in the rhizosphere. They provided evidence for their differential capacity in accessing recalcitrant inorganic P and promoting soil P cycling on alkaline, infertile deglacial moraine soil. These have implications in nutrient recycling under alkaline and saline conditions (Sun et al. 2025). Hence, the promising sources identified in this study for salinity tolerance at germination and seedling stage need to be studied under field condition to assess their response at differing levels of salinity in the pearl millet growing areas to identify salinity tolerant germplasm in field level at germination, seedling, and full maturity stage and ascertain their genetic basis for their further use in crop improvement. The availability of high levels of tolerance offers scope to integrate pearl millet crop into appropriate management programs to improve the productivity of the saline soils.

Conclusion

Pearl millet mini core collection comprising 238 accessions and eight controls were preliminarily evaluated under control and salinity stress (100 mM NaCl) for seed germination and seedling stage salinity tolerance traits under laboratory conditions. Identified 10 promising accessions were evaluated under control and higher levels of salinity stress (100, 150, 200, 250 mM NaCl) to confirm and assess the critical level of salinity tolerance among tolerant diverse germplasm. The promising accessions could tolerate 100 mM NaCl comfortably, but there will be drastic reduction from 150 mM through 250 mM NaCl, with the intensity differing among genotypes. At 150 mM, only two highly salinity tolerant accessions; i.e., IP14294 and IP21312 recorded more than 80% germination, suggesting it to be the critical limit of salinity that pearl millet can tolerate and anything beyond would not be tolerable at this stage and difficult to maintain enough plant stand in the field and ultimately productivity in pearl millet. This laboratory experiment is a starting point towards identification

of diverse germplasm sources for high level of salinity tolerance at germination and early seedling stage. These identified sources need to be confirmed in field experiments so that they can be employed in pearl millet improvement for salinity tolerance.

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Author contributions SKP and SRD planned this research, AUL conducted research and generated data as part of M.Sc. research work, SRD monitored research and data generation, AUL, SRD, SKP and THV analysed and interpreted data, prepared, read and confirmed manuscript for submission.

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Data availability The data presented in this study are available on request from the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

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