Assessment of Drought Tolerance in Rabi Sorghum Genotypes Under Polyethylene Glycol-Induced Osmotic Stress

Abstract

Sorghum is a vital cereal crop globally, especially in arid and semi-arid regions of India, where rabi sorghum is critical for food security. This study investigates the impact of polyethylene glycol (PEG 6000)-induced osmotic stress on the germination, seedling growth, and vigor of twenty rabi sorghum genotypes. PEG 6000, a non-toxic osmotic agent, was utilized to simulate drought conditions by reducing water potential. The genotypes were subjected to three osmotic stress regimes (0%, 0.5%, and 1% PEG) and evaluated for germination percentage, root and shoot length, seedling dry weight, and seedling vigor indices. Results demonstrated significant variations in germination and seedling performance across genotypes and osmotic stress levels. The highest germination percentage was observed in ICSV-15017 (86.67%) under control conditions, while the lowest was in Annigeri-1 (23.33%) under 1% PEG. Shoot and root lengths decreased with increasing PEG concentration, with ICSV-15017 and SVD-1419 consistently showing superior growth under stress. Seedling dry weight and vigor indices also varied significantly, with DSV-5 and SVD-1419 exhibiting higher values compared to others under osmotic stress. The study identifies ICSV-15017, ParbaniMotti, and DSV-4 as the most drought-tolerant genotypes, based on minimal reductions in germination percentage, seedling length, and vigour index under PEG-induced stress. In contrast, Annigeri-1 and ICSV-16006 showed limited drought tolerance. These findings highlight the effectiveness of PEG-induced osmotic stress for screening drought-tolerant sorghum genotypes and provide valuable insights for developing drought-resistant varieties in water-scarce regions.

Key words: Sorghum, PEG, osmotic stress,germination percentage, seedling dry weight.

1. Introduction

Sorghum [Sorghum bicolor (L.) Moench] is one of the most important crop in the world. It is known by various names such as Great millet and Guinea corn in West Africa, Kafir corn in South Africa, Dura in Sudan, and Jowar in India. The greatest diversity of cultivated and wild sorghum is found in Africa [4]. Sorghum ranks as the fifth most important cereal crop worldwide after wheat, corn, rice, and barley. It is cultivated in both kharif (rainy) and rabi (post-rainy) seasons in India, with rabi sorghum primarily used for human consumption and kharif sorghum for animal feed, starch, and alcohol industries. Only 5% of sorghum in India is irrigated, with over 48% of the cultivation area in Maharashtra and Karnataka. Rabi sorghum is a crucial post-rainy cereal crop in Peninsular India, especially in the semi-arid Deccan Plateau region, which includes Maharashtra, Karnataka, and Andhra Pradesh. However, rabi sorghum productivity is declining due to limited and erratic rainfall, causing moisture stress during critical growth stages and significant yield losses [9]. Therefore, adequate soil moisture is essential for successful crop production in arid regions.

Drought significantly impacts essential physiological and biochemical processes in plants, such as chlorophyll destruction, enzymatic activity, and protein synthesis. Minimizing crop yield losses in drought-prone areas is challenging, with genetic improvement for drought tolerance being a long-term strategy. Effective drought resistance strategies include drought escape or avoidance, as well as improving drought tolerance [12]. Key traits for characterizing drought-tolerant sorghum genotypes include root growth, leaf area development, epicuticular wax synthesis, and osmotic adaptation under stress [17]. Water stress affects various developmental stages, notably germination, shoot length, and root length, with significant damage observed during these critical phases [7]. Screening germplasm for drought tolerance during the seedling stage has been explored in crops like wheat [3], sorghum [19], maize [8], and sunflower [2].

Polyethylene glycol (PEG) with a molecular mass of 6000 and higher is a non-toxic, impenetrable osmotic substance used to reduce water potential and simulate drought stress in plant tissues. As a polymer, PEG is more effective than other chemicals in inducing water stress [10]. Increasing PEG concentrations have been shown to decrease germination and seedling vigor in certain crops [18]. Screening genotypes at the seedling stage offers several advantages, including low cost, ease of handling, reduced labor, and early elimination of susceptible genotypes. This study aims to assess the effect of osmotic stress on germination and vigor in rabi sorghum genotypes.

2. 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.

2.1 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, distilled 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. [14]

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

2.2 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).

2.3 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).

2.4 Seedling vigour indices

The seedling vigour index I was determined using the approach proposed by Abdul Baki and Anderson [1] and expressed numerically using the formula below.

$$\text{Seedling vigour index (I)} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

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.

$$\text{Seedling vigour index (II)} = \text{Germination (\%)} \times \text{Seedling dry weight (g)}$$

2.5 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).

2.6 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 [13]. 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

3. Result and discussion

3.1 Germination percentage

Water is essential for initiating metabolic processes in seeds. In the field, the seed environment is complex, and factors such as different genotypes and osmotic stress can negatively impact seed water uptake. Critical levels of water potential, especially at the onset of imbibition, adversely affect this uptake [15]. Table 1 displays the germination rates observed across various sorghum genotypes, osmotic concentrations, and their interactions. The mean germination percentage was highest at 0% PEG (69.50%) and lowest at 1% PEG (51.6%). Among the 20 sorghum genotypes tested, ICSV-15017 exhibited the highest mean germination percentage (86.67%), followed by SVD-1419, Giddmaladandi, and SVD-1353 (76.67%, 72.7%, and 72.67%, respectively). Conversely, the Annigeri-1 genotype showed the lowest average germination rate (23.33%). PEG, a hydrophilic polymer, hinders germination and seedling growth by reducing the availability of free water to seeds, thereby limiting their water uptake and subsequent imbibition [6]. Under control conditions (0% PEG), ICSV-15017 and SVD-1419 had the highest germination rates (90% and 86%, respectively). At 0.5% PEG, these genotypes maintained the highest germination rates (88% and 78%, respectively) and they continued to show the best performance under 1% PEG, with germination rates of 82% and 66%, respectively, compared to other genotypes. In contrast, ICSV-16006 and Annigeri-1 exhibited significantly lower germination rates of 16% under 1% PEG, which was considerably lower than the germination percentage observed in other genotypes.

3.2 Shoot length

Table 1 reveals that both genotypes and osmotic pressures significantly impact shoot length. The highest mean shoot length was recorded at 0% PEG (25.25 cm), followed by 0.5% PEG (18.73 cm), and the lowest mean shoot length at 1% PEG (13.66 cm). PEG disrupts cellular processes essential for seedling growth and development, affecting cell elongation and overall seedling length [16]. Among the 20 sorghum genotypes evaluated, Tansulwadi-L and SVD-1418 exhibited the longest mean shoot lengths (23.95 cm and 22.58 cm, respectively), while Annigeri-1 had the shortest mean shoot length (14.49 cm). Under control conditions (0% PEG), Tansulwadi-L and SVD-1418 had the longest shoot lengths (43.44 cm and 28.11 cm, respectively), whereas ICSV-16006 had the shortest (20.68 cm). At 0.5% PEG, SVD-1418 maintained the highest shoot length (23.24 cm). When exposed to 1% PEG, ParbaniMotti achieved the highest shoot length (16.8 cm), while Annigeri-1 exhibited the lowest shoot lengths at both 0.5% PEG and 1% PEG (14.69 cm and 7.74 cm, respectively).

3.3 Root length

According to Tsagoet al. [19], PEG 6000 influences germination percentage and morphophysiological traits such as root and aerial part lengths. Table 1 illustrates significant variation in root length across different sorghum genotypes and osmotic pressures. The maximum mean root length of 22.03 cm was observed under control conditions (0% PEG), followed by 17.22 cm at 0.5% PEG and 12.6 cm at 1% PEG. This study underscores substantial differences in root length among genotypes. DSV-5 exhibited the longest average root length of 20.01 cm, followed closely by ParbaniMotti (19.89 cm) and Giddmaladandi (19.76 cm). In contrast, Annigeri-1 displayed the shortest average root length at 12.99 cm. Significant genotype and osmotic stress interactions were observed, with SVD-1353 showing the highest root length under control (0% PEG) at 25.6 cm, while SVD-1325 had the lowest at 18.2 cm. At 0.5% PEG, Giddmaladandi recorded the highest root length (21.65 cm), and DSV-5 exhibited the highest root length at 1% PEG (17.45 cm). Conversely, Annigeri-1 consistently showed the lowest root lengths under both 0.5% and 1% PEG (13.03 cm and 7.53 cm, respectively). Islam et al. [5] similarly investigated PEG's impact on root parameters in maize cultivars, observing that all root parameters exhibited the highest values under control conditions and decreased significantly with increasing PEG concentrations.

3.4 Seedling dry weight:

PEG impacts seedling dry weight by limiting water availability crucial for metabolic processes, resulting in reduced biomass accumulation [6].Table 2 summarizes the influence of genotypes, osmotic pressures, and their interactions on seedling dry weight. Significant variation in mean seedling dry weight was evident across different osmotic stress levels. The highest mean seedling dry weight of 0.20 g was recorded under control conditions (0% PEG), followed by 0.17 g at 0.5% PEG and0.14 g at 1% PEG.Among the genotypes, DSV-5 had the highest mean dry weight of 0.22 g, followed by SVD-1325 (0.21 g) and CSV-22R (0.20 g). In contrast, Annigeri-1 had the lowest mean dry weight at 0.12 g.Significant differences were observed in genotype interactions with osmotic pressure. Under 1% PEG stress, DSV-5 (0.18 g) and SVD-1325 (0.18 g) maintained the highest seedling dry weights, whereas Annigeri-1 had the lowest seedling dry weight of 0.09 g.

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

	Germination percentage (%)	Root length (cm)	Shoot length (cm)

	Genotypes	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	ICSR-13043	78.00	62.00	56.00	65.33	21.26	13.41	12.99	15.89	23.18	16.22	13.74	17.71
2	ICSR-13004	64.00	52.00	46.00	54.00	20.87	19.93	13.61	18.14	23.43	16.66	15.29	18.46
3	ICSV-16006	40.00	30.00	16.00	28.67	18.97	14.84	10.73	14.85	20.68	18.71	10.80	16.73
4	ICSV-15016	70.00	66.00	60.00	65.33	19.64	15.76	11.95	15.78	21.88	17.60	12.39	17.29
5	ICSV-15017	90.00	88.00	82.00	86.67	20.13	15.44	13.25	16.27	21.97	17.70	13.02	17.56
6	SVD – 0813	54.00	46.00	38.00	46.00	23.27	16.39	11.12	16.93	24.25	19.18	16.53	19.99
7	SVD – 1325	54.00	46.00	36.00	45.33	18.20	16.45	13.10	15.92	25.28	18.66	14.56	19.50
8	SVD – 1353	84.00	72.00	62.00	72.67	25.60	16.00	13.92	18.51	24.60	17.44	11.82	17.95
9	SVD – 1418	76.00	68.00	58.00	67.33	25.14	17.24	11.92	18.10	28.11	23.24	16.40	22.58
10	SVD – 1419	86.00	78.00	66.00	76.67	20.91	15.87	12.86	16.55	25.67	23.18	16.50	21.78
11	SVD – 1603	54.00	48.00	38.00	46.67	18.50	13.47	7.68	13.22	23.43	17.71	12.04	17.73
12	CSV- 22R	82.00	74.00	58.00	71.33	24.48	19.09	14.00	19.19	26.82	20.97	15.59	21.13
13	DSV- 5	78.00	66.00	58.00	67.33	25.32	17.25	17.45	20.01	27.69	20.20	13.91	20.60
14	DSV- 4	78.00	66.00	58.00	67.33	23.47	20.83	14.35	19.55	22.51	16.95	13.83	17.76
15	Phulerevathi	58.00	44.00	36.00	46.00	22.27	18.65	13.12	18.01	23.49	19.07	12.92	18.49
16	Parbanimotti	80.00	72.00	64.00	72.00	23.73	19.60	16.33	19.89	26.04	20.33	16.80	21.06
17	Annigeri- 1	36.00	24.00	16.00	25.33	18.41	13.03	7.53	12.99	21.03	14.69	7.74	14.49
18	Giddmaladandi	82.00	72.00	64.00	72.67	24.74	21.65	12.88	19.76	25.19	19.23	13.14	19.19
19	PKV-Kranthi	74.00	68.00	60.00	67.33	21.87	19.08	11.06	17.34	26.28	20.10	14.35	20.24
20	Tansulwadi-L	72.00	66.00	60.00	66.00	23.84	20.42	12.18	18.81	43.40	16.68	11.76	23.95
Mean		69.50	60.40	51.60	60.50	22.03	17.22	12.60	17.28	25.25	18.73	13.66	19.21
		S.Em. ±		CD @5%		S.Em. ±		CD @5%		S.Em. ±		CD @5%	

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
Main plot (M)	0.227313		0.634332		0.064375		0.179643		0.073168		0.20418	
Sub Plot (P)	0.050829		0.141841		0.014395		0.040169		0.016361		0.045656	
Interaction	0.393717		1.098696		0.111501		0.311151		0.12673		0.35365	

3.5 Vigour index I

Osmotic stress reduces the seedling vigor index by impairing water uptake, which is crucial for growth processes and plant health. This stress leads to decreased cell turgor, reduced metabolic activity, and slower growth, resulting in a lower vigor index [3]. Significant variations in vigor index I were observed across different genotypes, osmotic stress levels, and their interactions (Table 2). The vigor index I declined with increasing osmotic pressure, with the highest mean vigor index I of 3379.33 recorded at 0% PEG. This value was significantly higher than at 0.5% PEG (2233.27) and 1% PEG (1428.28). Among the genotypes, SVD-1419, ParbaniMotti, and CSV-22R had the highest mean vigor index I values of 2994.15, 2992.29, and 2962.42, respectively. In contrast, Annigeri-1 and SVD-1603 had significantly lower mean vigor index I values of 760.16 and 978.99, respectively.Regarding genotype and osmotic pressure interactions, SVD-1419 (3038.29) followed by CSV-22R (2964.44) showed the highest vigor index I under 0.5% PEG. Under 1% PEG, ICSV-15017 exhibited the highest vigor index I of 2151.2, followed by ParbaniMotti, SVD-1419, and DSV-5 with vigor index I values of 2120.32, 1932.92, and 1818.88, respectively. Conversely, genotype Annigeri-1 recorded significantly lower vigor index I values of 665.28 under 0.5% PEG and 195.36 under 1% PEG.

3.6 Vigour index II

Table 2 showed the effects of genotype, osmotic concentration, and their interactions on vigor index II. Significant variations in vigor index II were observed across different osmotic stress levels, with the highest mean value recorded at 0% PEG (14.58), followed by 0.5% PEG (10.48). Vigor index II decreased markedly under 1% PEG, averaging 7.35. According to Magar et al. [11], key traits such as germination percentage, root length, and vigor index are critical for assessing drought tolerance during germination and seedling establishment. The seed vigor index is particularly useful for reflecting drought resistance and germination characteristics under stress.Among the genotypes studied, SVD-1419 recorded the highest mean vigor index II of 16.14, followed by DSV-5 (15.15) and ICSV-15017 (15.01). Conversely, genotype Annigeri-1 displayed the lowest vigor index II value of 5.74.Interaction effects further revealed significant differences: under 0% PEG, SVD-1419 recorded the highest vigor index II of 21.79, followed by DSV-5 with 20.75, and CSV-22R with 19.43. At 0.5% PEG, the highest vigor index II values were observed in SVD-1419 and

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	ICSR-13043	0.17	0.13	0.12	0.14	3466.32	1837.06	1496.88	2266.75	13.57	8.18	6.72	9.49
2	ICSR-13004	0.18	0.16	0.12	0.15	2835.20	1902.68	1329.40	2022.43	11.33	8.53	5.70	8.52
3	ICSV-16006	0.18	0.16	0.13	0.16	3319.68	2166.60	1516.32	2334.20	13.83	9.54	6.76	10.04
4	ICSV-15016	0.21	0.13	0.10	0.15	2903.78	2199.78	1461.71	2188.42	14.41	8.77	5.83	9.67
5	ICSV-15017	0.19	0.17	0.16	0.17	3792.41	2917.67	2151.20	2953.76	16.94	15.14	12.94	15.01
6	SVD – 0813	0.19	0.17	0.17	0.18	2566.08	1636.22	1050.70	1751.00	10.37	8.00	6.38	8.25
7	SVD – 1325	0.23	0.22	0.18	0.21	2347.92	1615.06	995.76	1652.91	12.64	9.94	6.62	9.73
8	SVD – 1353	0.20	0.19	0.14	0.18	4216.80	2407.68	1595.88	2740.12	16.88	13.46	8.74	13.03
9	SVD – 1418	0.25	0.18	0.16	0.20	4047.00	2752.64	1642.56	2814.07	19.30	12.38	9.34	13.67

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**
10	SVD – 1419	0.25	0.21	0.15	0.20	4011.24	3038.29	1932.92	2994.15	21.79	16.49	10.14	16.14
11	SVD – 1603	0.18	0.17	0.11	0.15	1586.00	1006.50	344.48	978.99	7.28	4.95	1.81	4.68
12	CSV- 22R	0.24	0.19	0.16	0.20	4206.60	2964.44	1716.22	2962.42	19.43	14.13	9.51	14.36
13	DSV- 5	0.27	0.22	0.18	0.22	4134.78	2471.70	1818.88	2808.45	20.75	14.26	10.44	15.15
14	DSV- 4	0.20	0.19	0.17	0.19	3586.44	2493.48	1634.44	2571.45	15.44	12.34	9.69	12.49
15	Phulerevathi	0.22	0.20	0.14	0.19	2654.08	1659.68	937.44	1750.40	12.93	8.93	4.97	8.94
16	Parbanimotti	0.19	0.15	0.13	0.16	3981.60	2874.96	2120.32	2992.29	15.20	10.94	8.00	11.38
17	Annigeri- 1	0.15	0.13	0.09	0.12	1419.84	665.28	195.36	760.16	5.40	3.00	1.41	3.27
18	Giddmaladandi	0.18	0.15	0.12	0.15	4094.26	2943.36	1665.28	2900.97	14.84	10.66	7.49	11.00
19	PKV-Kranthi	0.18	0.13	0.12	0.14	3563.10	2664.24	1524.60	2583.98	12.95	8.91	7.14	9.67
20	Tansulwadi-L	0.23	0.17	0.12	0.17	4853.38	2448.04	1435.20	2912.21	16.38	11.09	7.37	11.61
Mean		0.20	0.17	0.14	0.17	3379.33	2233.27	1428.28	2346.96	14.58	10.48	7.35	10.81
		S.Em. ±		CD @5%		S.Em. ±		CD @5%		S.Em. ±		CD @5%	
Main plot (M)		0.00064		0.001787		9.41168		26.26395		0.043151		0.120415	
Sub Plot (P)		0.000143		0.0004		2.104516		5.872798		0.009649		0.026926	
Interaction		0.001109		0.003096		16.30151		45.49049		0.074739		0.208564	

ICSV-15017 (16.49 and 15.14, respectively). Under 1% PEG, ICSV-15017 exhibited the highest vigor index II of 12.94, while Annigeri-1 demonstrated the lowest at 1.41.

4. Conclusion

The study assessed the impact of osmotic stress induced by polyethylene glycol (PEG 6000) on germination, seedling growth and seedling vigor in 20 rabi sorghum genotypes. The results highlight significant genotype-by-stress interactions, demonstrating the variability in drought tolerance among the genotypes. ICSV-15017, ParbaniMotti, SVD-0813, DSV-4, and ICSR-13004 emerged as the most promising genotypes, exhibiting superior drought resilience. These genotypes maintained relatively high germination percentages, seedling lengths, and vigor indices under varying levels of osmotic stress, compared to the control. Specifically, ICSV-15017 consistently showed high germination rates and vigor indices across all PEG concentrations, indicating its potential for breeding drought-tolerant varieties. Similarly, ParbaniMotti and DSV-4 performed well, suggesting their potential for enhancing drought resilience in rabi sorghum. In contrast, genotypes such as Annigeri-1 and ICSV-16006 demonstrated lower performance under osmotic stress, indicating their limited drought tolerance. This study underscores the utility of PEG-induced osmotic stress as an effective screening method for identifying drought-tolerant sorghum genotypes. The findings provide valuable insights for the development of sorghum varieties that can sustain productivity under water-limited conditions, crucial for enhancing food security in drought-prone regions.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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