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Screening sorghum genotypes for osmotic stress tolerance during seed germination and seedling growth

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Abstract
Drought has an impact on some critical physiological and biochemical processes in plants. The most difficult problem in drought-prone areas is minimizing crop yield losses. Inducing osmotic stress invitro with polyethylene glycol (PEG) is an alternative technique. Polyethylene glycols with molecular masses of 6000, which is impermeable, non-toxic osmotic polymer compound that can be utilized to reduce water potential and induce drought stress in plant tissues. In the current study, 20 sorghum genotypes were tested with two different osmotic levels using PEG and the control. Water stress influences practically every step of plant development. The data were collected 14 days after the treatment. The germination percent, seedling length, and vigour index were significantly reduced with PEG compared to control. The genotypes also exhibited significant variation for osmotic stress. The genotypes Chitapur L, Phule anuradha, M-35-1, Tadur L and Basavan pada showed minimum reduction with osmotic stress and can be recommended for future research on sorghum's drought tolerance.

Keywords: Osmotic stress, PEG, germination, seedling length and vigour index

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the world's most vital commodities. It is one of the most important cereals in the world and is commonly referred to as "King of Millets" or "Great Millet" due to its enormous grain size in comparison to other millets. In West Africa, it is known as Great millet and Guinea maize, in South Africa as Kafir maize, in Sudan as Dura, and in India as Jowar. Its origins are in Africa (Khaton *et al*., 2016) [7]. Africa contains the greatest variety of cultivated and wild sorghum (Doggett, 2018) [4]. It is the fifth most important cereal crop worldwide, following wheat, maize, rice, and barley (Ogbaga *et al*., 2016) [10]. As because of its higher fibre content (2.5-6%), sorghum is acquiring popularity as a healthy food. It contains 72.6 percent carbohydrates, 10 to 12 percent protein, 1.6% minerals, and 1.9% lipids. It is rich in amino acids, particularly lysine, thiamine, riboflavin, and folic acid, as well as vitamin B complexes, particularly niacin (vitamin B6). It contains significant amounts of nitrogen (212 mg), carbohydrate (5.6% to 7.3%), copper, zinc, and molybdenum. Its protein in bran and germ fractions has four times the amount of lysine and twice the amount of arginine and glycine as endosperm protein (Baldaniya *et al*., 2021) [2].

Important physiological and biochemical processes in plants, such as chlorophyll degradation, enzymatic activity, and protein synthesis, are affected by drought (Queiroz *et al*., 2019) [11]. The greatest difficulty in drought-prone regions is minimising crop yield losses. Low water potential and increased drought stress in plant tissues can be induced by using polyethylene glycols of molecular mass 6000 or greater, which are impermeable and non-toxic osmotic compounds (Sintayehu *et al*., 2018) [14]. Polyethylene glycol (PEG) has been found to be an effective chemical for inducing water stress (Kaur *et al*., 2018) [8]. Some crops have lower germination rates and less vigorous seedlings after being exposed to higher PEG concentrations (Khodarahmpour, 2011) [9]. There are many benefits to screening genotypes while they are still seedlings, including reduced costs, reduced labour, and early eradication of sensitive genotypes (Ram *et al*., 2020) [13]. The primary goal of this research was to screen advanced sorghum genotypes *in vitro* moisture stress generated by PEG 6000 in Rabi sorghum genotypes, an experiment was conducted in a controlled environment.

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) [11].

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

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 (%) x 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 (%) x 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).

Results

It was found through germination studies that the levels of osmotic stress had a major impact on germination %, seedling length, seedling dry weight, and vigour indexes I and II as described below.

Germination percentage

The table 1 below shows the wide range of germination rates we observed across genotypes, osmotic concentrations, and their interactions. When comparing the osmotic levels, the mean germination percentage was highest (76.80%) at 0% PEG, then lowest (59.20%) at 1% PEG. Among the 20 sorghum genotypes, SVD 1272 exhibited the highest mean germination percentage (92.67), followed by Chitapur L (90.67), SPV-486 (88.67), and SVD-1358R (88.67). In contrast, the average germination rate for the M138-148 genotype was only 25.33 percent. In the absence of PEG, germination rates for Basavan pad, Chitapur-L, and SVD-1272 were all above 98%. The SVD - 1272 genotype also had the highest germination rate in all three interaction conditions (PEG concentrations of 0%, 0.5, and 1%). The M148-138 genotype had a significantly lower germination rate (12%) under 1% PEG compared to the rest of the genotypes.

Shoot length

Table 1 makes it evident that genotypes, osmotic pressures, and their interactions have a considerable impact on shoot length. The highest reported mean shoot length was at 0% PEG (25.34 cm), followed by 0.5% PEG (20.40 cm), and the lowest was at 1% PEG (15.51 cm). The length of the seedlings varied significantly among sorghum genotypes as well. In comparison to Phule Annuradha and Basavan pad (23.09 cm and 23 cm, respectively), the genotype chitapur L had the longest mean shoot length (23.79 cm), followed by Tandur - L (23.54 cm). The M148-138 genotype, in contrast to other genotypes, had the smallest mean shoot length (11.39 cm). The genotypes BJV-44 and SVD-1528R also had the longest shoots under control (29.75 and 29.04 cm, respectively), among the interaction levels. When exposed to 1% PEG, genotype M148-138 exhibited the shortest shoot length (6.80 cm).

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	SVD-1272R	96.00	94.00	88.00	92.67	21.63	17.54	14.61	17.93	27.68	22.07	16.06	21.94
2	SVD-1358R	94.00	92.00	80.00	88.67	26.73	22.14	18.59	22.49	26.57	20.87	14.28	20.57
3	SVD-1528R	94.00	86.00	78.00	86.00	25.53	15.65	16.04	19.07	29.04	20.87	16.01	21.97
4	SVD-1403R	60.00	54.00	52.00	55.33	21.79	14.73	8.96	15.16	25.09	22.52	14.43	20.68
5	SPV-486	94.00	88.00	84.00	88.67	23.71	18.39	13.42	18.51	27.18	22.40	17.36	22.31
6	SPV-2217	86.00	74.00	66.00	75.33	24.88	22.18	16.95	21.34	26.33	20.76	17.80	21.63
7	CSV-216R	86.00	78.00	64.00	76.00	25.67	19.91	14.39	19.99	26.72	22.50	17.72	22.31
8	CSV-29R	64.00	54.00	40.00	52.67	21.71	17.88	10.08	16.56	26.17	21.98	14.74	20.96
9	ICSR-15001	88.00	84.00	82.00	84.67	23.38	17.03	15.20	18.54	23.71	19.15	15.00	19.29
10	Basavan pad	98.00	82.00	76.00	85.33	18.58	15.52	14.93	16.34	27.36	22.45	19.20	23.00
11	Tandur L	84.00	76.00	64.00	74.67	18.79	18.11	14.57	17.16	24.81	24.80	21.02	23.54
12	Phule annuradha	82.00	74.00	62.00	72.67	24.66	20.44	17.51	20.87	28.46	21.07	19.75	23.09
13	Chitapur – L	96.00	94.00	82.00	90.67	24.19	23.78	19.68	22.55	25.61	23.87	21.88	23.79
14	DKS- 35	78.00	66.00	58.00	67.33	25.32	17.25	17.45	20.01	27.69	20.20	13.91	20.60
15	M-35-1	80.00	72.00	64.00	72.00	23.96	18.01	18.68	20.22	25.29	19.46	15.74	20.16
16	M 148-138	36.00	28.00	12.00	25.33	11.82	7.62	6.29	8.58	15.51	11.85	6.80	11.39
17	Basavan motti	40.00	30.00	16.00	28.67	18.97	14.84	10.73	14.85	20.68	18.71	10.80	16.73
18	Phule vasudha	54.00	48.00	38.00	46.67	18.50	13.47	7.68	13.22	23.43	17.71	12.04	17.73
19	BJV-44	60.00	52.00	40.00	50.67	18.36	15.10	9.88	14.45	29.75	20.14	13.97	21.29
20	ICSR- 13025	66.00	50.00	38.00	51.33	19.65	13.82	10.16	14.54	19.74	14.63	11.67	15.35
Mean		76.80	68.80	59.20	68.27	21.89	17.17	13.79	17.62	25.34	20.40	15.51	20.42
		S.Em. +		LSD @1%		S.Em. +		LSD @1%		S.Em. +		LSD @1%	
Genotypes (G)		1.257021		4.304836		0.410515		1.301264		0.482439		1.425336	
Treatment (T)		0.251031		0.96755		0.114606		0.319817		0.120237		0.33436	
G x T		2.940214		7.591020		0.765436		2.370289		1.008814		2.615165	

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	SVD-1272R	0.26	0.21	0.20	0.22	4749.91	3735.96	2699.18	3728.35	24.95	19.81	17.60	20.79
2	SVD-1358R	0.31	0.22	0.20	0.24	5008.15	3952.24	2612.77	3857.72	28.66	20.49	16.06	21.74
3	SVD-1528R	0.30	0.25	0.22	0.26	5129.58	3140.72	2499.90	3590.07	28.39	21.07	17.24	22.23
4	SVD-1403R	0.28	0.25	0.15	0.23	2812.80	2011.50	1216.28	2013.53	17.04	13.50	7.90	12.81
5	SPV-486	0.28	0.26	0.24	0.26	4783.66	3589.52	2831.76	3734.98	26.60	22.44	22.17	23.74
6	SPV-2217	0.28	0.24	0.17	0.23	4404.06	3177.56	2293.50	3291.71	24.08	17.46	11.42	17.65
7	CSV-216R	0.26	0.21	0.16	0.21	4506.04	3298.62	2049.57	3284.74	22.53	15.94	10.34	16.27
8	CSV-29R	0.20	0.17	0.12	0.16	3064.32	2152.44	992.80	2069.85	12.54	9.34	4.60	8.83
9	ICSR-15001	0.20	0.19	0.17	0.18	4143.92	3039.12	2476.40	3219.81	17.16	15.96	13.86	15.66
10	Basavan pad	0.24	0.21	0.17	0.21	4502.12	3113.54	2593.88	3403.18	23.72	16.81	13.15	17.89
11	Tandur L	0.22	0.19	0.18	0.20	3662.40	3261.16	2277.76	3067.11	18.06	14.59	11.52	14.72
12	Phule annuradha	0.27	0.26	0.20	0.24	4355.84	2988.72	2310.12	3218.23	21.89	18.43	12.65	17.66
13	Chitapur – L	0.18	0.16	0.15	0.16	4780.80	4479.10	3407.92	4222.61	17.38	15.42	11.89	14.89
14	DKS- 35	0.19	0.18	0.13	0.17	4512.60	3470.04	2869.60	3617.41	17.85	14.70	10.08	14.21
15	M-35-1	0.23	0.21	0.19	0.21	3940.00	2697.84	2202.88	2946.91	18.00	14.98	11.90	14.96
16	M 148-138	0.13	0.10	0.07	0.10	816.48	403.20	103.80	441.16	4.50	2.69	0.80	2.66
17	Basavan motti	0.18	0.17	0.11	0.15	1586.00	1006.50	344.48	978.99	7.28	4.95	1.81	4.68
18	Phule vasudha	0.17	0.11	0.08	0.12	2264.22	1496.64	749.36	1503.41	8.91	5.28	3.04	5.74
19	BJV-44	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- 13025	0.17	0.13	0.09	0.13	2599.74	1422.50	829.54	1617.26	11.35	6.40	3.42	7.06
Mean		0.23	0.19	0.16	0.19	3725.46	2713.47	1915.78	2784.90	18.11	13.91	10.27	14.10
		S.Em. +		LSD @1%		S.Em. +		LSD @1%		S.Em. +		LSD @1%	
Genotypes (G)		0.005277		0.014725		87.64669		244.5842		0.44547		1.134728	
Treatment (T)		0.00118		0.003293		19.5984		54.69069		0.094932		0.224913	
G x T		0.00914		0.025504		151.8085		423.6323		0.695337		1.952009	

Root length

As can be observed in Table 1, there was a significant amount of variation in root length that was caused by genotypes, osmotic pressures, and the interactions between both of them. Maximum root length (21.89 cm) was found at 0% PEG, followed by 0.5% PEG (17.17 cm), and least root length (13.79 cm) was found at 1% PEG. There was also a big difference in root length between the different genotypes. The Chitapur-L genotype had the longest average root length (22.55 cm), followed by SVD-1358 (22.49 cm) and SPV-

2217 (21.34 cm). Compared to the other genotypes, the M148-138 genotype had the shortest average root length (8.58 cm). In addition, the Chitapur-L genotype had the largest root length (19.68 cm) at 1% PEG, followed by the M35-1 genotype (18.68 cm), the SVD - 1358 genotype (18.59 cm), and the Phule Annuradha genotype (17.51 cm). Genotype M148-138, however, had the shortest root length (6.29 cm) at the same osmotic level than the remaining genotypes. Additionally, the percentage loss in root length caused by 1% PEG was lowest for the Basavanpad genotype compared to the other genotypes, while the SVD-1403R genotype showed the biggest percentage reduction (almost 50%) at this osmotic stress level.

Seedling dry weight

Genotypes, osmotic pressures, and their interactions all had an impact on the dry weight of seedlings (Table 2). Depending on the level of osmotic stress, the mean dry weight of the seedlings differed significantly. 0% PEG was found to produce the highest mean seedling dry weight (0.23 g), which was followed by 0.5% PEG (0.19 g). The mean seedling dry weight (0.16 g) was much lower under 1% PEG, though. The genotypes with the highest mean seedling dry weight (0.26 g) were SVD-1528 and SPV-486, which were followed by Phule Annuradha and SVD-1358 (0.24 g). While the mean seedling dry weight of the genotype M148-138 (0.10 g) was much lower. Similar significant changes in seedling weight were found at interaction. The Phule Annuradha, SVD-1358, and SVD-1272 genotypes all had seedling dry weights of about 0.20 g, whereas the SPV-486 genotype had the maximum seedling dry weight of 0.24 g when grown in 1% PEG. Additionally, M148-138 (0.07 g) exhibited the least seedling dry weight when treated with 1% PEG.

Vigour Index I

Based on the vigour index I, significant variations in genotypes, osmotic levels, and their interactions have been detected (Table 2). The vigour index decreased as osmotic levels went up. The 0% PEG (3725.46) had a considerably higher mean vigour index I than the 0.5% PEG (2713.47). The mean vigour index I (1915.78) was significantly lower with 1% PEG. The genotypes SVD-1358, SPV-486, and DSK-35 (3857.72, 3734.98, and 3617.41, respectively) exhibited the greatest mean vigour index I of all the genotypes. The mean vigour index I value for genotype M148-138 (441.16) was significantly lower. Furthermore, among the interaction levels, the genotype Chitapur- L under 1% PEG exhibited the greatest vigour index I value (3407.92), followed by the genotypes DSK- 35, SPV- 486, SVD - 1272, and SVD - 1358 under the same osmotic level (2869.60, 2831.76, 2699.18, and 2612.77, respectively). Meanwhile, the genotype M148-138 (103.80) displayed a significantly lower vigour index I under 1% PEG. Furthermore, when compared to the control condition, M148-138 showed the maximum percentage reduction at 1% PEG concentration.

Vigour Index II

Table 2 displays the effect that genotype, osmotic concentration, and their interactions have on the vigour index II. The osmotic values for the vigour index II varied significantly. The mean vigour index II was highest at 0% PEG (18.11), followed by environments with 0.5% PEG (13.91). With 1% PEG, the average vigour index II value dropped to 10.27, a statistically significant decrease. SPV-486 exhibited the highest mean vigour index II value (23.74), followed by SVD-1528 (22.23), and SVD-1358 (21.74). The M148-138 (2.66) showed the lowest vigour index II value. Eventually, the interaction levels revealed significant differences in relation to the vigour index II. Under 0% PEG, the genotype with the highest vigour index II value was SVD-1358 (28.66), followed by SVD-1528 (28.39) and SPV-486 (26.66). Also, at 0.5% and 1% PEG, the SPV-486 genotype exhibited the highest vigour index II values (22.44 and 22.17, respectively). Under 1% PEG, however, the genotype M148-138 demonstrated a lower vigour index II (0.80).

Discussion

As predicted, different genotypes differed greatly in their resistance to PEG-induced drought stress. Seed germination is a key transition stage for crop plants from seeds to seedlings, and increased seed germination under stressful and benign climatic conditions allows plants to thrive and provide larger yields under both adverse and benign conditions. Osmotic stress during the germination process has been examined to determine whether plant species are drought-resistant. Since the main effects of crops and osmotic potentials interact significantly for most of the evaluated attributes, this suggests that plant species respond differently to varying degrees of osmotic stress. In order to identify reasonably tolerant genotypes of sorghum, we conducted tests on 20 different sorghum varieties during the germination and early seedling growth stages. At the germination stage, significant variance is seen across different genotypes. After being subjected to osmotic stress, the seeds' germination capacity dropped from 76.82% to 59.2% (from 0% PEG to 1% PEG). In contrast to tolerant sorghum genotypes, which showed little variation in germination ability over a range of osmotic stress concentrations, osmotic-sensitive sorghum genotypes had a low vigour index (Irawati *et al*. 2017) [6]. Similar findings demonstrated that sweet sorghum genotypes were susceptible to osmotic stress, which in turn affected germination and early growth of the seedlings (Rezende *et al*., 2017).

According to Bibi *et al*. 2010 [3], water stress in sorghum affects the majority of the morphological and physiological traits at the seedling stage. More than root growth was reduced by drought stress, and occasionally root growth even accelerated (Hind *et al*, 2016) [5]. Since drought stress directly inhibits growth by reducing cell division and elongation, reduced seedling growth is the result of restricted cell division and enlargement (Rajani *et al*, 2018) [12]. In the current work, reduced seed germination, seedling length (Fig 1) and vigour index (Fig 2) under higher negative osmotic potential is associated with poorer water imbibition and, as a result, reduced enzyme activity required for seed germination. For upcoming research on sorghum's ability to withstand drought, the genotypes SVD1272 and SVP486 can be suggested based on germination percentage and seedling length for 1% PEG concentration.

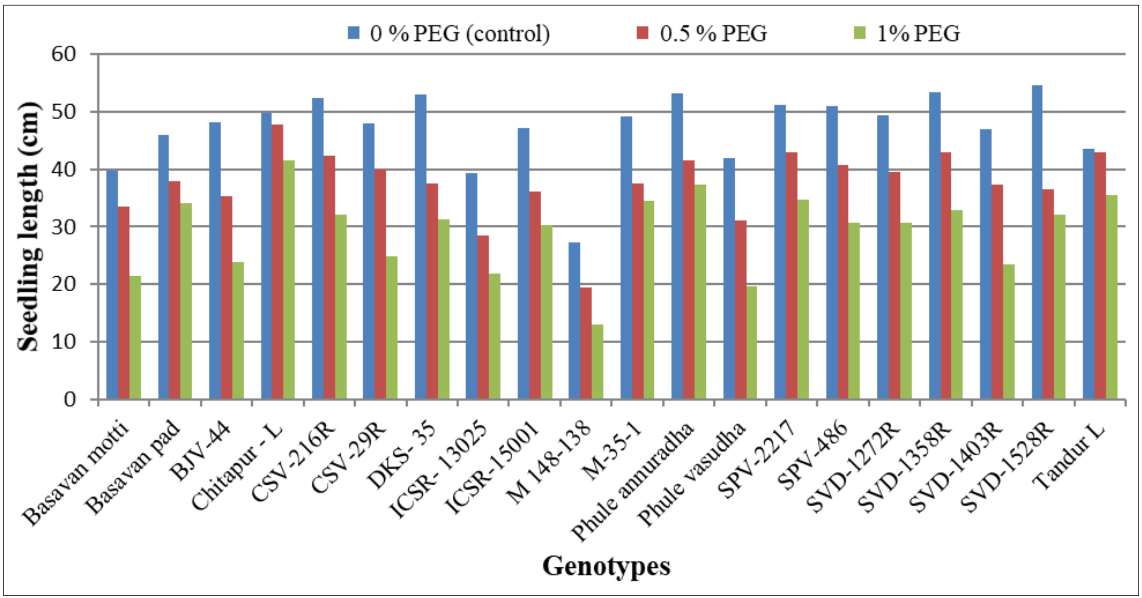

Fig 1: Effect of PEG on seedling length of sorghum genotypes

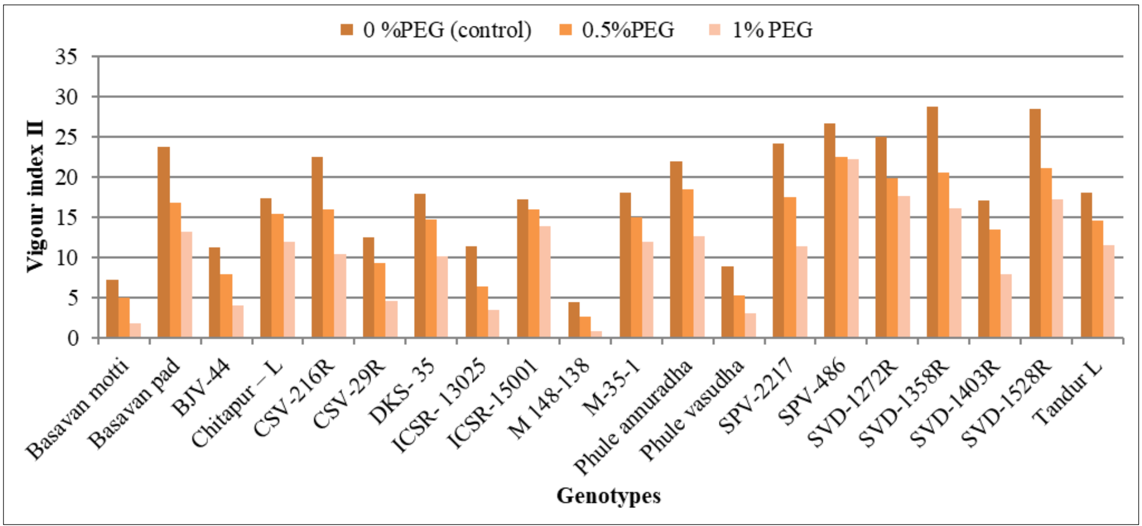

Fig 2: Impact of polyethylene glycol (PEG)-induced stress on sorghum seed vigour index

Conclusion

The tolerance of different genotypes to PEG-induced moisture stress varied significantly. When compared to the control treatment, the highest reduction in seed germination and growth parameters was observed at 1% PEG osmotic potential. The sorghum genotypes Chitapur L, Phule anuradha, and Tandur L showed higher resistance to moisture stress than the other genotypes studied. SVD 1358 and SVP2217 were considered as sensitive genotypes because they showed a lot of variation in germination and seedling growth under osmotic stress.

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