Plant Archives Vol. 24, No. 2, 2024 pp. 891-898 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.125

EVALUATING SORGHUM GENOTYPES FOR TOLERANCE TO OSMOTIC STRESS DURING GERMINATION AND EARLY SEEDLING DEVELOPMENT

R. Navyashree[1]*, U.V. Mummigatti[1], P. Nethra[1], B. Basavaraj[2] and N.G. Hanamaratti[3]

[1]Department of Crop Physiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India.

[2]Department of Molecular Biology and Biotechnology, University of Agricultural Sciences, Dharwad, Karnataka, India.

[3]AICRP on Sorghum, MARS, University of Agricultural Sciences, Dharwad, Karnataka, India.

*Corresponding author E-mail : navyashreeshakti@gmail.com

(Date of Receiving-09-05-2024; Date of Acceptance-20-07-2024)

ABSTRACT

This study evaluates the tolerance of twenty sorghum genotypes to osmotic stress during germination and early seedling development using polyethylene glycol (PEG 6000) to simulate drought conditions. The genotypes were subjected to three levels of PEG-induced osmotic stress (0%, 0.5% and 1%) to assess their germination percentage, seedling length, seedling dry weight and vigor indices. The results revealed significant variability among genotypes. Tolerant genotypes, such as Savadatti-L and Murkibhavi-L, demonstrated superior performance, maintaining higher germination percentages, longer seedling lengths and greater seedling dry weights compared to sensitive genotypes like IS-4515. The mean germination percentage decreased with increasing PEG concentration, from 79.00% at 0% PEG to 60.09% at 1% PEG. Similarly, seedling length, dry weight and vigor indices were adversely affected by higher osmotic stress. Notably, Savadatti-L exhibited minimal reductions in root length and maintained the highest vigor indices under stress conditions. These findings underscore the potential of selecting drought-tolerant sorghum genotypes to enhance crop sustainability in water-limited environments, providing a foundation for future research on improving drought resistance in sorghum.

Key words : Sorghum, PEG, Seedling length, Vigour indices, Osmotic stress.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] stands as one of the most crucial cereal crops globally, earning the titles “King of Millets” and “Great Millet” due to its large grain size among the millets. This versatile crop is primarily cultivated for both food and fodder purposes, prized for its high yielding capacity, superior quality and palatability (Abderhim *et al.*, 2017). Its uses extend beyond food and fodder to include the preparation of alcoholic beverages, fibers, sugar, and syrup. Sorghum’s resilience and adaptability make it particularly valuable in arid and semi-arid regions, where other crops might fail.

Drought poses a significant threat to sorghum production by disrupting key physiological and biochemical processes such as chlorophyll synthesis, enzymatic activity and protein formation (Sanjari *et al.*, 2021). The challenge in drought-prone areas is to minimize yield losses, and genetic improvement for drought tolerance emerges as a long-term solution. Strategies for enhancing drought resistance include drought escape, drought avoidance and drought tolerance, with a focus on traits like root growth, leaf area development, epicuticular wax synthesis and osmotic adjustment under stress (Masuka *et al.*, 2017; Sanchez *et al.*, 2011).

Research on the soil-plant-water relationship in crops, including sorghum has demonstrated that growth and yield are directly influenced by the plant’s water deficit, which is affected by soil moisture. Water stress and dehydration impact canopy development, assimilation rates through the canopy, and the distribution of assimilates within plants (Mickelbart *et al.*, 2015). In sorghum, water stress reduces both the number of grains and grain size, with more severe yield reductions occurring during the flowering and grain-filling phases (Rattunde *et al*., 2016).

Screening genotypes at the seedling stage offers several benefits, including reduced costs, labor and early elimination of sensitive genotypes (Ram *et al*., 2020). Polyethylene glycol (PEG) with a molecular mass of 6000 or higher is an impenetrable, non-toxic osmotic agent that can lower water potential and simulate drought stress in plant tissues. PEG is considered a superior chemical for inducing water stress compared to others (Li *et al*., 2017). Increasing PEG concentration has been shown to decrease germination and seedling vigor in various crops (Sani and Boureima, 2014). Screening for drought tolerance at the seedling stage has been exploited in crops such as wheat (Boutraa *et al*., 2010), sorghum (Tsago *et al*., 2018), maize (Khodarahmpour, 2011) and sunflower (Ahmad *et al*., 2009). Given the critical importance of early-stage drought resistance, this research aims to screen advanced sorghum genotypes under in vitro moisture stress conditions generated by PEG 6000, specifically targeting Rabi sorghum genotypes. This study seeks to identify and develop sorghum varieties that can withstand drought stress, ensuring sustainable production in water-limited environments.

Materials and Methods

Twenty selected sorghum genotypes were subjected for comparison of germination and seedling growth in PEG 6000 solutions of three osmotic stress regimes in the department of crop physiology at the College of Agriculture in Dharwad. Polyethylene glycol (PEG) is a natural polymer with a molecular weight of 6000 that is both water-soluble and non-ionic. The water potential is lowered by PEG 6000 in a way that is similar to drought due to osmotic stress. In a completely randomised design (CRD), two replicated tests were conducted. Twenty distinct genotypes, three osmotic conditions, and a control were used in this experiment. The data was collected 14 days after the sorghum seeds were germinated after being treated. The following is a detailed description of the observations that were recorded.

Seed germination (%)

Sorghum seeds were surface sterilised with sodium hypochlorite solution (2%, v/v) for 5 minutes. After that, different concentrations of polyethylene glycol 6000 (PEG 6000) were applied to the seedlings. In order to maintain a control, distillated water was used. Two replicates of 50 seeds from each genotype are evenly distributed across two sheets of germination paper (Germitest®), which have been moistened with various PEG solutions in a volume equal to 2.5 times the paper's dried mass and rolled. The rolls are then sealed in plastic containers to prevent evaporation and maintain a humidity level close to 100 percent. 14 days of germination were conducted in a germinator at a constant temperature of 25°C (24-26°C) in the light. When the radicle length exceeds 5.0 mm, seeds are considered to have germinated. (Queiroz *et al*., 2019).

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Number of seeds put for germination}} \times 100$$

Root length (cm)

On the fourteenth day after the germination test, ten normal seedlings were chosen at random from all replications in each treatment. The root length was measured with a scale from the tip of the primary root to the base of the hypocotyl and the mean root length was expressed in centimetres (cm).

Shoot length (cm)

Shoot length was measured using ten normal seedlings that had previously been used to determine root length. Shoot length was measured from the tip of the primary leaf to the base of the hypocotyl and represented in centimetres (cm).

Seedling vigour indices

The seedling vigour index I was determined using the approach proposed by Abdul Baki and Anderson (1973) and expressed numerically using the formula below.

Seedling vigour index (I) = Germination (%) × Seedling length (cm)

The seedling vigour index II was calculated by multiplying the germination % by the dry weight of the seedlings and expressing the result as a whole number.

Seedling vigour index (II) = Germination (%) × Seedling dry weight (g)

Seedling dry weight (g)

After being air dried, ten normal seedlings that were still attached to their cotyledons were placed in a butter paper pocket and then placed in a hot air oven at a temperature of 70 degrees Celsius for twenty-four hours. These were used to measure the root and shoot lengths. The seedlings' dry weight was measured and recorded, and the result was given in grams (g).

Statistical analysis and Interpretation of data

The analysis and interpretation of data was done using the Fisher's method of analysis and variance technique as given by Panse and Sukhatme. The level of significance used in "F" and "t" test was at 5% probability level and wherever "F" test was found significant, the "t" test was performed to estimate critical differences among various treatments. Two factorial CRD (complete randomized design) was used to analyze data of germination studies

Results

Germination studies revealed that varying levels of osmotic stress significantly influenced several key parameters, including germination percentage, seedling length, seedling dry weight and vigor indexes I and II. These findings are detailed below.

Germination percentage

Table 1 displays the germination rates observed across different sorghum genotypes, osmotic concentrations and their interactions. The mean germination percentage was highest at 0% PEG (79.00%) and lowest at 1% PEG (60.09%). Among the 20 sorghum genotypes tested, Barsi Jawar and Lakamapur-L exhibited the highest mean germination percentages, both recorded 82.00%. These were followed by ICSR-13042, Ichangi-L and Murkibhavi-L (81.33%, 80% and 80%, respectively). In contrast, the IS-4515 genotype showed the lowest average germination rate (50.67%). In the absence of PEG (0% PEG), Ichangi-L and Barsi Jawar had the highest germination rates (96% and 94%, respectively). Under 1% PEG, the genotypes ICSR-13042 and Lakamapur-L maintained the highest germination rates, both showed 74%. Conversely, the IS-4515 genotype had a significantly lower germination rate of 40% under 1% PEG, which was notably lower compared to the other genotypes.

Shoot length

Table 1 made it evident that genotypes, osmotic pressures, and their interactions had a considerable impact on shoot length. The highest reported mean shoot length was observed at 0% PEG (24.52 cm), followed by 0.5% PEG (19.53 cm), with the lowest mean shoot length at 1% PEG (15.83 cm). The length of the seedlings varied significantly among the sorghum genotypes. Among the 20 sorghum genotypes, Savadatti-L and Murkibhavi-L had the longest mean shoot lengths (25.03 cm and 21.44 cm, respectively), while the genotype ICSR-13042 exhibited the shortest mean shoot length (12.32 cm). Under control conditions (0% PEG), the genotypes IS-4515 and Savadatti-L also had the longest shoots (29.60 cm and 29.03 cm, respectively). When exposed to 1% PEG, the genotype Savadatti-L had a shoot length of 21.4 cm, while ICSR-13042 showed the shortest shoot length of 9.42 cm.

Root length

Significant variation in root length was observed across different genotypes and osmotic pressures, as detailed in Table 1. Maximum root length of 20.91 cm was recorded at 0% PEG, with subsequent measurements of 16.61 cm at 0.5% PEG and 13.02 cm at 1% PEG. This study highlights notable differences in root length among genotypes. The Kalagunda-L genotype exhibited the longest average root length of 20.27 cm, followed closely by Lakamapur-L (20.21 cm) and EP-15 (18.71 cm). Conversely, the IS-4515 genotype had the shortest average root length (14.45 cm). At 1% PEG, Kalagunda-L maintained the greatest root length of 19.68 cm, while Savadatti-L recorded 15.70 cm, Lakamapur-L 15.28 cm, and EP-15 14.56 cm. In contrast, EP-19 had the shortest root length of 7.78 cm under the same osmotic stress. The percentage loss in root length at 1% PEG varied among genotypes, with Savadatti-L showing the lowest reduction at 8%, and EP-19 demonstrating the highest percentage decrease of nearly 64%.

Seedling dry weight

The impact of genotypes, osmotic pressures, and their interactions on the dry weight of seedlings is summarized in Table 2. There was a significant variation in mean seedling dry weight across different levels of osmotic stress. The highest mean seedling dry weight was observed at 0% PEG, measuring 0.18 g. This was followed by a mean dry weight of 0.15 g at 0.5% PEG. The mean seedling dry weight decreased to 0.13 g under 1% PEG. Among the genotypes, Savadatti-L exhibited the highest mean seedling dry weight of 0.22 g, followed by Barsi Jawar and Murkibhavi-L, both with a mean dry weight of 0.18 g. In contrast, the genotype ICSR-13042 had the lowest mean seedling dry weight of 0.09 g. Significant differences were also observed in seedling dry weight due to genotype and osmotic pressure interactions. Specifically, Savadatti-L (0.19 g) and Murkibhavi-L (0.16 g) achieved the highest mean seedling dry weight under 1% PEG stress. Conversely, ICSR-13042 had the lowest mean seedling dry weight of 0.07 g under the same osmotic condition.

Vigour Index I

Significant variations in vigour index I were observed across different genotypes, osmotic stress levels and their interactions (Table 2). The vigour index I decreased with increasing osmotic pressure. The highest mean vigour index I was recorded at 0% PEG, with a value of 3475.57, which was significantly greater than the 0.5% PEG level (2429.04). The vigour index I values were lowest under 1% PEG, reaching a mean value of 1653.82. Among the

Table 1 : Effect of osmotic stress on germination percentage, shoot length and root length in sorghum genotypes.

	Genotypes	Germination percentage (%)				Root length (cm)				Shoot length (cm)			
		0 %PEG (Control)	0.5% PEG	1 % PEG	Mean	0 %PEG (Control)	0.5% PEG	1 % PEG	Mean	0 %PEG (Control)	0.5% PEG	1 % PEG	Mean
1	Murkibhavi - L	92.00	78.00	70.00	80.00	26.09	13.01	12.46	17.19	27.90	20.18	16.25	21.44
2	Lakamapur- L	88.00	84.00	74.00	82.00	24.36	20.99	15.28	20.21	25.63	20.83	16.53	21.00
3	Billigunda- L	90.00	80.00	58.00	76.00	19.60	16.99	14.18	16.92	25.98	20.47	14.11	20.19
4	Barsi Jawar	94.00	86.00	66.00	82.00	16.24	15.84	13.96	15.35	23.37	21.06	17.24	20.56
5	Dagadi sokapur	90.00	78.00	64.00	77.33	22.22	20.26	13.20	18.56	23.04	20.47	16.66	20.06
6	Savadatti- L	82.00	74.00	58.00	71.33	17.13	17.80	15.70	16.88	29.03	24.66	21.40	25.03
7	Ichangi – L	96.00	78.00	66.00	80.00	20.70	16.52	13.34	16.85	24.69	19.78	17.48	20.65
8	Kalagunda- L	80.00	70.00	68.00	72.67	22.76	19.48	18.56	20.27	24.21	20.26	18.96	21.14
9	NRD- L	76.00	60.00	52.00	62.67	21.20	17.20	13.42	17.27	22.70	19.10	15.90	19.23
10	EP-75	60.00	56.00	46.00	54.00	17.56	16.44	12.44	15.48	23.08	18.00	13.41	18.17
11	EP-95	70.00	66.00	64.00	66.67	19.42	14.34	12.35	15.37	26.11	22.11	16.08	21.43
12	EP-117	66.00	62.00	60.00	62.67	22.91	18.90	14.13	18.65	22.95	18.13	14.58	18.56
13	EP-80	66.00	54.00	50.00	56.67	18.90	14.59	11.77	15.09	23.25	18.23	16.70	19.39
14	EP-19	76.00	60.00	56.00	64.00	21.69	15.35	7.78	14.94	22.63	17.62	16.03	18.76
15	EP-15	82.00	76.00	72.00	76.67	24.75	16.82	14.56	18.71	24.40	19.40	16.12	19.97
16	RSLG-23	78.00	72.00	60.00	70.00	25.13	15.43	11.23	17.26	23.20	17.71	16.77	19.23
17	IS-4698	74.00	60.00	54.00	62.67	19.47	14.20	10.07	14.58	28.39	19.58	15.04	21.00
18	IS-5094	74.00	66.00	58.00	66.00	21.31	16.64	13.77	17.24	25.29	20.48	13.95	19.91
19	IS-4515	60.00	52.00	40.00	50.67	18.36	15.10	9.88	14.45	29.60	20.04	13.90	21.18
20	ICSR-13042	86.00	84.00	74.00	81.33	18.49	16.20	12.39	15.69	15.00	12.54	9.42	12.32
	Mean	79	69.8	60.5	69.77	20.91	16.61	13.02	16.85	24.52	19.53	15.83	19.96
		S.Em. ±		CD @5%		S.Em. ±		CD @5%		S.Em. ±		CD @5%	
	Main plot (M)	0.250104		0.697932		0.062013		0.173051		0.036287		0.101263	
	Sub Plot (P)	0.055925		0.156062		0.013866		0.038695		0.008114		0.022643	
	Interaction	0.433192		1.208853		0.107409		0.299734		0.062852		0.175392	

Table 2 : Effect of osmotic stress on seedling dry weight, vigour index I and vigour index II in sorghum genotypes.

	Genotypes	Seedling dry weight (g)				Vigour index I				Vigour index II			
		0 %PEG (Control)	0.5% PEG	1 % PEG	Mean	0 %PEG (Control)	0.5% PEG	1 % PEG	Mean	0 %PEG (Control)	0.5% PEG	1 % PEG	Mean
1	**Murkibhavi - L**	0.20	0.19	0.16	0.18	4979.96	2553.72	2053.80	3195.83	18.40	14.59	11.48	14.82
2	**Lakamapur- L**	0.17	0.11	0.08	0.12	2264.22	1496.64	749.36	1503.41	8.91	5.28	3.04	5.74
3	**Billigunda- L**	0.19	0.16	0.13	0.16	4113.90	3004.80	1644.88	2921.19	16.65	13.12	7.25	12.34
4	**Barsi Jawar**	0.21	0.18	0.14	0.18	3734.62	3182.86	2065.14	2994.21	19.55	15.74	9.50	14.93
5	**Dagadi sokapur**	0.18	0.17	0.14	0.16	4063.50	3169.14	1905.92	3046.19	16.56	13.03	8.90	12.83
6	**Savadatti- L**	0.24	0.22	0.19	0.22	3797.42	3150.92	2158.18	3035.51	19.68	16.50	10.79	15.66
7	**Ichangi – L**	0.17	0.15	0.14	0.15	4368.96	2839.20	2040.06	3082.74	16.13	11.62	9.11	12.29
8	**Kalagunda- L**	0.18	0.14	0.14	0.15	3767.20	2788.80	2558.16	3038.05	14.48	10.08	9.18	11.25
9	**NRD- L**	0.20	0.17	0.12	0.16	3064.32	2152.44	992.80	2069.85	12.54	9.34	4.60	8.83
10	**EP-75**	0.16	0.13	0.12	0.14	2445.60	1933.68	1192.32	1857.20	9.60	7.00	5.38	7.33
11	**EP-95**	0.20	0.16	0.14	0.17	3178.00	2398.44	1814.40	2463.61	13.93	10.30	9.09	11.11
12	**EP-117**	0.20	0.17	0.14	0.17	3019.50	2290.28	1718.40	2342.73	13.33	10.54	8.64	10.84
13	**EP-80**	0.19	0.16	0.14	0.16	2773.98	1767.42	1419.50	1986.97	12.61	8.75	7.10	9.49
14	**EP-19**	0.20	0.13	0.11	0.15	3376.68	1983.60	1337.84	2232.71	14.82	7.80	6.27	9.63
15	**EP-15**	0.18	0.14	0.10	0.14	4040.14	2760.32	2214.72	3005.06	14.76	10.26	7.06	10.69
16	**RSLG-23**	0.18	0.13	0.10	0.14	3760.38	2379.60	1675.20	2605.06	13.81	8.78	6.00	9.53
17	**IS-4698**	0.18	0.16	0.14	0.16	3531.28	2020.80	1352.16	2301.41	13.02	9.30	7.34	9.89
18	**IS-5094**	0.15	0.13	0.10	0.13	3458.02	2456.52	1611.82	2508.79	11.10	8.71	5.80	8.54
19	**IS-4515**	0.19	0.15	0.10	0.15	2886.60	1832.48	954.00	1891.03	11.28	7.96	4.00	7.75
20	**ICSR-13042**	0.11	0.09	0.07	0.09	2887.02	2419.20	1617.64	2307.95	9.03	7.81	5.03	7.29
	Mean	**0.18**	**0.15**	**0.13**	**0.15**	**3475.57**	**2429.04**	**1653.82**	**2519.47**	**14.01**	**10.33**	**7.28**	**10.54**
		S.Em. ±		**CD @5%**		**S.Em. ±**		**CD @5%**		**S.Em. ±**		**CD @5%**	
	Main plot (M)	**0.000569**		**0.001587**		**9.39777**		**26.22513**		**0.039378**		**0.109886**	
	Sub Plot (P)	**0.000127**		**0.000355**		**2.101405**		**5.864118**		**0.008805**		**0.024571**	
	Interaction	**0.000985**		**0.002748**		**16.27742**		**45.42326**		**0.068204**		**0.190329**	

genotypes, Murkibhavi-L, Ichangi-L and Dagadi Sokapur exhibited the highest mean vigour index I values of 3195.83, 3082.74, and 3046.19, respectively. In contrast, genotype Lakamapur-L had a significantly lower mean vigour index I of 1503.41. In terms of genotype and osmotic pressure interactions, Kalagunda-L showed the highest vigour index I under 1% PEG, with a value of 2558.16. This was followed by EP-15, Savadatti-L, Barsi Jawar, and Murkibhavi-L, which had vigour index I values of 2214.72, 2158.18, 2065.14 and 2053.80, respectively. Conversely, genotype Lakamapur-L had a significantly lower vigour index I of 749.36 under 1% PEG. Additionally, when compared to the control condition, genotype NRD-L demonstrated the highest percentage reduction in vigour index I, approximately 67%, at 1% PEG concentration.

Vigour Index II

Table 2 displays the influence of genotype, osmotic concentration, and their interactions on vigour index II. Significant variations in vigour index II were observed across different osmotic stress levels. The highest mean vigour index II was recorded at 0% PEG with a value of 14.01, followed by 0.5% PEG at 10.33. The vigour index II decreased markedly under 1% PEG, with an average value of 7.28, indicating a statistically significant reduction. Among the genotypes, Savadatti-L exhibited the highest mean vigour index II of 15.66, followed by Barsi Jawar at 14.93, and Murkibhavi-L at 14.82. In contrast, genotype Lakamapur-L showed the lowest vigour index II value of 5.74.

Interaction effects revealed further significant differences. Under 0% PEG, Savadatti-L had the highest vigour index II of 19.68, followed by Barsi Jawar with 19.55 and Murkibhavi-L with 18.40. At 0.5% PEG, the highest vigour index II was observed in Savadatti-L (16.5). At 1% PEG, Murkibhavi-L exhibited the highest vigour index II of 11.48, while Lakamapur-L demonstrated the lowest vigour index II of 3.04.

Discussion

The present study aimed to assess the drought stress tolerance among different sorghum genotypes during the germination and early seedling stages. Our findings confirm significant variability in the responses of sorghum genotypes to osmotic stress induced by PEG. As observed, seed germination rates declined from 79.00% under control conditions (0% PEG) to 69.5% under severe osmotic stress (1% PEG). This reduction underscores the impact of drought stress on sorghum's early developmental stages, a critical phase for crop establishment and yield potential (Hind *et al.*, 2016). The ability of sorghum genotypes to maintain consistent germination rates across varying osmotic stress levels highlights their differing levels of tolerance. Tolerant genotypes such as Savadatti-L and Murkibhavi-L exhibited minimal reductions in seed germination (Fig. 1), shoot length, root length (Fig. 2) and vigour index (Fig. 3) under higher osmotic potentials, suggesting robust

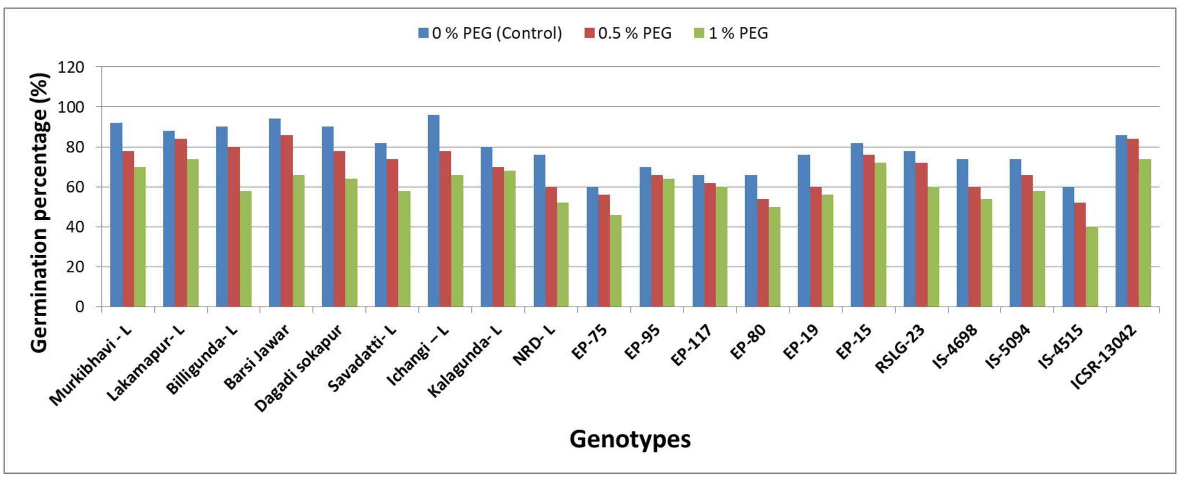

Fig. 1 : Effect of osmotic stress on germination percentage of sorghum genotypes.

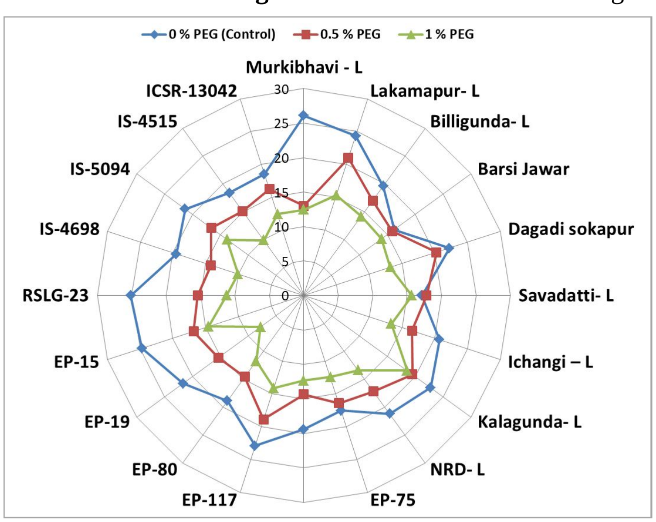

Fig. 2 : Effect of osmotic stress on root growth of sorghum seedlings.

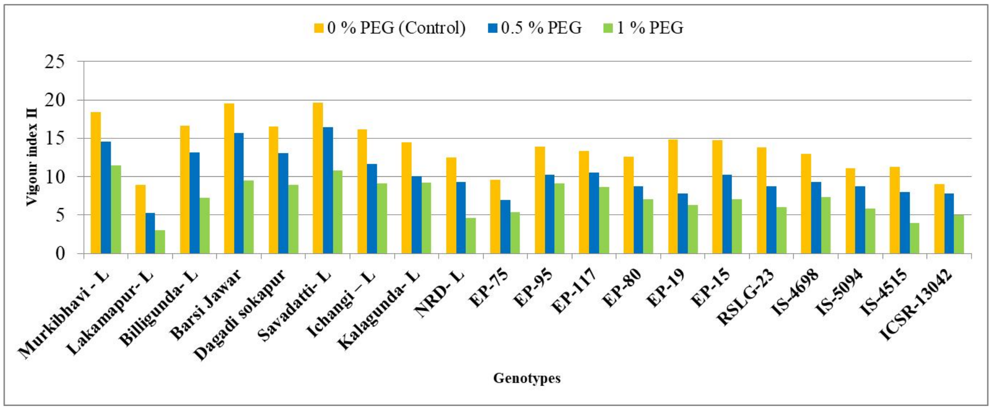

Fig. 3 : Effect of osmotic stress on vigour index II of sorghum genotypes.

performance under adverse conditions (Irawati *et al.*, 2017; Rezende *et al.*, 2017). Conversely, sensitive genotypes displayed pronounced decreases in these parameters, indicating susceptibility to drought-induced osmotic stress.

Our results align with previous studies indicating that drought stress negatively impacts various morphological and physiological traits during the seedling stage of sorghum (Bibi *et al.*, 2018). The observed reduction in shoot and root growth under severe osmotic stress can be attributed to impaired water imbibition and reduced enzymatic activity critical for germination (Rajani *et al.*, 2018). Among the genotypes tested, Savadatti-L and Murkibhavi-L demonstrated superior drought tolerance, with the highest seedling dry weights (0.19g and 0.16g, respectively) under 1% PEG. Savadatti-L also showed the highest vigour index II at 0.5% PEG (16.5), while Murkibhavi-L achieved the highest vigour index II (11.48) at 1% PEG. For future research on drought tolerance in sorghum, genotypes such as Kalagunda-L, Savadatti-L, EP-95, EP-117, and Barsi Jawar are recommended based on their minimal reductions in germination percentage, seedling length, and vigour index under 1% PEG concentration compared to control conditions. Future investigations focusing on the molecular and physiological mechanisms underlying the drought tolerance of these genotypes could provide valuable insights for enhancing sorghum resilience to water stress in agricultural settings.

Conclusion

The study demonstrates significant variability among sorghum genotypes in their response to PEG-induced osmotic stress during germination and early seedling growth. Germination capacity decreased as PEG concentration increased, highlighting the adverse effects of osmotic stress. Tolerant genotypes like Savadatti-L and Murkibhavi-L maintained higher seedling dry weight and vigour index II values, showcasing their resilience. Savadatti-L exhibited the highest tolerance with minimal root length reduction at 1% PEG. Genotypes such as Kalagunda-L, Savadatti-L, EP-95, EP-117, and Barsi Jawar showed promising drought resilience based on minimal reductions in germination percentage, seedling length, and vigour index. These findings underscore the importance of selecting drought-tolerant sorghum genotypes to ensure sustainable production in the face of climatic challenges. Further research on these resilient genotypes is recommended to enhance drought resistance strategies.

Acknowledgement

We express our sincere gratitude to all contributors who played a pivotal role in the successful completion of our research article on the "Evaluating sorghum genotypes for tolerance to osmotic stress during germination and early seedling development". Our heartfelt thanks go to the University of Agricultural Sciences, Dharwad, for providing essential infrastructure and unwavering support throughout the study. The Department of Crop Physiology, College of Agriculture, Dharwad, deserves special recognition for their valuable guidance, resources and a conducive research environment.

Conflict of interest

Authors have declared that no conflict of interest exist.

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