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Assessment of Finger Millet (*Eleusine coracana* L.) Genotypes for Salt Tolerance at Seedling Stage using Fuzzy Membership Function Value

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Abstract

Salinity is an important abiotic stress that affects the productivity of cereals. Of the cereals, finger millet is a staple food and fodder crop in India and Africa. The seedling stage is most sensitive to salinity, which affects the plant stand and productivity. Hence, the present study envisaged to identify salinity-tolerant accessions compared to the popular variety GPU-28 at the seedling stage, using fuzzy membership function value (MFV). Results revealed that at 200 mM sodium chloride (NaCl) concentration, 50 per cent reduction in seedling growth was noticed. Using 200 mM NaCl, 49 genotypes were evaluated and found that the shoot length was more affected than the root. The relationship of mean MFV with mean salinity tolerance index (r=0.969***) and mean percent reduction in seedling length (r=-0.969***) was significant. However, the MFV-based classification of genotypes was comprehensive and stringent. Based on the MFV, the popular variety GPU-28 was found moderately tolerant (MFV, 0.56). The salt tolerant genotypes with MFV value >0.581 to 0.759 and better than GPU-28, are GE-290, GE-4596, GE-1662, GE-3741 and GE-1915. The genotypes Poorna and GE-4729 (MFV, >0.759) were classified as highly saline tolerant. This study confirms that the finger millet is moderately tolerant to salinity and the MFV approach is effective for classifying genotypes to salinity tolerance at the seedling stage.

Keywords : Finger millet, Salinity tolerance index, Membership function value

Salinity is one of the predominant abiotic stresses that severely affect the seeding establishment and productivity (Bahari & Sharifi, 2014; Hema *et al*., 2014 and Kumar & Sharma, 2020). The rising temperatures and altered precipitation patterns in climate change are accumulating salt, particularly in arid regions (Gebreyohannes *et al*., 2024). In addition, the population threat with increasing food demand, decreasing forest area, increasing surface evaporation, frequent droughts, excess rainfall, indiscriminate use of agrochemicals and leaching of ions (chlorides and sulfates of sodium, calcium and magnesium) to the soil subsurface are increasing the salinization (Kumar & Sharma, 2020 and Murthy & Raut, 2024). Saline soils are classified by having the electrical conductivity of 4 dSm^{-1} (40 mM NaCl) and exchangeable sodium of 15 per cent (Joshi *et al*., 2021). With climate change, exploiting ground water from deeper layers and flooding irrigation are leading to an increasing the saline areas all over the world (Machado and Serralheiro, 2017) with a decrease in fresh water availability for agriculture (Gupta *et al*., 2020). In India, the saline-alkaline area is 6.74 mha and 0.15 mha in Karnataka (Kumar and Sharma, 2020). Such salinity stress affects relative water content, cell membrane integrity and crop yield

(Krishnamurthy *et al*., 2014; Mukami *et al*., 2020 and Divya *et al*., 2022). Morphologically, salinity increases the leaf thickness, abscission of leaves and necrosis of leaves and roots (Parida and Das, 2005). Hence, to combat salinity stress, identifying saline-tolerant genetic resources could be one of the best approaches (Krishnamurthy *et al*., 2014).

Among the cereal food crops, finger millet (*Eleusine coracana*) is a suitable crop for climate change with its higher water use efficiency, adaptation to drought, heat and degraded soils, with a storage period of more than 2-3 years (Hema *et al*., 2014; Onyango, 2016; Nanja Reddy *et al*., 2021; Nanja Reddy *et al*., 2022; Nanja Reddy and Priya Reddy, 2023). It occupies an area of 1.004 million hectare, with a production of 1.755 million tones and productivity of 1747 kg ha^{-1} in India. In India, the major states are Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, Maharashtra, Uttar Pradesh, Bihar, Gujarat and Uttarakhand. In Karnataka, it was 0.641 million hectare, 1.164 million tone production and productivity of 1816 kg ha^{-1} during 2019-20 (www.indiaagristat.com). It is cultivated as a rainfed crop in arid and semi-arid regions of the world, mainly in India and Africa (Davis *et al*., 2019; Anuradha & Rao, 2001 and Bhagyashree *et al*., 2023). It is a staple food crop in India and Africa with a rich source of minerals, phenolic compounds and anti-oxidants (Chivenge *et al*., 2015 and Hiremath *et al*., 2018). However, finger millet is a glycophyte (Hema *et al*., 2014) and is classified as a salt-sensitive crop (Bray *et al*., 2000). For instance, even 30 mM NaCl (1.8 $g.L^{-1}$) has significantly decreased the root and shoot length (Venothini *et al*., 2024). Further, a moderate concentration of 100 mM NaCl reduced the grain yield by 27 per cent (Krishnamurthy *et al*., 2014). In this context, several management techniques are in practice to mitigate salt stress (Singh *et al*., 2019 and Mbinda & Mukami, 2021) and they are laborious, cost-intensive and short-lived. Hence, exploring genetic resources in identifying salt-tolerant genotypes could be the best approach (Shailaja and Thirumeni, 2007). The seedling stage is the most sensitive stage of the crop (Huang *et al*., 2006 and Krishnamurthy *et al*., 2014). Additionally, the early seedling establishment is critical for achieving an adequate plant population that suppresses the weed growth, reduces soil evaporation and favours the water availability for crop growth under rainfed conditions (Anuradha & Rao, 2001; Fahad *et al*., 2017 and Nanja Reddy *et al*., 2021). Salinity negatively impacts both root and shoot development in finger millet seedlings by reducing the root and shoot lengths, fresh weight, dry weight and seedling vigor index, thus seedling establishment and plant health (Divya *et al*., 2022). Therefore, identifying the tolerant genotypes for salinity stress at the seedling stage would be appropriate.

Several techniques and computations are available for classifying the genotypes to salt tolerance (Singh *et al*., 2019 and Mbinda & Mukami, 2021). However, computing the salt stress tolerance following the fuzzy membership function value (MFV) could be a reliable and comprehensive approach (Ding *et al*., 2018) in *Sorghum bicolor*; (Wu *et al*., 2019) in *Brassica napus* and (Sikder *et al*., 2020 in *Gossypium hirsutum*). A higher mean MFV at seedling stage indicates that the genotype is more tolerant to moisture stress and vice versa (Bangari *et al,* 2024), hence it can be extended to finger millet as a prospective crop for saline soils (Krishnamurthy *et al*., 2014). With this background, the present study was conducted to identify the NaCl concentration required for reducing the seedling growth by 50 per cent, evaluate the set of 49 genotypes for salt tolerance using the identified NaCl concentration and identify the salt-tolerant genotypes using the MFV approach.

Material and Methods

The experiments were conducted from August to October 2023, at the Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bengaluru, situated at 13^{0} 05"N latitude, 77^{0} 34"E longitude and 924 m above MSL.

Determination of NaCl Concentration Required for Reducing the Seedling Length by 50 per cent

A popular high-yielding variety (GPU-28) occupying >60 per cent finger millet area (Gowda *et al*., 2009) was used as a reference genotype to determine the concentration of NaCl to induce nearly 50 per cent reduction in seedling length. The NaCl concentration used in the experiment ranged from 0 mM (distilled water as a control) through 100 mM, 150 mM, 200 mM, 250 mM, 300 mM and 350 mM in three replications (Petri plates) and the preparation of different concentrations is detailed below (Table 1). Seeds were soaked overnight in water for pro truberance of radical, placed on petri plates lined with two-layered blotting paper wetted sufficiently with the respective NaCl concentrations and incubated in the dark under ambient laboratory conditions (approximate temperature, 25-26 °C and RH, 65%) for seven days. Each Petri plate had five seedlings and was uniformly treated with NaCl solutions every two days and the seedling length was measured on the 7th day after incubation.

Table 1

Preparation of different NaCl concentrations

NaCl (g/ 100 mL)	Concentration (mM)	Concentration (%)
0	0	0
0.584	100	0.58
0.876	150	0.88
1.168	200	1.17
1.460	250	1.46
1.752	300	1.75
2.044	350	2.04

Screening of Germplasm Accessions for Salinity (NaCl) Stress Response

The experiment was conducted in a completely randomized design with two treatments, namely control (Water) and salinity treatment (NaCl, 200mM) in three replications. A total of 49 finger millet entries (germplasm accessions and a few released varieties, GPU-28, PR-202) were obtained from the All-India Coordinated Small Millets Improvement Project, GKVK, Bengaluru and used in the experiment. The experiment was conducted as explained above, except for the treatments. Ten pre-germinated seeds were placed in the Petri plates of 15 cm diameter lined with two-layered blotting paper. The seedling length was measured using measuring scale on the 7th days after incubation and the per cent reduction in the root, shoot, total seedling length and the shoot to root ratio due to salinity were computed.

$$\text{Reduction in root growth (\%)} = \frac{\text{(Root length in control - Root length in NaCl treatment)}}{\text{(Root length in control)}} \times 100$$

$$\text{Reduction in shoot growth (\%)} = \frac{\text{(Shoot length in control - Shoot length in NaCl treatment)}}{\text{(Shoot length in control)}} \times 100$$

$$\text{Reduction in total seedling length (\%)} = \frac{\text{(Seedling length in control - Seedling length in NaCl treatment)}}{\text{(Seedling length in control)}} \times 100$$

Analysis of Genotypic Response to Salinity Tolerance using Membership Function Value (MFV)

The salinity stress tolerance index (SSTi) : Root length (cm), shoot length (cm) and total seedling length (cm) were used to calculate SST_i for each accession. The SSTi is the ratio of a given parameter under salinity stress to that of the control seedling.

SSTi = (V_{is} / V_{ic}), where, salinity stress tolerance index (SSTi) is for the ith trait and V_{is} and V_{ic} represent the original value of a given genotype for ith trait in salinity and control, respectively. To derive the differential response of each genotype to salinity stress tolerance, the shoot length (SST_{SL}), root length (SST_{RL}) and total seedling length (SST_{TSL}) were computed.

Membership Function Value (MFV)

The stress tolerance level of finger millet genotypes was evaluated using a fuzzy comprehensive evaluation method using the MFV. The salinity stress tolerance MF value was calculated using the following equation (Bangari *et al*., 2024).

$X_i = (X - X_{min}) / (X_{max} - X_{min})$

Where, Xi represent the MFV of the i[th] genotype in a given trait, X is the SSTi value of a given genotype for the given parameter and X_{max} and X_{min} are the maximum and minimum values of the SSTi of i[th] trait across the genotypes, respectively. Hence, MFV will range from 0 to 1 for each trait. To classify the salinity stress tolerance level of genotype in each trait, the tolerance capacity was categorized into five groups considering on three variables, (1) the MFV (Xi) for the genotype, (2) the grand mean (X) and (3) the standard deviation across the genotypes (Bangari *et al*., 2024). The categories are, (1) $Xi < X - 1.64$ SD, highly salinity sensitive (HSS); (2) $Xi \geq X - 1.64$ SD and $<X - 1.0$ SD, salinity sensitive (SS); (3) $Xi \geq X - 1.0$ SD and $< X + 1.0$ SD, moderately salinity tolerant (MST); (4) $Xi \geq X + 1.0$ SD and $< X + 1.64$ SD, salinity tolerant (ST); and (5) $Xi \geq X + 1.64$ SD, high salinity tolerance (HST) as evidenced for moisture stress tolerance (Chen *et al*., 2012 and Bangari *et al*., 2024). Finally, the mean MFV across traits was computed to classify the germplasm accessions as tolerant or susceptible.

Statistical Analysis

The collected data were statistically analyzed for ANOVA, Duncan Multiple Range Test and correlations by using OPSTAT statistical software (Sheoran *et al*., 1998).

RESULTS AND DISCUSSION

The seedling stage is the most sensitive stage to the salt stress (Krishnamurthy *et al*., 2014 and Fahad *et al*., 2017). One of the predominant millet under arid and semi-arid regions of Africa and India is the finger millet with a relatively higher productivity compared to other millets (www.indiaagristat.com) and is classified as sensitive (Bray *et al*., 2000) and moderately tolerant to salt stress (Krishnamurthy *et al*., 2014 and Satish *et al*., 2016). Therefore, identifying salt-tolerant genotypes would be relevant in the increasing trend of salinity affected area under climate change scenarios (Krishnamurthy *et al*., 2014).

Standardization of Appropriate NaCl Concentration for 50 Per cent Reduction in Seedling Growth

Screening more genotypes for abiotic stress tolerance at the seedling stage would require an appropriate technique for easy and rapid evaluation. In the salinity context, identifying the selection of NaCl concentration that inhibits the seedling growth by 50 per cent would be ideal. Results revealed that concentrations of NaCl up to 150 mM, the shoot length and total seedling length did not differ significantly compared to the control; rather a marginal increase of 14.2 and 8.4 per cent, respectively at 100 mM and -2.7 and 4.3 per cent increase, respectively at 150 mM (Table 2). The higher root growth up to 150 mM could be due to a moderate increase in the osmotic potential of the seedling and thus increased water uptake in view of mild stress in the medium (Mukami *et al*., 2020). Where, the roots play an important role in salt tolerance and are less affected compared to the shoot (Table 2), this could be ascribed to translocation of salts from the root to the shoot system to maintain lower salt levels in the root (Mukami *et al*., 2020).

However, a higher concentration of 200 mM and above significantly decreased the root, shoot and total seedling length compared to the control (Table 2). Higher salinity affected the shoot growth and is demonstrated through a decreased shoot/root ratio with increasing salt stress (Table 2), which could be due to a higher accumulation of salts in the shoot. The reduction in root and shoot length at 250 mM was severe by more than 53.8 and 71.6 per cent, respectively (Table 2). The salt stress leads to increased Na^+ ions with a reduction in K/Na ratio (Mukami *et al*., 2020). The higher salt concentrations in the medium restrict the uptake of water due to the relatively lower water potential in the medium compared to the seedling, thus retarded the seedling growth or complete suppression of seedling growth, as evidenced through phenotypic expression (Plate 1). Similarly, increased NaCl concentration from 0 to 1 per cent has been reported to decrease the root, shoot length and seedling vigour in foxtail millet (Suvarna

TABLE 2

Effect of salt stress on seedling growth of finger millet, Cv. GPU-28

NaCl (mM)	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	% Red. (RL)	% Red. (SL)	% Red. (TSL)	Root/ shoot ratio
Control	5.11^{a}	3.66^{b}	8.77^{a}	-	-	-	1.40
100 mM	5.33^{a}	4.18^{a}	9.51^{a}	-4.3	-14.2	-8.4	1.27
150 mM	5.59^{a}	3.56^{b}	9.15^{a}	-9.4	2.7	-4.3	1.57
200 mM	3.15^{b}	1.61^{c}	4.76^{b}	38.3	56.1	45.7	1.96
250 mM	2.36^{b}	1.04^{d}	3.40^{c}	53.8	71.6	61.2	2.27
300 mM	1.17^{c}	0.47^{e}	1.64^{d}	77.2	87.1	81.3	2.46
SEm+	0.27	0.17	0.38				
CD @ 0.5%	0.84	0.52	1.17				
CV (%)	12.3	11.9	10.5				

Note : Superscript alphabets indicate the statistical significance between treatments by Duncan Multiple Range Test

Plate 1. Effect of NaCl concentrations (soaking for 6h, 0 to 350 mM) on root, shoot and total seedling length of finger millet (cv. GPU-28)

et al., 2019). Amongst the root and shoot, the mean shoot growth across the treatments was more affected (40.7%) compared to the root length (31.1%) probably a survival mechanism, as evidenced by a lower shoot/ root ratio (Table 2) Mukami *et al.*, 2020 and Divya *et al.*, 2022).

Although finger millet was classified as susceptible (Bray *et al.*, 2000), the results of the present study infer that finger millet is moderately tolerant, especially with popular varieties like GPU-28 (Plate 1) (Krishnamurthy *et al.*, 2014). The threshold salinity level for finger millet was reported to be 6 dSm^{-1} (60 mM); (Shailaja and Thirumeni, 2007), above which a decrease in yield parameters and yield will be observed (Bahari and Sharifi, 2014) in wheat and (Divya *et al.*, 2022) in finger millet). A NaCl concentration of 100-125 mM could be appropriate for genotypic differentiation in finger millet with which the percent reduction in yield varies between -29 to 78 per cent (Krishnamurthy *et al*., 2014). The shoot length was decreased up to 150 mM but the root length was increased up to 150 mM, hence 200 mM could be apt to differentiate for salt tolerance at seedling stage (Satish *et al*., 2016). In the present study, 250 mM or higher concentration resulted in a highly deleterious effect on root and shoot length. Hence, 200 mM would be appropriate for screening genotypes to salt tolerance and expected to exhibit a higher genotypic variation.

Differential Response of Genotypes to the Salinity Stress

The mean shoot length decreased significantly (by 39.2%) from 4.30 cm in control to 2.59 cm under salinity treatment, correspondingly the mean total seedling length also reduced from 10.08 cm to 8.95 cm (9.9%) and the shoot-to-root ratio decreased from 0.77 in control to 0.42 under salinity. Contrastingly, the mean root length increased significantly from 5.78 to 6.37 cm (14.4%; Table 3). The reduction in seedling growth could be due to direct contact with the saline medium (Munns and Tester, 2008), Na^+ and Cl^- creating higher osmotic stress in the medium, decreased uptake of water, less turgidity, lower cell expansion (Cramer & Bowman, 1991 and Parihar *et al*., 2015), and with increased membrane damage, decreased epidermal surface area, size of epidermal cells and accumulation of peroxides, leading to cell death (Satish *et al*., 2016). Furthermore, the Na^+ and Cl^- enter into the transpiring stream and injure transpiring leaves, hence the higher effect of salinity on shoot length (Parihar *et al*., 2015). A similar effect of salinity on shoot length compared to root length has been reported (Mukami *et al*., 2020; Divya *et al*., 2022), suggesting that for survival under salinity stress, plants maintain better root system to acquire water and minerals from the medium.

Significant genotypic variations in root, shoot and seedling length due to 200 mM NaCl were observed (Table 3). A similar significant genotypic variation in seedling length is reported even at lower NaCl

TABLE 3

Genetic diversity for seedling parameters due to salinity stress (200 mM) in finger millet at 7 days after sowing

Genotype	Shoot length (cm)			Root length (cm)			Total seedling length (cm)			Shoot/ Root ratio	
	Control	NaCl	% Red	Control	NaCl	% Red	Control	NaCl	% Red	Control	NaCl
GE-4714	4.46	2.23	49.9	5.94	6.38	-7.4	10.40	8.62	17.2	0.75	0.35
GE-4596	4.34	2.91	33.0	5.71	8.22	-43.9	10.05	11.13	-10.7	0.76	0.35
GE-4685	4.73	2.16	54.3	3.78	5.03	-33.0	8.51	7.19	15.5	1.25	0.43
GE-4437	3.93	2.28	42.0	7.28	5.56	23.6	11.21	7.85	30.0	0.54	0.41
GE-4829	4.81	2.56	46.8	7.65	8.17	-6.8	12.46	10.73	13.9	0.63	0.31
GE-4404	4.73	2.69	43.1	5.74	7.51	-30.8	10.47	10.20	2.6	0.82	0.36
GE-4129	4.72	2.19	53.6	7.05	4.38	37.9	11.77	6.57	44.2	0.67	0.50
GE-4478	4.84	2.23	53.9	5.24	4.60	12.2	10.09	6.84	32.2	0.92	0.49
GE-4729	3.75	2.31	38.5	3.44	7.20	-109.2	7.19	9.50	-32.2	1.09	0.32
GE-3752	4.13	2.74	33.7	7.14	5.92	17.2	11.28	8.66	23.2	0.58	0.46
GE-3369	5.39	3.23	40.1	6.07	7.71	-27.0	11.46	10.93	4.6	0.89	0.42
GE-3741	4.17	3.13	25.0	5.52	7.78	-41.0	9.69	10.91	-12.6	0.75	0.40
GE-3179	5.03	2.42	51.8	7.38	4.51	38.9	12.40	6.93	44.1	0.68	0.54
GE-3454	4.91	3.35	31.8	6.17	7.96	-29.0	11.08	11.31	-2.1	0.80	0.42

Continued....

TABLE 3 Continued....

Genotype	Shoot length (cm)			Root length (cm)			Total seedling length (cm)			Shoot/ Root ratio	
	Control	NaCl	% Red	Control	NaCl	% Red	Control	NaCl	% Red	Control	NaCl
GE-3638	4.40	3.10	29.7	7.14	8.12	-13.6	11.55	11.21	2.9	0.62	0.38
GE-3767	3.82	2.95	22.8	5.74	6.06	-5.6	9.56	9.01	5.8	0.67	0.49
GE-2866	4.59	2.61	43.1	7.43	4.97	33.2	12.02	7.57	37.0	0.62	0.53
GE-2644	4.19	2.73	34.8	5.53	6.84	-23.7	9.72	9.57	1.5	0.76	0.40
GE-2329	4.85	2.86	41.1	5.88	6.49	-10.5	10.73	9.35	12.8	0.83	0.44
GE-2073	3.59	3.28	8.7	6.21	5.20	16.2	9.80	8.48	13.5	0.58	0.63
GE-1915	4.04	2.61	35.4	4.03	7.04	-74.6	8.07	9.65	-19.6	1.00	0.37
GE-1634	4.25	2.70	36.4	5.80	6.14	-5.9	10.05	8.84	12.0	0.73	0.44
GE-1662	4.75	2.70	43.3	4.61	8.11	-75.9	9.36	10.80	-15.4	1.03	0.33
GE-1684	4.73	2.11	55.4	5.27	4.73	10.3	10.00	6.84	31.6	0.90	0.45
GE-1673	4.61	2.01	56.5	3.92	5.36	-36.6	8.53	7.36	13.7	1.18	0.37
GE-1537	4.25	3.02	29.0	6.79	8.13	-19.8	11.04	11.15	-1.0	0.63	0.37
GE-1417	3.22	2.17	32.5	4.86	6.21	-27.9	8.07	8.38	-3.8	0.66	0.35
GE-1596	3.78	2.18	42.2	4.14	6.14	-48.4	7.92	8.33	-5.2	0.91	0.36
GE-1274	4.03	2.92	27.6	5.05	3.88	23.3	9.08	6.80	25.2	0.80	0.75
GE-1235	4.73	3.00	36.6	8.05	7.12	11.5	12.77	10.12	20.8	0.59	0.42
GE-1200	4.65	2.53	45.6	7.15	6.66	6.8	11.80	9.19	22.1	0.65	0.38
GE-1172	4.48	2.82	36.9	6.72	5.53	17.7	11.20	8.36	25.4	0.67	0.51
GE-1090	3.66	2.50	31.8	5.53	6.68	-20.7	9.19	9.17	0.2	0.66	0.37
GE-1240	4.90	2.88	41.1	6.45	6.74	-4.4	11.35	9.62	15.2	0.76	0.43
GE-1034	3.76	2.51	33.2	5.33	7.05	-32.2	9.09	9.56	-5.1	0.71	0.36
GE-942	3.59	2.25	37.2	6.57	7.03	-6.9	10.16	9.28	8.6	0.55	0.32
GE-902	4.57	2.99	34.6	6.66	6.58	1.3	11.23	9.57	14.8	0.69	0.45
GE-838	3.69	2.43	34.2	6.03	5.98	0.9	9.72	8.41	13.5	0.61	0.41
GE-834	4.80	2.65	44.9	6.16	6.49	-5.2	10.96	9.13	16.7	0.78	0.41
GE-665	4.30	2.30	46.4	4.04	4.99	-23.6	8.33	7.29	12.5	1.06	0.46
GE-616	4.91	2.55	48.1	4.87	6.54	-34.2	9.79	9.09	7.1	1.01	0.39
GE-568	4.40	2.66	39.6	5.11	6.64	-30.0	9.50	9.29	2.2	0.86	0.40
GE-492	4.58	2.23	51.3	5.84	5.29	9.5	10.42	7.52	27.9	0.78	0.42
GE-290	4.08	2.46	39.6	5.17	8.03	-55.3	9.25	10.50	-13.5	0.79	0.31
GE-5252	3.79	1.85	51.3	4.90	4.22	13.9	8.69	6.07	30.2	0.77	0.44
PR-202	3.69	2.04	44.6	7.03	5.33	24.1	10.72	7.38	31.2	0.52	0.38
VR-708	3.94	2.34	40.8	4.09	5.83	-42.5	8.04	8.17	-1.6	0.96	0.40
GPU-28	3.87	2.68	30.8	6.71	8.67	-29.1	10.58	11.34	-7.2	0.58	0.31
Poorna	3.28	2.70	17.6	4.23	6.19	-46.4	7.50	8.89	-18.5	0.78	0.44
Mean	4.30	2.59	39.2	5.78	6.37	-14.4	10.08	8.95	9.9	0.77	0.42
T	G	TxG	T	G	TxG	T	G	TxG			
SEm+	0.04	0.20	0.28	0.06	0.30	0.42	0.08	0.41	0.58		
CD @ 5%	0.11	0.55	0.78	0.17	0.83	1.18	0.23	1.13	1.60		
CV (%)		14.1			12.1			10.5			

concentrations of 60 mM (Sujatha and Pushpalatha, 2019), 100 mM NaCl (Thrupthi *et al*., 2023), 120 mM NaCl (Shailaja and Thirumeni, 2007) and 160 mM (Prabhu and Ganesan, 2014). Based on the seedling length, genotypes TNAU-1008 and GS-59 were identified as salt tolerant at 160 mM (Prabhu and Ganesan, 2014) and Co-Ra (14) at 200 mM NaCl (Satish *et al*., 2016). In the present study, among the accessions, except the GE-2073, the shoot length was significantly affected by 200 mM NaCl (Table 3). Regarding root length, out of 49 accessions, 32 accessions differed significantly between the control and 200 mM NaCl, of which 23 accessions showed a higher root length under salinity than the control (Table 3). With regard to total seedling length, 17/49 accessions differed significantly due to salt stress, of which only one accession, GE-4729, has shown an increased seedling length with salt stress compared to the control (Table 3). The accessions, GE-4596, GE-3741, GE-1915, GE-1662, GE-290 and Poorna have shown >10 per cent higher seedling length over the control and these genotypes could be tolerant to salinity levels of 200 mM (Table 3). The tolerant genotypes might develop adaptive mechanisms like extrusion or tolerance to ionic concentration (Huang *et al*., 2006). In addition, the tolerant genotypes might possess an extensive increase in root porosity with the presence of aerenchyma cells in the cortex region for aerobic respiration and restricting the Na^+ entry into cortical cells by well-developed endodermis, double-layered pericycle and xylem-surrounded by companion cells (Krishnamurthy *et al*., 2014). The tolerant genotypes might have higher expression of SOS1, which drives Na^+/H^+antiporter for extrusion of excess Na^+ from the cytosol to apoplast, thus reducing the toxic effect of Na^+ (Pushpa *et al*., 2019). In this respect, a mtlD gene expressing transgenic finger millet seedlings was found tolerant over the wild type at 400 mM NaCl (Hema *et al*., 2014).

Use of Fuzzy Membership Function Value (MFV) for Classifying Genotypes to Salinity Tolerance

The stress tolerance indices (STI), in response to salt stress (200 mM NaCl) for each genotype in each trait were used to compute the MFV. A higher STI represents a high tolerance to salinity stress and vice versa. The mean STI_{RL} was high (1.14) compared to STI_{SL} (0.61) and STI_{TSL} (0.90; Fig. 1). The correlation between percent reductions in seedling parameters under salinity with MFV was exactly similar to that of STI but with a negative sign (Table 4).

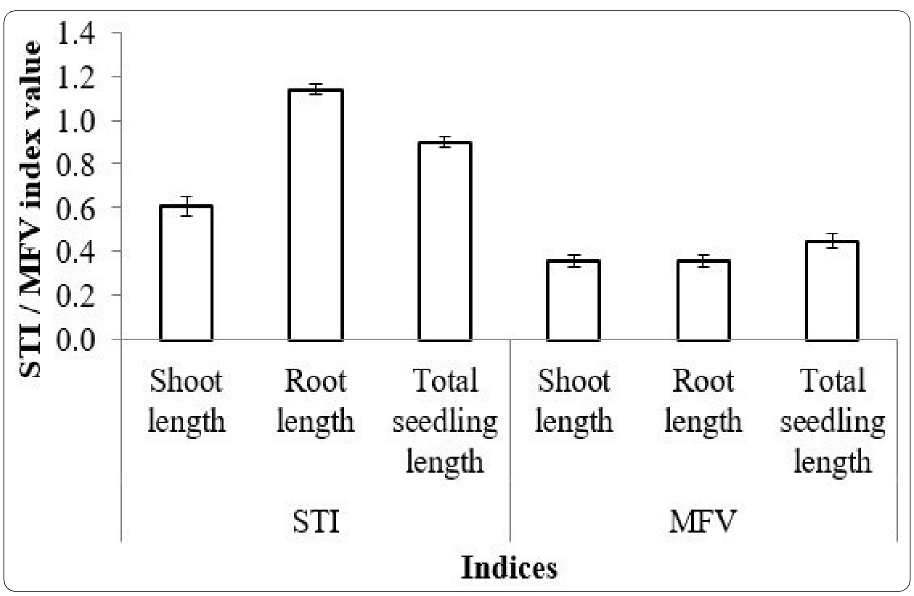

Fig. 1 : The mean stress tolerance index (STI) and membership function value (MFV) due to NaCl stress (200 mM) in finger millet accessions (The lines above the bars indicates are the standard error of mean)

TABLE 4

Correlation between mean membership functional value (MFV) and percent reduction and salinity tolerance indices in finger millet

Salinity tolerance indices	'r' value	Significance
% reduction in shoot length	-0.665	***
% reduction in root length	-0.842	***
% reduction in seedling length	-0.975	***
% reduction in shoot length	-0.962	***
STI_{SS}, Shoot length	0.665	***
STI_{RL}, Root length	0.842	***
STI_{TSL}, Total seedling length	0.975	***
STI_{M}, Mean	0.962	***
Shoot / ratio	-0.391	**

Note : ** and *** represents P= 0.01 and 0.001 respectively

The MFV is a multivariate index that includes multiple variables to assess the performance of genotypes against a given stress. A higher MFV indicates the higher tolerance to salinity. The mean MFV for total seedling length was higher (MFV_{TSL}, 0.449) compared to root length (MFV_{RL}, 0.360) and shoot length (MFV_{SL}, 0.357) and is relatively different from the percent reduction or STI for a given trait (Fig. 1). The correlations between these parameters show that, the mean MFV across the traits had a significantly positive correlation with each STI and negatively with per cent reduction in each trait (Table 4). The relationship of the mean MFV with mean STI (r = 0.969***) and mean per cent reduction (r = -0.969***) was highly significant. This suggests that, although the per cent reduction or STI or the MFV can be used to select the genotypes for salt tolerance, the better is the MFV, as a comprehensive measure for appropriate classification of genotypes.

The MFV classification showed a normal distribution of genotypes for salinity stress response, with highly susceptible (2), susceptible (7), moderately tolerant (33), tolerant (5) and highly tolerant (2) (Table 5). The MFV has shown promising differentiation of genotypes in different crops also like, *Sorghum bicolour* (Ding *et al*. 2018), *Brassica napus* (Wu *et al*. 2019) and *Gossypium hirsutum* (Sikder *et al*. 2020). Present experiment is an attempt in case of finger millet at seedling stage. The previous studies with respect to grain yield have shown a significant reduction in grain yield ranging from -27 to 78 per cent at a lower level of 125 mM NaCl in different finger millet accessions (Krishnamurthy *et al*., 2014). Further, even at 100 mM NaCl, the finger millet varieties, GPU-28, ML-365, GPU-67, Trichy-1 were identified as tolerant based on the yield *per se* (Divya *et al*., 2022 and Rahman *et al*., 2014). In the present study at seedling level, 200 mM NaCl found to distinguish and identify genotypes for salt tolerance (Table 3 and Divya *et al*., 2022). Hence, the present classification is worth while and comprehensive for selection of salinity tolerant genotypes. The classification of crops based on yield reduction with salt concentration have shown that up to 8 (80 mM), 16 (160 mM), 24 (240 mM), 32 (320 mM) and > 32 dSm^{-1} are classified as sensitive, moderately sensitive, moderately tolerant, tolerant and most tolerant, respectively (www.fao.org.). Considering the said classification the released finger millet varieties, falls into the category of moderately tolerant and sustain up to 240 mM or more depending up on the genotype.

Among the 49 accessions, the accessions with a higher mean STI than the cv. GPU-28 (1.02), are GE-1596, GE-4596, GE-3741, GE-290, Poorna, GE-1662, GE-1915 and GE-4729 are termed as salinity tolerant in the increasing order. The genotypes GE-4129 (0.55), GE-3179 (0.55), GE-2866 (0.62) and PR-202 (0.67) were highly susceptible to salinity. The response of genotypes to salinity was similar for STI and the percent reduction in each trait. By using the mean MFV across the traits, the cv. GPU-28 (0.56) was moderately tolerant. The salt tolerant genotypes over the GPU-28, (MFV >0.581 to 0.759) are GE-290, GE-4596, GE-1662, GE-3741 and GE-1915 and the genotypes Poorna and GE-4729 with higher MFV (>0.759) were classified as highly saline tolerant. The highly susceptible genotypes with <0.095 MFV are GE-4129 and GE-3179 (Table 3). The MFV selection found to be stringent,

TABLE 5

Classification of finger millet germplasm accessions based on membership function value (MFV)

Class	Description	MFV value	No. of genotypes
HSS	Highly salinity susceptible	<0.095	2
SS	Salinity susceptible	0.096 to 0.210	7
MST	Moderately salinity tolerant	0.211 to 0.568	33
ST	Salinity tolerant	0.569 to 0.683	5
HST	Highly salinity tolerant	>0.684	2
Total			**49**

hence MFV based classification and selection would be highly effective and finger millet found to be moderately tolerant to salinity with 200 mM NaCl (Satish *et al*., 2016).

The present study identified that 200 mM NaCl could be apt for screening finger millet genotypes for salt tolerance at seedling level. For classifying genotypes to salinity response, the MFV approach is appropriate than the salt tolerance index or percent reduction. The selected accessions can serve as donors for improvement of salt tolerance in finger millet.

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