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Effect of polyethylene glycol induced water stress on germination and seedling development of rabi sorghum genotypes

PN Bobade, SB Amarshettiwar, TH Rathod, RB Ghorade, NV Kayande and YM Yadav

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PN Bobade
Ph.D. Student Department of Botany, PGI, Dr. PDKV, Akola, Maharashtra, India

SB Amarshettiwar
Professor and Asso. Dean College of Agriculture, Gadchiroli, Maharashtra, India

TH Rathod
Professor, Department of Botany, PGI, Dr. PDKV, Akola, Maharashtra, India

RB Ghorade
Senior Research Scientist, Sorghum Research Unit, Dr. PDKV, Akola, Maharashtra, India

NV Kayande
Assistant Cotton Breeder, Cotton Research Unit, Dr. PDKV, Akola, Maharashtra, India

YM Yadav
Ph.D. Student Department of Botany, PGI, Dr. PDKV, Akola, Maharashtra, India

Abstract

Thirteen different genotypes of *rabi* sorghum (*Sorghum bicolor* var. Moench) were evaluated for moisture stress tolerance at seedling stage using -0 (control), -0.066, -0.10, -0.13 and -0.16 MPa non-ionic water soluble polymer polyethylene glycol of molecular weight 6000 and the data was recorded on various seedling parameters like germination percent, root length, RLSTI, shoot length, SLSTI, seedling dry weight, SDWSTI and seed vigour. The experiment was carried out in two replicates under Complete Randomized Design. Different levels of moisture stress induced by PEG-6000 had significantly affected seed germination. Data indicated that increase in osmotic stress caused a significant decrease in germination percentage in all genotypes under study. Maximum germination was (55%), root length (5.32 cm), shoot length stress tolerant index (41.89 %) and seed vigour (521.4) was observed in genotype CSV-29R and shoot length (4.46 cm), shoot length stress tolerant index (21.55 %) and seedling dry weight (0.033 mg) was highest recorded in Elongvan-19 genotype at highest PEG concentration -0.16 MPa. From the overall results it was concluded that the promising genotypes CSV-29R, Elongvan-19, M 35-1 and Phule Maulee showed comparatively better performance than other genotypes under higher concentration of PEG induced water stress. The Parbhani Moti and CSV-22R genotypes were highly affected in comparison to other genotypes at higher PEG concentration. Hence these better genotypes may categorized as stress tolerant during seedling stage.

Keywords: *Sorghum bicolor* L., polyethylene glycol (PEG), water stress, germination per cent, seedling traits

Introduction

Sorghum (*Sorghum bicolor* L.) is one the most important cereal grain crop in the world, popularly known as “King of Millets” or “Great Millet” as an account of its large grain size among millets. Sorghum has been in India since prehistoric times and there is a record of sorghum being growing in Assynia as early as 700 BC. Evidence indicates that centre of origin of sorghum is East Africa in Ethiopia (Southern border west word to Chand) and then it spread to India, China, Middle East and Europe. Sorghum ranks fifth, among the world cereal food crops after rice, wheat, maize and barley (Anonymous, 2012) [6]. Sorghum is main cereal crop of Maharashtra. The state has total area of 25.31 Lakh hectares (2016-17) of which area 4.92 Lakh hectare and 20.21 Lakh hectares are grown in *kharif* and *rabi* season with production 4.95 Lakh tonnes in *kharif* and 17.40 Lakh tonnes in *rabi* and productivity of 1007 kg per hectare in *kharif* and 861 kg per hectare in *rabi* season. (Anonymous, 2017) [7].

Rabi sorghum is the most important post rainy season cereal crop in peninsular India, grown predominantly under rainfed conditions. There is decline in the productivity of *rabi* sorghum under rainfed areas. Limited and erratic rainfall in the rainfed area creates moisture stress conditions during the various critical growth stages of crop life, resulting in severe yield reduction (Massacci *et al*., 1996) [21]. They limit the photosynthesis and consequently, limited availability of photosynthetic assimilates and energy to the plant. Apparently, under drought stress conditions, an urgent need for plants would be to increase the uptake of water, which is usually more available deep down in the soil (Xiong *et al*. 2006) [29]. Water stress affects almost every developmental stage of the plant. However, damaging effects of this stress was more noted when it coincided with various growth stages such as germination; seedling shoot length, root length and flowering (Bibi, *et al*. 2012) [9]. Rauf (2008) [23] narrated several benefits of screening genotypes at seedling stages such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at earliest.

Correspondence
PN Bobade
Ph.D. Student Department of Botany, PGI, Dr. PDKV, Akola, Maharashtra, India

Field experiments related to water stress has been difficult to handle due to significant environmental or drought interactions with other abiotic stresses. An alternate approach to field experiments related to moisture stress is to induce stress using polyethylene glycol (PEG) *in vitro* condition. Polyethylene glycols with molecular mass of 6000 and above are non-penetrable and non-toxic osmotic substance which can be used to lower the water potential and stimulates drought stress in plant tissue. PEG is polymer and considered as better chemical than other to induce water stress (Kaur *et al.*, 1998) [16].

Materials and Methods

Thirteen genotypes of sorghum (*Sorghum bicolor* L.) including three checks were chosen on the basis of their morphological and agronomic diversity. The healthy and uniform seeds were washed thoroughly in water, surface sterilized with 0.1 % (w/v) $HgCl_2$ for about 15 min and then washed 2-3 times with distilled water. The seeds were then kept for germination on germination paper in sterilized petri plates, containing different concentrations of PEG-6000 solution such as 0 (distilled water), -0.066, -0.10, -0.13 and -0.16 MPa., which were prepared as per the method of Sairam and Kumari (1998) [25]. Five ml of these solutions of respective concentration was added in each petri plate. The control was maintained with distilled water. All the sets were arranged in duplicate under uniform condition in seed germination chamber and observation on germination percent, shoot length, root length, seedling dry weight and seed vigour were recorded by using standard method on eight days after sowing. The data were statistically analysed by using completely randomize design.

Results and Discussion

Water stress due to drought is one of the most significant abiotic factors that limit the seed germination, seeding growth, plants growth and yield (Hartmann *et al.*, 2005, Van den Berg and Zeng, 2006) [15, 27]. Several methods have been developed to screen drought tolerant germplasm in plant species. Based on the literature available, PEG is considered as a superior chemical to induce water stress (Kaur *et al.*, 1998) [16]. The PEG inhibited the germination of the susceptible lines and caused them in record low germination percentage. Almaghrabi (2012) [3] reported that environmentally confined seedlings in laboratory experiments would appear to be suitable for screening large population to improve drought tolerance prior to yield testing.

Data indicated that increase in osmotic stress caused a significant decrease in germination percentage in all genotypes (table.1). Under non stress (0 MPa) genotype E-19 recorded maximum (100.0 %) and genotype Parbhani Moti recorded minimum (85.0%) germination per cent and under stress (-0.066, -0.10 and -0.13 MPa) condition. Genotypes CSV-29R (90.0%, 85.0% and 75.0%, respectively) recorded highest per cent germination and lowest germination was recorded in Parbhani Moti (75.0%, 60.0%, 30.0% and 20.0%, respectively). At higher concentration, -0.16 MPa genotype CSV-29 reported maximum germination per cent (55.0%) and being at par with E-19 (50.0%) and Phule Maulee (40.0%) followed by remaining genotypes.

The results of Table 1 showed that the speed of germination was reduced with the increment of water deficit stress but the degree of reduction in rate of germination was not similar for all genotypes at moderate and higher water deficit stress compared to control. The PEG inhibited the germination of the susceptible genotypes and caused them in record low germination percentage. Usually the drought tolerant genotype will have the highest germination rate and better survival. The higher germination rates of the tolerant germplasm may be due to their capability to absorb water even under PEG induced water stress. Dodd and Donavon, (1999) [11] stated that PEG induced reduction in germination percentage was because of reduction in the water potential gradient between seeds and their surroundings.

Results of the current study were in agreement with Mut and Akay (2010) [22] in oat and Govindaraj *et al.* (2010) [14]. Almansouri *et al.* (2001) [4] in wheat it was observed that germination percentage with decreasing water potential of the environment probably was caused by the low hydraulic conductivity of the environment, where PEG 6000 makes water unavailable to seeds, affecting the imbibitions process of the seed which is fundamental for germination. Check M-35-1 a drought tolerant genotype had germination percentage comparatively less than CSV-29R and E-19 at all osmotic potential and more to germinate at -0.16 MPa.

In present investigation all the genotypes showed common trend i.e. reduction rate in shoot length with increasing concentration of PEG. Increases osmotic potential lead to an increase in shoot length only at -0.066 MPa in some genotype, but later on it decreases in all genotypes with increasing osmotic potential.

At higher (-0.16 MPa) osmotic stress condition, highest shoot length was recorded by E-19(4.46 cm) being comparable with CSV-29R (4.16 cm), M 35-1 (4.13 cm) and M 35-1 (3.83 cm) as well as highest SLSTI was noted with the genotype CSV-29R (21.48 %) and found on par with E-19 (21.56%) and M 35-1 (20.25 %). Whereas, the genotypes CSV-22R and Parbhani Moti were recorded least shoot length and shoot length stress tolerance index (Table2).

The root length in all the genotypes decreased from -0.66 to -0.16 MPa PEG induced water stress. The maximum root length and RLSTI noted under CSV-29R (5.32 cm and 41.89%) followed by remaining genotypes and found comparable with E-19 (7.35 cm, 38.31%), Phule Maulee (6.85cm and 38.09%) and M 35-1 (4.45 cm and 35.35%), respectively. Lowest root length and RLSTI was recorded in genotypes E-42 and Parbhani Moti.

A strong negative correlation between shoot length and PEG concentration and a positive correlation between shoot length and root length noted as well as it clearly indicated that increase in root length helps in increase of shoot length reported by Basha *et.al.* (2015) [8] in sorghum. Drought stress greatly affected the hypocotyl length rather than growth of radical which indicates that the length of hypocotyl was more sensitive to drought stress (Kumar *et al.*, 2011) [20]. Under drought stress condition, the root develops faster than the hypocotyls to acclimatize the drought stress (Kumar *et al.*, 2011) [20]. Therefore, the growth of radical and hypocotyls reflects the adaptability of plant to drought stress (Zhu *et al.*, 2006) [30]. Thus, root plays a role in plant survival during drought and also drought tolerant can be characterized by extensive root growth and reduction of shoot growth. The variability in the decreasing trend of osmotic regulation of the genotypes indicates the genotypic variability in response to water deficit stress. Similar finding were reported by Takele (2000) [26], Ambika *et al.* (2011) [5] and Khodarahumpour (2011) [19] in sorghum.

As osmotic stress increases seedling dry weight decreased. At highest concentration of -0.16 MPa osmotic stress condition genotype CSV-29R (0.032 mg) recorded highest dry weight seedling^{-1} followed by remaining genotypes and found at par with E-19 (0.033 mg), Phule Maulee (0.031 mg) and M 35-1 (0.029 mg) and genotype Phule Maulee (67.39%) recorded highest seedling dry weight stress tolerance index followed by CSV-29R (61.54%), M 35-1 (60.42%) and E-19 (60.0%) over general mean and the lowest seedling dry weight and SDWSTI was recorded in genotype CSV-22R and Parbhani Moti.

Where only in one genotype (CSV-29R) showed consistently increase in seedling dry weight under osmotic stress condition that may be attributed to the accumulation of organic and inorganic solutes and due to the higher growth because of osmotic adjustment. Reduced biomass under drought stress tolerance index was observed in several plant species. The present outcomes are in agreement with the results of Ahmad *et al.* (2009) [1], Saensee *et al.* (2012) [24] in sunflower and Vanaja *et al.* (2011) [28] in maize.

Genotype CSV-29R (521.4) recorded highest seedling vigour followed by remaining genotypes and being at par with E-19 (461.5) and Phule Maulee (341.2). Lowest values were recorded in Parbhani Moti (100.4) and CSV-22R (106.8) over general mean noted at -0.16 MPa osmotic stress condition. The degree and percentage of seed establishment are enormously key factors in deciding yield and period of maturity (Rauf, 2008) [23]. In many parts of the Maharashtra, water stress mainly at seedling phase is a major determinant to *rabi* sorghum production.

Table 1: Effect of PEG 6000 induced osmotic stress on per cent germination of *rabi* sorghum genotypes.

Sr. No.	Genotypes	% Germination				
		0 MPa	**-0.066 MPa**	**-0.10 MPa**	**-0.13 MPa**	**-0.16 MPa**
1	M-35-1	95 (77.08)	85 (67.21)	75 (60.00)	60 (50.77)	35 (36.27)
2	Phule Anuradha	90 (71.57)	85 (67.21)	70 (56.79)	50 (45.00)	30 (33.21)
3	Ringni	90 (71.57)	80 (63.43)	70 (56.79)	50 (45.00)	30 (33.21)
4	CSV-22R	90 (71.57)	80 (63.43)	65 (53.73)	40 (39.23)	20 (26.57)
5	CSV-26R	90 (71.57)	80 (63.43)	65 (53.73)	45 (42.13)	25 (30.00)
6	CSV-29R	95 (77.08)	90 (71.57)	85 (60.21)	75 (60.00)	55 (47.87)
7	Parbhani Moti	85 (67.21)	75 (60.00)	60 (50.77)	30 (33.21)	20 (26.57)
8	PKV-Kranti	90 (71.57)	85 (67.21)	70 (56.79)	55 (47.87)	35 (36.27)
9	Phule Maulee	95 (77.08)	90 (71.57)	75 (60.00)	65 (53.73)	40 (39.23)
10	Elongvan-19	100 (90.00)	90 (71.57)	80 (63.43)	70 (56.79)	50 (45.00)
11	Elongvan-42	90 (71.57)	80 (63.43)	65 (53.73)	45 (42.13)	25 (30.00)
12	Elongvan-227	95 (77.08)	85 (67.21)	70 (56.79)	60 (50.77)	35 (36.27)
13	Elongvan-277	95 (77.08)	85 (67.21)	75 (60.00)	60 (50.77)	35 (36.27)
Mean		92.31	83.85	71.15	54.23	33.46
S.E (m).±		9.29	8.12	7.02	5.88	4.41
CD at 1%		NS	NS	NS	NS	19.05

Table 2: Effect of PEG 6000 induced osmotic stress on shoot length seedling^{-1} and shoot length stress tolerance index of *rabi* sorghum genotypes

Sr. No.	Genotypes	Average Shoot length seedling^{-1} (cm)					Shoot length stress tolerance index (%)			
		0 MPa	**-0.066 MPa**	**-0.10 MPa**	**-0.13 MPa**	**-0.16 MPa**	**-0.066 MPa**	**-0.10 MPa**	**-0.13 MPa**	**-0.16 MPa**
1	M-35-1	20.4	17.86	13.91	8.75	4.13	87.55	68.19	42.89	20.25
2	Phule Anuradha	18.7	16	12.53	7.7	3.21	85.56	67.01	41.18	17.17
3	Ringni	17.89	15.83	12.11	7.03	2.88	88.49	67.69	39.30	16.10
4	CSV-22R	16.13	13.81	10.73	6.25	1.95	85.62	66.52	38.75	12.09
5	CSV-26R	18.03	15.33	11.6	7.01	2.61	85.02	64.34	38.88	14.48
6	CSV-29R	19.37	18.98	15.38	9.85	4.16	97.99	79.40	50.85	21.48
7	Parbhani Moti	15.87	13.08	9.63	6.06	1.93	82.42	60.68	38.19	12.16
8	PKV-Kranti	18.87	16.63	12.68	8.03	3.23	88.13	67.20	42.55	17.12
9	Phule Maulee	21.07	17.71	14.45	8.85	3.83	84.05	68.58	42.00	18.18
10	Elongvan-19	20.7	18.53	15.01	9.71	4.46	89.52	72.51	46.91	21.55
11	Elongvan-42	16.97	14.7	11.48	6.68	2.38	86.62	67.65	39.36	14.02
12	Elongvan-227	19.53	17.25	13.63	8.46	3.61	88.33	69.79	43.32	18.48
13	Elongvan-277	20.47	17.48	13.76	8.68	3.65	85.39	67.22	42.40	17.83
General mean		18.77	16.40	12.84	7.93	3.23	87.28	68.21	42.04	16.99
S.E (m).±		0.710	0.650	0.548	0.384	0.273	3.23	3.22	2.615	1.414
CD at 1%		2.12	1.920	1.630	1.140	0.80	9.71	9.66	7.830	4.230

Table 3: Effect of PEG 6000 induced osmotic stress on root length seedling^{-1} and root length stress tolerance index of *rabi* sorghum genotypes

Sr. No.	Genotypes	Average Root length seedling^{-1} (cm)					Root length stress tolerance index (%)			
		0 MPa	**-0.066 MPa**	**-0.10 MPa**	**-0.13 MPa**	**-0.16 MPa**	**-0.066 MPa**	**-0.10 MPa**	**-0.13 MPa**	**-0.16 MPa**
1	M-35-1	12.59	11.95	9.22	6.30	4.45	94.92	73.23	50.04	35.35
2	Phule Anuradha	13.94	10.55	8.05	5.47	3.82	75.68	57.75	39.24	27.40
3	Ringni	13.89	10.52	8.02	5.25	3.67	75.74	57.74	37.80	26.42
4	CSV-22R	12.32	10.17	7.60	4.37	3.39	82.55	61.69	35.47	27.52
5	CSV-26R	12.10	10.45	7.97	5.00	3.45	86.36	65.87	41.32	28.51
6	CSV-29R	12.70	12.30	10.19	8.09	5.32	96.85	80.24	63.70	41.89
7	Parbhani Moti	11.45	9.92	7.57	3.84	3.09	83.43	63.67	32.30	25.99

8	PKV-Kranti	12.22	11.17	8.30	5.92	4.02	91.41	67.92	48.45	32.90
9	Phule Maulee	12.34	11.67	9.42	6.85	4.70	94.57	76.34	55.51	38.09
10	Elongvan-19	12.45	11.74	9.79	7.35	4.77	94.30	78.63	59.04	38.31
11	Elongvan-42	16.12	10.24	7.72	4.74	3.40	63.52	47.89	29.40	21.09
12	Elongvan-227	13.54	11.20	8.49	6.12	4.14	82.72	62.70	45.20	30.58
13	Elongvan-277	13.14	11.64	9.04	6.17	4.20	88.58	68.80	46.96	31.96
General mean		13.02	11.04	8.57	5.81	4.03	85.43	66.44	44.69	31.23
S.E (m).±		0.768	0.398	0.37	0.336	0.312	2.01	1.91	1.729	1.606
CD at 1%		2.30	1.16	1.09	0.990	0.930	6.05	5.74	5.180	4.810

Table 4: Effect of PEG 6000 induced osmotic stress on dry weight seedling^{-1} and dry weight stress tolerance index of *rabi* sorghum genotypes

Sr. No.	Genotypes	Average Dry weight seedling^{-1}(mg)					Dry weight stress tolerance index (%)			
		0 MPa	-0.066 MPa	-0.10 MPa	-0.13 MPa	-0.16 MPa	-0.066 MPa	-0.10 MPa	-0.13 MPa	-0.16 MPa
1	M-35-1	0.048	0.041	0.04	0.033	0.029	85.42	83.33	68.75	60.42
2	Phule Anuradha	0.044	0.037	0.033	0.027	0.024	84.09	75.00	61.36	54.55
3	Ringni	0.04	0.036	0.032	0.025	0.022	90.00	80.00	62.50	55.00
4	CSV-22R	0.045	0.033	0.028	0.022	0.019	73.33	62.22	48.89	42.22
5	CSV-26R	0.042	0.035	0.029	0.024	0.02	83.33	69.05	57.14	47.62
6	CSV-29R	0.052	0.046	0.042	0.036	0.032	88.46	80.77	69.23	61.54
7	Parbhani Moti	0.039	0.032	0.027	0.021	0.018	82.05	69.23	53.85	46.15
8	PKV-Kranti	0.046	0.038	0.034	0.027	0.024	82.61	73.91	58.70	52.17
9	Phule Maulee	0.046	0.044	0.04	0.035	0.031	95.65	86.96	76.09	67.39
10	Elongvan-19	0.055	0.045	0.041	0.035	0.033	81.82	74.55	63.64	60.00
11	Elongvan-42	0.041	0.033	0.028	0.023	0.019	80.49	68.29	56.10	46.34
12	Elongvan-227	0.054	0.038	0.034	0.029	0.025	70.37	62.96	53.70	46.30
13	Elongvan-277	0.047	0.039	0.035	0.029	0.025	82.98	74.47	61.70	53.19
General mean		0.0461	0.0382	0.0341	0.0282	0.0247	83.12	73.90	60.90	53.30
S.E (m).±		0.002	0.002	0.002	0.002	0.002	2.45	2.07	2.30	1.96
CD at 1%		0.005	0.006	0.007	0.006	0.005	7.35	6.23	6.91	5.89

Table 5: Effect of PEG 6000 induced osmotic stress on seedling vigour of *rabi* sorghum genotypes

Sr. No.	Genotypes	Seedling vigour				
		-0 MPa	-0.066 MPa	-0.10 MPa	-0.13 MPa	-0.16 MPa
1	M-35-1	3134.1	2533.9	1734.8	903.0	300.3
2	Phule Anuradha	2937.6	2256.8	1440.6	658.5	210.9
3	Ringni	2391.3	2108.0	1409.1	614.0	196.5
4	CSV-22R	2560.5	1918.4	1191.5	424.8	106.8
5	CSV-26R	2711.7	2062.4	1272.1	540.5	151.5
6	CSV-29R	3074.2	2815.2	2173.5	1345.5	521.4
7	Parbhani Moti	2322.2	1725.0	1032.0	297.0	100.4
8	PKV-Kranti	2798.1	2363.0	1468.6	767.3	253.8
9	Phule Maulee	3136.0	2644.2	1790.3	1020.5	341.2
10	Elongvan-19	3315.0	2724.3	1984.0	1194.2	461.5
11	Elongvan-42	2978.1	1995.2	1248.0	513.9	144.5
12	Elongvan-227	3141.7	2418.3	1548.4	874.8	271.3
13	Elongvan-277	3193.0	2475.2	1710.0	891.0	274.8
General mean		2899.5	2310.8	1538.7	772.7	256.5
S.E (m).±		354.79	287.25	144.12	119.24	61.720
CD at 1%		NS	NS	431.33	356.20	184.23

Conclusion

The present research work was conducted to evaluate germination per cent and early seedling growth stage and genetic potential of thirteen *rabi* sorghum genotypes through artificially created water stress by PEG of molecular weight 6000 in laboratory conditions followed by selection of genotypes based on easily measurable and inherited seedling traits contributing to drought tolerance. The genotypes CSV-29R, E-19, Phule Maulee, E-277, E-227 and PKV-Kranti and among checks genotype, M 35-1 found superior and might be productive in further breeding programmes for drought tolerance. Selection can be made on the basis of these characters at early growth stage to screen a large population for drought stress. It would be cost effective, less time consuming and less laborious to screen the genotype at early stage. So is suggested that the findings may be helpful and fruitful for selection of moisture stress in sorghum under the discussed traits.

Moreover, distinct genetic differences were found among the genotypes with respect to germination, seedling growth and seedling dry weight subjected to PEG.

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