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Screening of fodder pearl millet (*Pennisetum glaucum* L.) for drought tolerance using polyethylene glycol 6000

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Abstract

Fodder scarcity remains a major constraint to India's livestock sector, and pearl millet (*Pennisetum glaucum* L.), valued for its drought resilience and high-quality green fodder, offers a promising solution. This study screened thirty fodder pearl millet accessions for drought tolerance at the seedling stage using polyethylene glycol (PEG) 6000-induced osmotic stress at five water potentials (0.0, -3.0, -5.0, -7.5, and -10.0 bars) in a two factorial CRD with three replications. Germination percentage, shoot length, root length, seedling length, root/shoot ratio and seedling vigour were recorded ten days after treatment. Analysis of variance revealed highly significant effects ($P \leq 0.01$) of PEG concentration, genotype, and their interaction on all six traits, with concentration contributing the largest share of variation. Mild osmotic stress (-3.0 bars) stimulated root elongation in half the genotypes, while all other traits declined progressively with increasing stress and germination, seedling length, and vigour showed the sharpest reductions at -10.0 bars. The significant genotype $\times$ concentration interaction indicated differential genotypic responses across the stress gradient. Genotypes IIMR AVS 93, IIMR AVS 16, IIMR AVS 57, IIMR AVS 31, IIMR AVS 49 and IIMR AVS 52 consistently performed best across traits. Germination percentage, seedling length and seedling vigour emerged as effective indirect selection criteria for early-stage drought screening. These findings identify promising drought-tolerant genotypes for further evaluation and breeding in fodder pearl millet improvement programs.

Keywords: Pearl millet, drought tolerance, PEG 6000, osmotic stress, genotype screening

Introduction

Agriculture and animal husbandry in India are closely interlinked, with livestock rearing playing a vital role in rural livelihoods. India possesses one of the world's largest livestock populations, and the livestock sector has emerged as a major component of the agricultural economy, contributing 30.38% to the agricultural Gross Value Added (GVA) and 5.50% to the total Gross Value Added (GVA) of the country in 2022–23 (DAHD, 2024) [5]. Fodders are the mainstay of animal wealth and their production is the backbone of the livestock industry. India has a fodder deficit, according to Union Minister Rajiv Ranjan Singh's written response to the Rajya Sabha on July 31, 2024. The ICAR-Indian Grassland and Fodder Research Institute (IGFRI), Jhansi, estimates a deficit of 23% for dry fodder and 11.24–32% for green fodder nationwide. Pearl millet (*Pennisetum glaucum* L.), is a significant crop that is cultivated for food and feed to satisfy the demands of both human and animal populations. It is an important crop in dryland agriculture because of its high nutritional profile and remarkable resistance to drought (Bhavani *et al.*, 2025) [4]. Pearl millet outperforms all other cereals, including wheat, maize, rice, sorghum, and barley, due to its special qualities as a C4 plant with high photosynthetic efficiency, greater capacity to produce dry matter, and ability to survive in harsh agroclimatic conditions with fewer inputs and higher economic returns (Satyavathi *et al.*, 2021) [14]. For cattle, the lush, tasty, and extremely nutritious green fodder of bajra ensures a high milk output. Compared to sorghum, it has no HCN and may be given to cattle at any point throughout the growing season (Balodariya *et al.*, 2022) [2].

As a result of the recent introduction of several improved cultivars of fodder pearl millet, it is essential to find out how these cultivars respond to drought, particularly during the seedling stage. The goal of the current study was to determine better genotypes for drought tolerance and to evaluate the impact of polyethylene glycol 6000 on root and shoot characteristics in seedlings of a pearl millet population.

Materials and Methods

Thirty pearl millet accessions were examined for germination rate, shoot and root growth at various polyethylene glycol (PEG) 6000 concentrations. The concentrations of water potential values are 0.0 (control), -3.0, -5.0, -7.5, and -10.0 bars. The experiment was conducted as a two-factor factorial experiment under a completely randomized design (CRD) with three replications at the Department of Genetics and Plant Breeding, College of Agriculture, Vellayani, in November 2025. The external water-stress treatment was the first factor, and the genotype was the second. Ten seeds from each of the thirty genotypes were chosen, sterilised in 1% sodium hypochlorite, and twice cleaned in distilled water. Ten days following treatment, observations were made while the seeds germinated in roll paper. When the emergent radicle grew to a length of 2 mm, the seed was said to have germinated (ISTA, 2024) [9]. At the end of the tenth day, germination percentage, shoot length, root length, seedling length, root/shoot length ratio and seedling vigour (Seedling vigour = Germination percent $\times$ Seedling length) were measured. Data were statistically analysed using the RAISINS (R and AI Solutions for Inferential Statistics) platform (Hisham *et al.*, 2025) [8].

Results and Discussion

Analysis of variance (Table 1) revealed highly significant differences ($P \leq 0.01$) for all six characters across the three sources of variation — PEG 6000 concentration, genotype, and their interaction. For every trait the mean squares due to concentration were far larger than those due to genotypes, which in turn exceeded the concentration $\times$ genotype interaction mean squares. Imposed water stress was therefore the dominant source of variation, although significant genotypic differences and a genotype-specific response to stress were also evident. Error mean squares were low relative to treatment mean squares, and coefficients of variation were small (5.6–17.0%) for all traits except the root/shoot ratio (59.1%), reflecting the higher variability of that derived character.

Response of Root Length to PEG 6000 Treatment

Early and fast root extension is a significant predictor of drought resistance, since a long, deep root system helps water extraction under upland conditions (Kim *et al.*, 2001; Narayan, 1991) [10]. Root length differed significantly among concentrations ($F = 4358.81$; Cohen's $f = 7.62$, a large effect) and among genotypes ($F = 270.52$). Mild osmotic stress (-3.0 bars) increased root length in 15 of the 30 genotypes, so the highest concentration mean occurred at -3.0 bars (24.64^a) rather than the control (23.79^b); the lowest was at -10.0 bars (7.34^e), and all five levels carried distinct letters, indicating each differed significantly from the others (Table 2). Among genotypes (Table 3), IIMR AVS 93 had the highest mean (25.12^a) while Giant Bajra (9.20^o) and IIMR AVS 50 (9.38^o) were statistically on par at the lowest end. A comparable stress-induced increase in root length has recently been reported among pearl millet inbred lines screened with PEG 6000 (Bazie *et al.*, 2025) [3]. The stimulation of root growth under mild stress is consistent with an osmotic-adjustment response that promotes deeper rooting to forage for water (Shrestha *et al.*, 2023) [15].

Response of Shoot Length to PEG 6000 Treatment

Shoot length is agronomically important because the harvestable fodder is largely vegetative shoot biomass. It declined steadily and significantly with increasing water stress, from the control (11.07^a) to -10.0 bars (0.53^e), each level differing significantly

from the next (Table 2; $F = 2680.80$, large effect). Genotype IIMR AVS 31 recorded the highest mean (8.50^a, on par with IIMR AVS 93 at 8.09^{ab}) and TSFB-1610 the lowest (2.67^f) (Table 3). The progressive suppression of shoot elongation reflects reduced water uptake and cell expansion under low external water potential.

Response of Root / Shoot Ratio to PEG 6000 Treatment

The root/shoot ratio reflects the relative partitioning of growth between roots and shoots and is a useful selection criterion, as drought-tolerant types often maintain a balanced or root-favoured allocation. The ratio rose from the control (2.18^d) to a maximum at -7.5 bars (5.31^a) and then fell sharply at -10.0 bars (1.98^d), where it was statistically on par with the control (Table 2). This was the most variable trait measured (CV = 59.1%; genotype $F = 8.06$, the lowest among the characters), partly because many seedlings produced roots but no measurable shoot under severe stress, leaving the ratio undefined or extreme in those cases — a point to weigh when interpreting the -7.5 and -10.0 bar means. Among genotypes, IIMR AVS 57 had the highest ratio (6.96^a) and Giant Bajra the lowest (0.71^o) (Table 3). The increase under moderate stress indicates preferential maintenance of root over shoot growth, a recognised drought-avoidance response, while the collapse at -10.0 bars coincides with near-complete arrest of both organs.

Response of germination percentage to PEG 6000 Treatment

Germination percentage decreased consistently with declining osmotic potential, from 74.89^a in the control to 5.78^e at -10.0 bars, with every level significantly different (Table 2; $F = 1717.83$). The most stress-tolerant genotypes for germination were IIMR AVS 16 (66.00^a) and IIMR AVS 57 (64.00^a), statistically on par, whereas IIMR AVS 94 germinated poorest (18.67^o) (Table 3). Reduced germination under PEG-induced stress is attributable to restricted water imbibition needed to initiate and sustain germination (Hadas, 1976) [6].

Response of seedling length to PEG 6000 Treatment

Seedling length, the sum of root and shoot length, declined significantly with increasing PEG concentration, from 34.86^a (control) to 7.87^e (-10.0 bars), each level distinct (Table 2; Cohen's $f = 9.51$, the largest concentration effect among all traits). IIMR AVS 93 produced the longest seedlings (33.21^a) and Giant Bajra the shortest (12.43ⁱ) (Table 3). Because it integrates both root and shoot performance, seedling length is a robust composite indicator of seedling-stage drought tolerance.

Response of seedling vigour to PEG 6000 Treatment

Seedling vigour, which combines germination with seedling growth, showed the steepest relative decline under stress, falling from 2629.10^a in the control to 83.32^e at -10.0 bars (Table 2; $F = 2605.25$). IIMR AVS 16 expressed the highest vigour (2165.09^a, with IIMR AVS 31 on par at 2051.99^{ab}) and IIMR AVS 83 the lowest (591.65ⁱ) (Table 3). As vigour captures the cumulative impact of osmotic stress on both germinability and seedling extension, it is particularly valuable for ranking genotypes.

Concentration $\times$ genotype interaction

The significant concentration $\times$ genotype interaction for all six traits (Table 1) shows that genotypes did not respond uniformly to increasing stress, in agreement with the genotypic variation in drought response reported for pearl millet under PEG-induced stress (Radhouane, 2007; Bazie *et al.*, 2025) [12, 3]. The highest individual treatment combinations came from superior genotypes

under no or mild stress (for example, control $\times$ IIMR AVS 71 for root length and -3.0 bars $\times$ IIMR AVS 93 for shoot length), whereas the most severe levels reduced many genotypes to zero growth, producing large blocks of statistically on-par low values. Because the genotype ranking shifted with the level of imposed stress, as also observed when grass species were screened across a PEG gradient (Van den Berg and Zeng, 2006) [17], the presence of this interaction justifies evaluating genotypes across the full stress gradient rather than at a single level.

Identification of drought-tolerant genotypes

Considering all traits together, several genotypes ranked consistently among the best: IIMR AVS 93 (highest root and seedling length and second-highest shoot length), IIMR AVS 16 (highest germination and vigour), IIMR AVS 57 (highest root/shoot ratio and among the highest germination), IIMR AVS

31 (highest shoot length and high vigour), and IIMR AVS 49 and IIMR AVS 52 (high germination and vigour). These combine sustained germination with vigorous root and seedling growth under osmotic stress and are promising candidates for drought tolerance, consistent with the use of seedling-stage PEG screening to identify tolerant genotypes (Kim *et al.*, 2001; Radhouane, 2007; Bazie *et al.*, 2025) [10, 12, 3]. A deep, vigorous root system in particular is associated with better water capture under moisture-limited conditions (Narayan, 1991) [11]. In contrast, Giant Bajra, IIMR AVS 50, IIMR AVS 94 and IIMR AVS 83 were repeatedly among the poorest performers. Because germination percentage, seedling length and seedling vigour discriminated genotypes strongly and are simple to record, they can serve as effective indirect selection criteria for early-stage drought screening (Kim *et al.*, 2001; Van den Berg and Zeng, 2006) [10, 17].

Table 1: Treatment and error mean squares (ANOVA)

Source of variation	DF	Root Length	Shoot Length	Root/Shoot Ratio	Seedling Length	Germination%	Seedling Vigour
Concentration	4	4849.91**	1851.92**	194.1**	12125.31**	74439.11**	105381865.62**
Genotypes	29	301**	32.65**	32.27**	458.93**	2648.61**	3452602.66**
Conc. $\times$ Gen.	116	62**	7.21**	26.32**	76.84**	413.25**	559218.65**
Error	300	1.11	0.69	4.00	1.79	43.33	40449.78

DF = degrees of freedom. Mean squares are given for each character. * significant at 5%; ** significant at 1%; NS = non-significant.

Table 2: Concentration means with multiple comparisons

Concentration	Root Length	Shoot Length	Root/Shoot Ratio	Seedling Length	Germination%	Seedling Vigour
Control	23.79 ^b	11.07 ^a	2.18 ^d	34.86 ^a	74.89 ^a	2629.10 ^a
-3.0 bar	24.64 ^a	9.10 ^b	2.93 ^c	33.74 ^b	60.67 ^b	2061.98 ^b
-5.0 bar	21.40 ^c	4.95 ^c	4.53 ^b	26.34 ^c	34.00 ^c	1004.01 ^c
-7.5 bar	14.37 ^d	1.84 ^d	5.31 ^a	16.21 ^d	18.44 ^d	412.47 ^d
-10.0 bar	7.34 ^e	0.53 ^e	1.98 ^d	7.87 ^e	5.78 ^e	83.32 ^e
F stat	4358.81**	2680.80**	48.49**	6776.38**	1717.83**	2605.25**
p value	0.00	0.00	0.00	0.00	0.00	0.00
CD (A)	0.31	0.24	0.59	0.39	1.93	59
MSE	1.11	0.69	4.00	1.79	43.33	40449.78
SE(m)	0.11	0.09	0.21	0.14	0.69	21.20
SE(d)	0.16	0.12	0.3	0.2	0.98	29.98
CV(%)	5.76	15.12	59.11	5.62	16.99	16.24
Cohen's F	7.62	5.98	0.80	9.51	4.79	5.89

Within each column, means sharing a common superscript letter are not significantly different (LSD / protected Fisher's LSD).

Table 3: Genotype means with multiple comparisons

Genotype	Root Length	Shoot Length	Root/Shoot Ratio	Seedling Length	Germination%	Seedling Vigour
Giant Bajra	9.20 ^o	3.23 st	0.71 ^o	12.43 ^t	27.33 ^{klm}	621.21 ^l
GAFB-4	17.05 ^k	5.17 ^{klm}	3.25 ^{efghij}	22.22 ⁿ	33.33 ^{hij}	926.54 ^{hij}
NDFB-2	12.02 ⁿ	4.17 ^{opq}	2.11 ^{klmno}	16.19 ^q	38.00 ^{fgh}	988.28 ^{hi}
NDFB-3	17.63 ^{jk}	3.82 ^{qrs}	2.60 ^{hijklm}	21.45 ^{no}	24.00 ^{lmn}	732.61 ^{kl}
TSFB-1610	11.99 ⁿ	2.67 ^t	1.37 ^{mno}	14.66 ^r	28.67 ^{ijkl}	844.79 ^{ijk}
TSFB-15-8	15.24 ^l	4.29 ^{opq}	4.58 ^{bcd}	19.53 ^p	32.00 ^{ijk}	1051.19 ^h
BAIF Bajra	18.86 ^{hi}	4.44 ^{nop}	2.21 ^{ijklmn}	23.30 ^m	35.33 ^{hi}	1302.52 ^g
IIMR AVS 75	17.96 ^j	3.49 ^{rs}	3.03 ^{fghijk}	21.45 ^{no}	28.67 ^{ijkl}	872.77 ^{ijk}
IIMR AVS 94	19.33 ^{gh}	6.78 ^{cd}	3.47 ^{efghij}	26.11 ^{ij}	18.67 ^o	636.01 ^l
IIMR AVS 77	20.04 ^{fg}	7.84 ^b	3.89 ^{defgh}	27.87 ^{fg}	47.33 ^d	1469.91 ^{ef}
IIMR AVS 63	18.22 ^{ij}	7.06 ^c	3.24 ^{efghij}	25.28 ^{jk}	52.67 ^c	1534.54 ^e
IIMR AVS 62	19.25 ^h	5.50 ^{kl}	3.62 ^{defghi}	24.76 ^{kl}	44.00 ^{de}	1301.51 ^g
IIMR AVS 79	15.14 ^l	3.90 ^{pqr}	4.26 ^{cdefg}	19.04 ^p	22.67 ^{mno}	617.27 ^l
IIMR AVS 53	14.61 ^l	6.15 ^{efghi}	3.08 ^{fghijk}	20.76 ^o	34.00 ^{hi}	963.66 ^{hi}
IIMR AVS 50	9.38 ^o	4.05 ^{pqr}	0.92 ^{no}	13.43 ^s	25.33 ^{lmn}	862.68 ^{ijk}
IIMR AVS 55	12.16 ⁿ	4.35 ^{opq}	2.12 ^{klmno}	16.51 ^q	25.33 ^{lmn}	661.88 ^l
IIMR AVS 49	21.15 ^e	5.54 ^{kl}	4.98 ^{bcd}	26.69 ^{hi}	58.00 ^b	1716.25 ^d
IIMR AVS 4	21.18 ^e	5.99 ^{ghij}	1.55 ^{lmno}	27.17 ^{gh}	37.33 ^{gh}	1315.73 ^g
IIMR AVS 48	20.65 ^{ef}	5.69 ^{ijk}	5.55 ^{abc}	26.34 ^{hi}	57.33 ^{bc}	1598.41 ^{de}
IIMR AVS 77	13.56 ^m	5.86 ^{hij}	1.65 ^{klmno}	19.42 ^p	26.67 ^{lm}	807.69 ^{jk}
IIMR AVS 20	24.15 ^b	6.41 ^{defgh}	3.79 ^{defgh}	30.56 ^{bc}	40.67 ^{efg}	1519.25 ^{ef}

IIMR AVS 52	22.94 ^{cd}	6.01 ^{fghij}	4.41 ^{cdef}	28.95 ^{de}	54.67 ^{bc}	1983.45 ^{bc}
IIMR AVS 61	22.94 ^{cd}	6.53 ^{cdefg}	4.08 ^{defg}	29.48 ^d	42.67 ^{def}	1388.65 ^{fg}
IIMR AVS 25	20.12 ^f	6.41 ^{defgh}	4.21 ^{cdefg}	26.53 ^{hi}	41.33 ^{efg}	1272.73 ^g
IIMR AVS 71	24.22 ^b	6.68 ^{cde}	4.67 ^{bcde}	30.91 ^b	36.67 ^{ghi}	1562.97 ^e
IIMR AVS 83	17.64 ^{jk}	6.60 ^{cdef}	2.93 ^{ghijkl}	24.24 ^{lm}	20.67 ^{no}	591.65 ^l
IIMR AVS 16	22.69 ^d	4.99 ^{lmn}	5.89 ^{ab}	27.68 ^{fg}	66.00 ^a	2165.09 ^a
IIMR AVS 57	23.47 ^{bc}	4.66 ^{mno}	6.96 ^a	28.14 ^{ef}	64.00 ^a	1921.91 ^{bc}
IIMR AVS 93	25.12 ^a	8.09 ^{ab}	3.52 ^{efghij}	33.21 ^a	44.67 ^{de}	1862.15 ^c
IIMR AVS 31	21.35 ^e	8.50 ^a	2.87 ^{ghijkl}	29.84 ^{cd}	54.67 ^{bc}	2051.99 ^{ab}
F stat	270.52 ^{**}	47.26 ^{**}	8.06 ^{**}	256.48 ^{**}	61.12 ^{**}	85.36 ^{**}
p value	0.00	0.00	0.00	0.00	0.00	0.00
CD (B)	0.76	0.6	1.44	0.96	4.73	144.52
MSE	1.11	0.69	4.00	1.79	43.33	40449.78
SE(m)	0.27	0.21	0.52	0.35	1.70	51.93
SE(d)	0.39	0.3	0.73	0.49	2.4	73.44
CV(%)	5.76	15.12	59.11	5.62	16.99	16.24
Cohen's F	5.11	2.14	0.88	4.98	2.43	2.87

Within each column, means sharing a common superscript letter are not significantly different (LSD / protected Fisher's LSD)

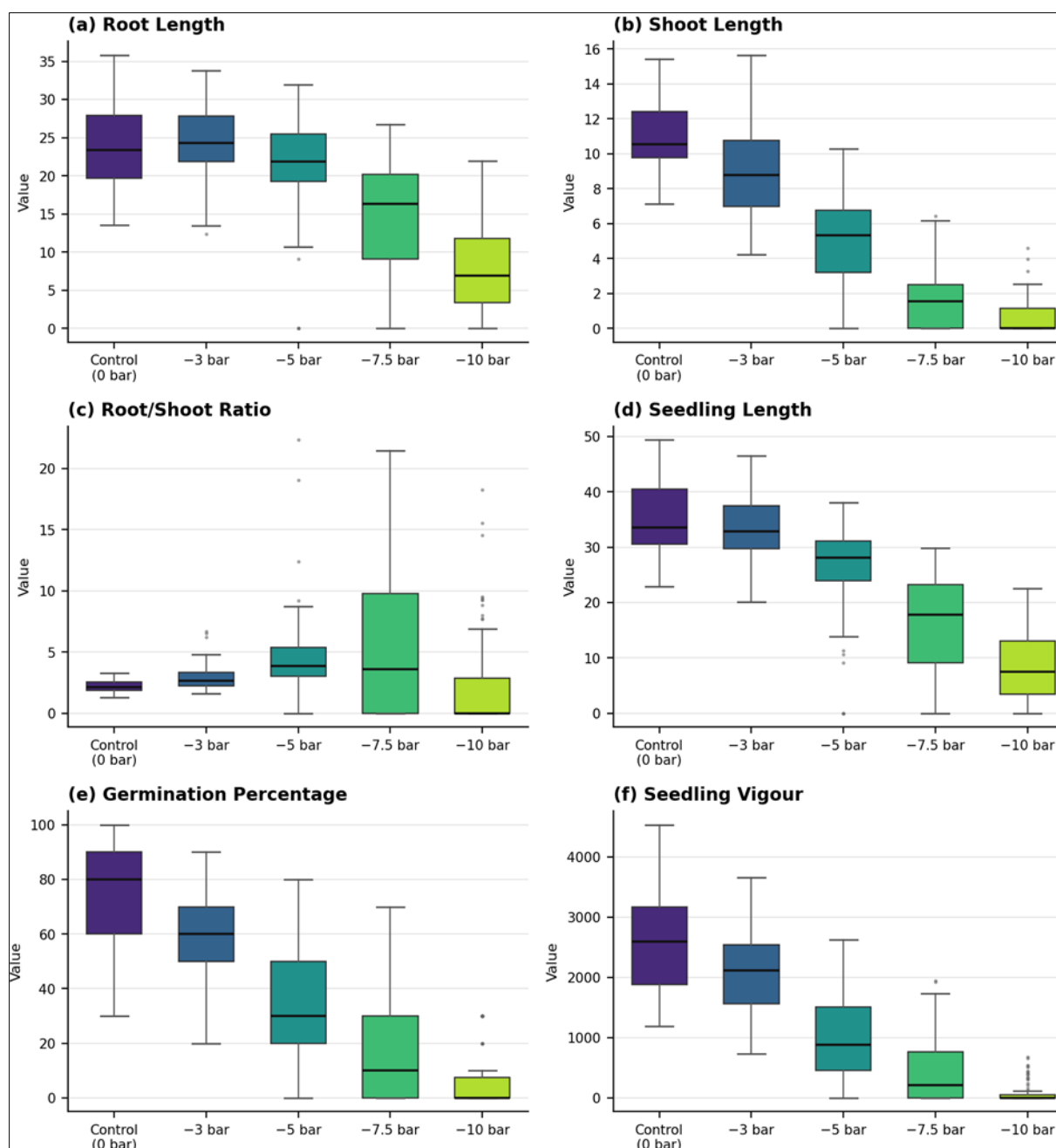


Fig 1: Studied traits of pearl millet under control and different levels of osmotic potential (PEG 6000): (a) root length, (b) shoot length, (c) root/shoot ratio, (d) seedling length, (e) germination percentage, (f) seedling vigour.

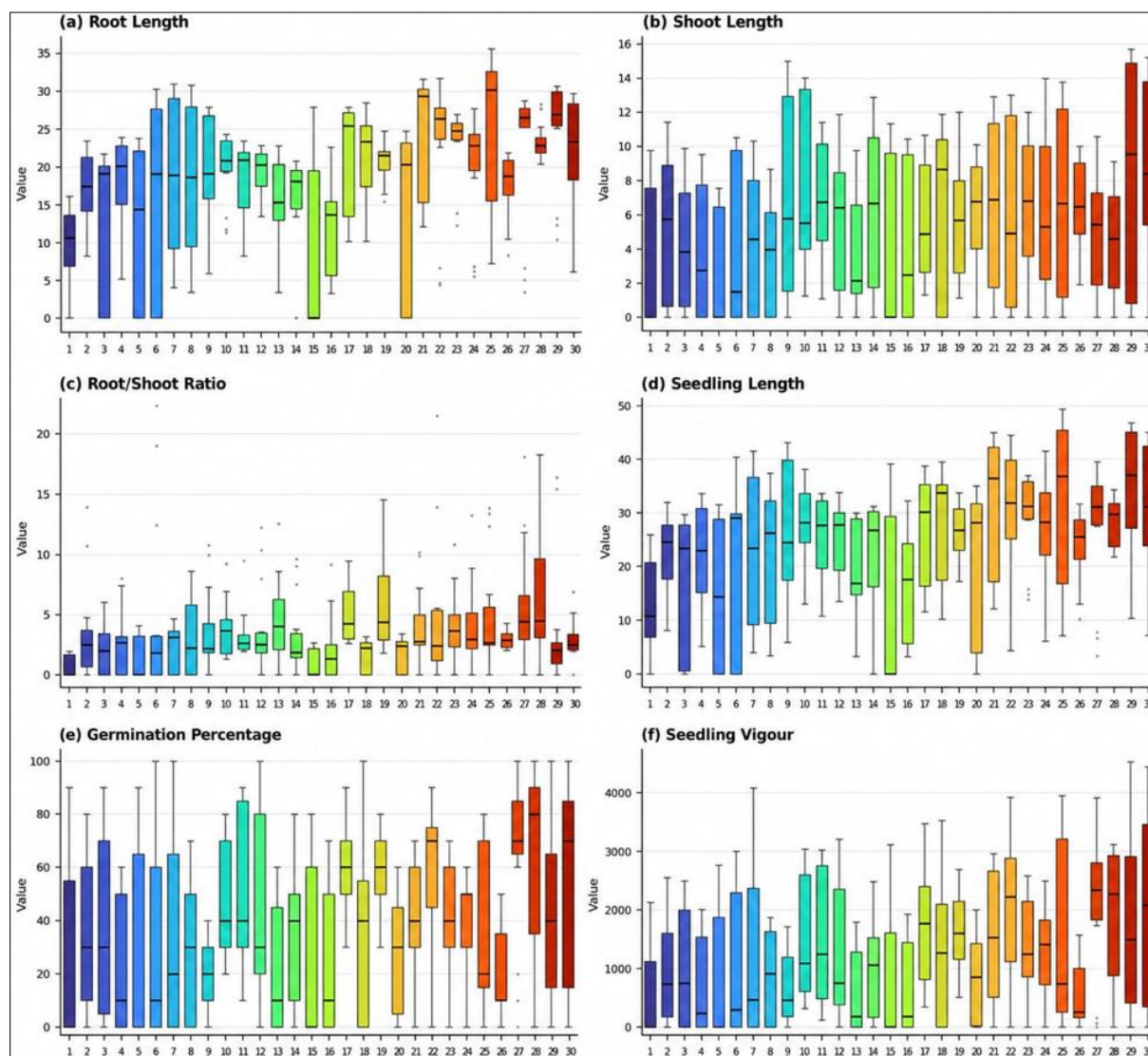


Fig 2: Studied traits for the thirty pearl millet genotypes: (a) root length, (b) shoot length, (c) root/shoot ratio, (d) seedling length, (e) germination percentage, (f) seedling vigour

Conclusion

It takes a lot of area and labour to screen for drought tolerance in the field and is influenced by environmental effects on the phenotypic expression of genotypes. *In vitro* screening is a good way to screen a wide range of germplasm correctly and with minimal effort (Kim *et al.*, 2001; Shrestha *et al.*, 2023) [10, 15]. Studying the impact of water stress on seed germination and seedling development characteristics has proven to be highly successful when using PEG to regulate external water potential (Hadas, 1976; Aquila *et al.*, 1984; Kim *et al.*, 2001; Van den Berg and Zeng, 2006; Radhouane, 2009) [6, 1, 10, 17, 12]. The study's findings showed that while modest PEG-induced drought stress was advantageous for growing root length, severe PEG-induced drought stress adversely affected all observable traits. According to reports, PEG-induced osmotic stress can lead to storage chemical hydrolysis, which further reduces the seed's internal osmotic potential (Sithole *et al.*, 2025; Hampson and Simpson, 1990) [16, 7].

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