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Invitro analysis of seed germination parameter in blackgram(*Vigna mungo* L. Hepper) by using Polyethylene glycol (PEG)

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Abstract—*The present invitro study carried out with eleven blackgram genotypes including T-9 used as check were collected from NBPGR regional centre Hyderabd and ICAR-CRIDA Hyderabad respectively. Seeds that were healthy and uniformly sized were surface sterilized for two to three minutes using 0.1% mercuric chloride (HgCl2) before being thoroughly washed with distilled water. The sterilized (10) seeds were sown in petri plates with moistened germination paper and various water potentials, namely, 0.0 (control), 5%, 10%, 15%, 20%, 25% and 30% of PEG 6000 in 100 ml of distilled water. The ANOVA results revealed sgnificant variability due to treatment, genotype and treatment verses genotype for the trait seed grmination at all the percentage of PEG 6000 studied. The results showed that among eleven genotypes IC426766 and IC382811 recorded higher germination percentage at higher PEG concentration and upto 20% PEG very lower percentage of germination was observed. It indicates that the variety IC426766 with hold the water even at higher concentration and showing water stress condition.*

Keywords— ***Blackgram, Germination%, Polyethylene glycol***

I. INTRODUCTION

The expected reduction in food crop yield over the next 50 years is due to global climate change, which expresses as higher temperatures and decreasing soil moisture. Lack of soil moisture has a direct impact on a plant's ability to germinate seeds and produce seedlings. A practical technique for evaluating how seeds respond to low water potential during germination is to germinate them in solutions with varying water potentials. Polyethylene glycol (PEG) molecules have been utilized to maintain consistent water potentials by simulating the effects of osmotic stress in vitro studies has shown that PEG 6000 is an effective solution to induce osmotic stress without harming plant cells (Datta *et al.*, 2011). PEG 6000 molecules are sufficiently large to prevent plant absorption but sufficiently small to affect the osmotic potential. Also, PEG simulates dry soil by drawing water out of the cell without entering the apoplast (Hatem Zgallai *et al.*, 2005 and Radhouane 2007). The results mentioned previously suggest that PEG solution may be utilized in laboratory conditions to screen for drought-tolerant cultivars. In order to protect the germplasm of ten blackgram genotypes from moisture stress during the germination and seedling phases in vitro, the current experiment was designed. One of the most valuable pulse crops, blackgram is grown practically everywhere in India. Because of its delicious flavor and many other health benefits, it has unavoidably established itself as the most popular pulse and is most appropriately referred to as the "king of pulses."Blackgram has an abundance of nutrients, including proteins (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids, and vitamins. It is either cooked and consumed immediately or broken into dhal. It is advised for diabetics and widely utilized in a variety of culinary recipes. The

green pods are used as a nutrient-dense vegetable. Cattle are fed the hulls or the outermost coating of straw and green (Baroowa and Gogoi 2015). The primary barrier to improved production for blackgram, which is mostly cultivated in marginal and rain fed areas, is inadequate moisture. Therefore, developing genotypes that can withstand drought is essential for agriculture in these regions. The purpose of this study was to determine which blackgram genotypes were more suited for drought tolerance and to evaluate the impact of polyethylene glycol on the germination ability of certain genotypes.

II. MATERIALS AND METHODS

The research work was carried out at experimental farm, Department of Genetics, Osmania University, Hyderabad during 2022. The ten blackgram accessions (IC261182, IC382811, IC426766, IC436524, IC436628, IC476753, IC476784, IC519620, IC546452, IC546472) along with T-9(check) were collected from NBPGR regional center, Hyderabad and ICAR-CRIDA, Hyderabad respectively were used in the present study. Seeds that were healthy and uniformly sized were surface sterilized for two to three minutes using 0.1% mercuric chloride (HgCl2) before being thoroughly washed with distilled water. According the Hadas (1976) technique, sterilized (10) seeds were sown in petri plates with moistened germination paper and various water potentials, namely, 0.0 (control), 5%, 10%, 15%, 20%, 25% and 30% of PEG 6000 in 100 ml of distilled water. Three replications were kept for each treatment. The germination criterion was established as the appearance of a 2 mm radicle (Kaur *et al.*, 2017 and Yadav *et al.,* 2013). Numbers of seeds germinated were counted from day-3 to day-6 after sowing to determine the germination percentage.

The following formula.

$$\text{Germination percentage (\%)} = \frac{\text{Numberofseedsgerminated}}{\text{Totalnumberofseeds}} \text{x} 100$$

III. RESULTS AND DISCUSSION

The ANOVA results The ANOVA results revealed sgnificant variability due to treatment, genotype and treatment verses genotype for the trait seed grmination at all the percentage of PEG 6000 studied (Table 1).

Table 1. Analysis of variance in blackgram for the trait seed germination at various concentration of PEG.

Source of Variation	DF	MSSQ					
		Characters					
		5%	10%	15%	20%	25%	30%
Replications	**2**	0.797	0.624	0.225	1.100	0.660	0.66
Treatments	**1**	136.627**	235.91**	1727.898**	6790.556**	18639.266**	27121.78**
Genotypes	**10**	219.303**	205.182**	224.335**	366.831**	497.46**	497.46**
Treatments vs Genotypes	**10**	14.323**	11.373**	31.294**	94.979**	210.946**	210.946**
Error		1.39	0.805	0.603	0.971	1.497	1.497
SD		0.290	0.221	0.191	0.243	0.301	0.301
CV%		1.37%	1.05%	0.94%	0.01	1.73%	1.95%

Highest germination percentage genotype IC382811 was recorded (94.16%), and lowest genotype IC476784recorded as (76.18%) fallowed by IC426766 (93.82%), IC261182 (93.03%), T-9 (88.38%), IC436524 (83.58%), IC476753 (82.34%), IC546472 (82.20%), IC436628 (81.00%), IC519620 80.85%), IC546452 (77.59%) and IC476784 (76.18%) respectively were observed at 5%PEG 6000concentration**.**The genotype IC426766 (93.20%) was shown higher whereas lower genotype IC546452was recorded (77.17%). Fallowed by IC382811 (91.93%), IC261182 (90.62%), T-9 (89.27%), IC436524 (81.52%), IC546472 (80.96%), IC519620 (80.57%), IC476753(80.50%), IC436628 (79.83%), IC476784 (77.63%), IC546452 (77.17%) respectively were observed at 10% PEG 6000 concertation.

Table 2: Mean values of seed germination% in blackgram at different PEG concentrations

Genotypes	5%	10%	15%	20%	25%	30%	Control
IC261182	93.03	90.62	79.45	71.63	58.9	42.9	95.34
IC382811	94.16	91.93	87.82	81.56	77.77	61.77	94.6
IC426766	93.82	93.2	91.69	90.37	77.28	61.28	93.82
IC436524	83.58	81.52	74.3	63.72	54.5	38.5	85.13
IC436628	81.00	79.83	73.01	64.99	54.52	38.52	81.36
IC476753	82.34	80.5	74.27	55.69	44.37	28.37	87.06
IC476784	76.18	77.63	71.44	54.4	46.86	30.86	80.4
IC519620	80.85	80.57	70.78	60.86	30.74	14.74	89.84
IC546452	77.59	77.17	74.25	67.87	50.6	34.6	82.73
IC546472	82.2	80.96	72.7	59.92	41.41	25.41	80.73
T-9	88.38	89.27	82.52	70.61	58.12	42.12	93.78
SD	***6.482***	***6.026***	***7.023***	***10.866***	***14.153***	***14.153***	***5.990***
SE	***1.954***	***1.817***	***2.117***	***3.276***	***4.267***	***4.267***	***1.806***

Higher genotype IC426766 was recorded 91.69% whereas lower germination genotype IC519620 recorded as 70.78% fallowed by genotypes IC382811 (87.82%), T-9 (82.52%), IC261182 (79.45%), IC436524 (74.30%), IC476753 (74.27%), IC546452 (74.25%), IC436628 (73.01%), IC546472 (72.70%), IC476784 (71.44%) respectively were observed at 15% PEG6000 concentration. Germination at 20% PEG 6000 concentration was observed the highest genotype IC426766 was 90.37% and lowest germination percentage genotypes IC476784 was observed 54.40% whereas germination percentage range fallowed by IC382811 (81.56%), IC261182 (71.63%), T-9 (70.61%), IC546452 (67.87%), IC436628 (64.99%), IC436524 (63.72%), IC519620 (60.86%), IC546472 (59.92%), IC476753 (55.69%), IC476784 (54.40%) respectively were observed at 20% PEG 6000 concentration. Seed germination percentage was recorded the genotype IC382811 (77.77%) and lower genotype IC519620 was observed 30.74% fallowed by genotypes were IC426766 (77.28%), IC261182 (58.90%), T-9 (58.12%), IC436628 (54.52%), IC436524 (54.50%), IC546452 (50.60%), IC476784 (46.86%), IC476753 (44.37%), IC546472 (41.41%) and IC519620 (30.74%) respectively were observed at 25% PEG6000 concertation. The germination percentage was highest genotype IC382811was recorded as 61.77% fallowed by IC426766(61.28%), IC261182(42.90%), T-9(42.12%), IC436628(38.52%), IC436524(38.50%), IC546452(34.60%), IC476784(30.86%), IC476753(28.37%), IC546472 (25.41%) and IC519620(14.74%) respectively were observed at 30% PEG 6000 concentration (Table 2).

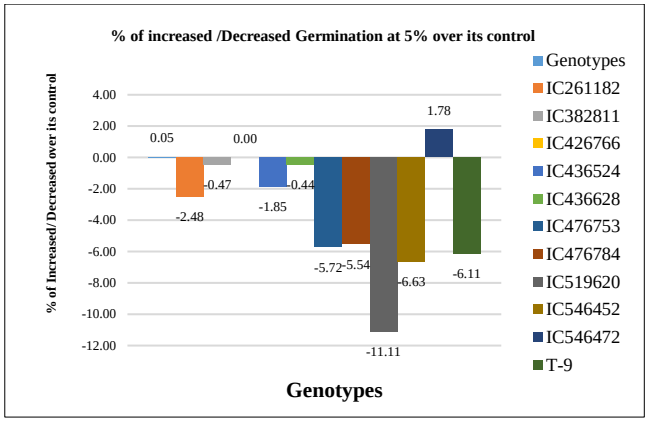
In the present study it was observed that with an increase in water stress (5%– 30%), there was a gradual depletion in rate of water uptake by blackgram seeds of all genotypes. This reduction might be due to the fact that water moves from high potential to low potential due to differences in the free energy content. The gradient of water potential between dry seeds and pure water (0.0 Mpa) decrease rapidly with the addition of any soluble substances such as polyethylene glycol in water. All the genotypes were observed seed germination at 5% over respective controls under PEG 6000 concentration the genotype IC546472 was presented 1.78% increased percentage and the genotype IC426766 showed 0.00 no significant over its control at 5% PEG 6000 concentration. In our study the highest reduction was observed genotype IC519620 result revealed -11.11%, followed by genotypes results were revealed IC546452 (-6.63%), T-9 (-6.11%), IC476753 (-5.72%), IC476784 (-5.54%), IC261182 (-2.48%), IC436524 (-1.85%), IC382811 (-0.47%), IC436628 (-0.44%) respectively decreasing over its control at 5% PEG concentration. Similar results were found in blackgram by Mohanlal *et al*., 2020; Ghanbari MohebSeraj *et al.*, 2021; Shobanadevi *et al.*, 2022; Bordoloi *et al.*,2023.

Table 3: Increased/ decreased seed germination percentage of blackgram at various concentration of PEG over its control

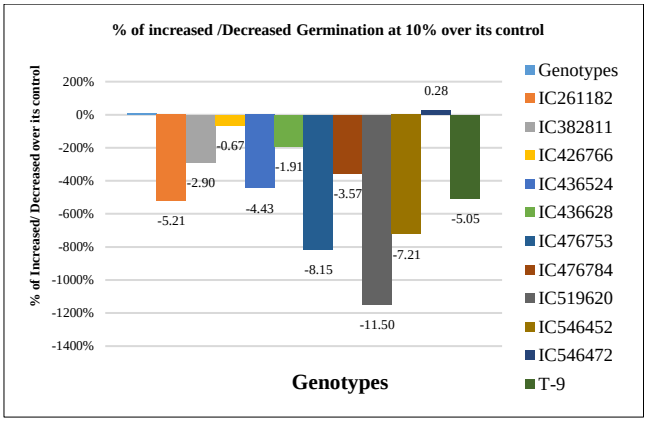
Genotypes	5%	10%	15%	20%	25%	30%
IC261182	-2.48	-5.21	-20.00	-33.09	-61.86	-122.22
IC382811	-0.47	-2.90	-7.72	-15.98	-21.65	-53.16
IC426766	0.00	-0.67	-2.32	-3.82	-21.41	-53.11
IC436524	-1.85	-4.43	-14.59	-33.60	-56.21	-121.13
IC436628	-0.44	-1.91	-11.43	-25.18	-49.22	-111.21
IC476753	-5.72	-8.15	-17.22	-56.33	-96.22	-206.90
IC476784	-5.54	-3.57	-12.55	-47.79	-71.56	-160.50
IC519620	-11.11	-11.50	-26.93	-47.60	-192.22	-509.34
IC546452	-6.63	-7.21	-11.43	-21.90	-63.49	-139.09
IC546472	1.78	0.28	-11.04	-34.73	-94.95	-217.68
T-9 (check)	-6.11	-5.05	-13.65	-32.81	-61.36	-122.65

The percentage of increased calculated at 10% PEG 6000 the genotype IC546472 was presented 0.28%. The genotype IC426766 was observed low value -0.67% seed germination percentage at 10% PEG 6000 concentration over its control(irrigated) was observed earlier in blackgram by Shobanadevi *et al.*, 2022; Mohanlal *et al.*, 2023. Also similar results showed in legumes by Swathi *et al.*, 2017; Priyadharshini *et al.*, 2021; Aadarsha and Shrestha. 2024.The decreased percentage calculated at 10% PEG 6000 concentration presented values lower in genotypes fallowed by IC436628(-1.91%), IC382811(-2.90%), IC476784(-3.57%), IC436524(-4.43%), T-9(-5.05%), IC261182(-5.21%), IC546452(-7.21%), IC476753(-8.15%) and IC519620(-11.50%) respectively condition over its control(irrigated) was observed earlier in blackgram by Priyadharshini *et al.*, 2021; Aadarsha and Shrestha. 2024.The decreased percentage of seed germination at 15% PEG 6000 concentration was observed low reduction genotype IC426766 was (-2.32%) and the genotype IC382811(-7.72%) over its control(irrigated). Lowest seed germination percentage was exhibited by 15% PEG 6000 concentration genotypes fallowed by IC546472(-11.04%), IC546452(-11.43%), IC436628(-11.43%), IC476784(-12.55%), T-9(-13.65%), IC436524(-14.59%), IC476753(-17.22%), IC261182(-20.00%) and IC519620(-26.93) respectively over its control was observed earliest in blackgram by Hellal *et al.*, 2018; Mohanlal *et al.*, 2023.The present results are in accordance with the reports of Sabesan and Saravanan 2016; Partheeban *et al.*,2017; Swathi *et al.*, 2017 in legume plants. The decreased percentage of seed germination at 20% calculated low in genotype IC426766 was -3.82% over its control(irrigated). The lowest decreased percentage of seed germination significant results followed genotypes byIC382811(-15.98%), IC546452(-21.90%), IC436628(-25.18%), T-9(-32.81%), IC261182(-33.09%), IC436524(-33.60%), IC546472(-34.73%), IC519620(-47.60%), IC476784(-47.79%) and IC476753(-56.33%) respectively over its control(irrigated) was observed earlier in blackgram by Hellal *et al.*, 2018; Jothimani and Arulbalachandran. 2020; Shobanadevi *et al.*, 2022; Khulal *et al.*, 2022; Samita *et al.*, 2022; Kumawat *et al.*, 2023; Aadarsha and Shrestha, 2024.The percentage of decreased in seed germination at 25% PEG compared to control(irrigated) low values observed in genotype IC426766 was -21.41% and the genotype IC382811 was -21.65% *In vitro* condition, reduction in germination percentage respectively over its control (irrigated). *In vitro* screening of blackgram genotypes using PEG 6000 concentration found that the genotype IC426766(-21.41%) expressed tolerance to drought at seed germination level same results were found in blackgram by Dong and Beckles, 2019; Aman *et al.*, 2022.At the 25% seed germination percentage of decreased was lowest values observed genotypes fallowed byIC436628(-49.22%), IC436524(-56.21%), T-9(-61.36%), IC261182(-61.86%), IC546452(-63.49%), IC476784(-71.56%), IC546472(-94.95%), IC476753(-96.22%), and IC519620 (-192.22%) respectively over its control(irrigated).Also have reported that increase in the osmotic concentration decreased energy of seed germination percentage in blackgram by Swathi *et al.*, 2017; Kaur *et al.*, 2017. Seed germination was observed PEG 6000 concentration earlier in blakgram by Jothimani and Arulbalachandran. 2020; Shobanadevi *et al.*, 2022; Aadarsha and Shrestha. 2024.The percentage of decreased in seed germination low result revealed in genotype IC426766 was -53.11% and IC382811 observed -53.16% over its control(irrigated) at

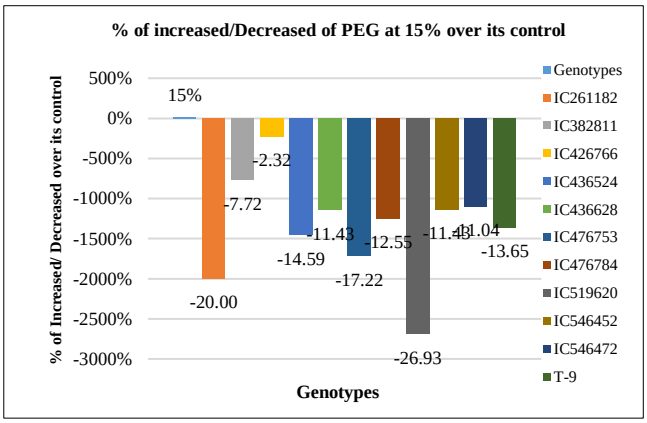
30% PEG concentration. The lower water potential during germination inhibits the seed germination or suppress the growth and development of seedlings in blackgram by Kaur *et al.*, 2017. Same results revealed significant in all genotypes fallowed by IC436628(-111.21%), IC436524(-121.13%), IC261182(-122.22%), T-9(-122.65%), IC546452(-139.09%), IC476784(-160.50%), IC476753(-206.90%), IC546472(-217.68%), and IC519620(-509.34%) respectively over its control(irrigated) was observed earlier in blackgram by Sridhar *et al*., 2020; Mohanlal *et al.*, 2023.A significant delay in the initiation and completion of germination was also observed in PEG 6000 treated varieties. The decreased germination in response to water stress is not uniform in all cultivars. A significant decrease in the germination percentage was observed at 30% concentration the germination percentage was higher decline in germination percentage due to lower water potential have been reported earlier in blackgram by Babu and Rosaiah. 2017; Rana *et al.*, 2017; Swathi*et al.*, 2017; Hellal*et al.*, 2018; Shobanadevi *et al.*, 2022; Aadarsha and Shrestha.2024 (Table 3).



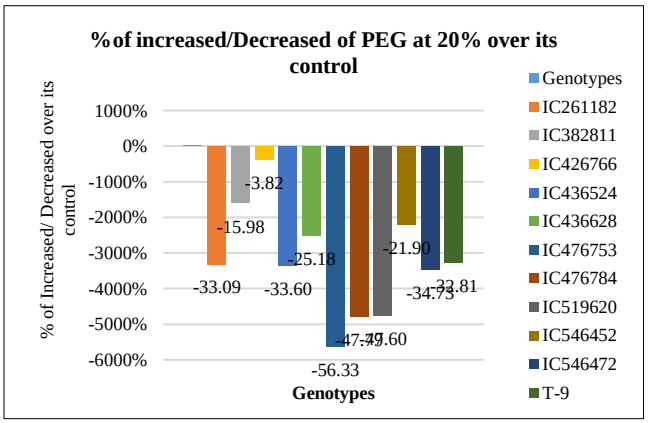
(a) % of increased /Decreased Germination% at 5% over its control



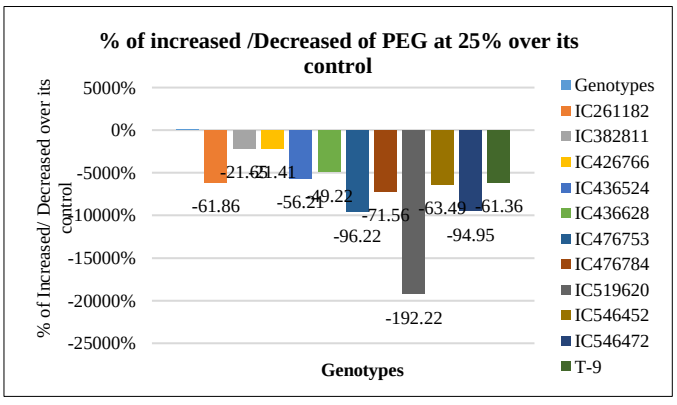
(b) % of increased /Decreased Germination% at 10% over its control



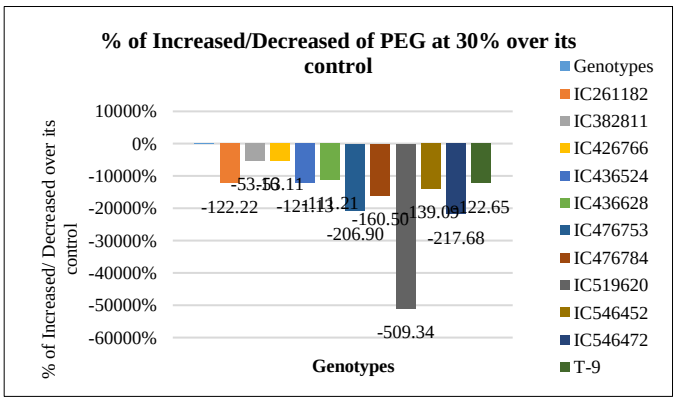
(c) % of increased /Decreased Germination% at 15% over its control



(d) % of increased /Decreased Germination% at 20% over its control



e) % of increased /Decreased Germination% at 25% over its control



(f) % of increased /Decreased Germination% at 30% over its control

Fig. 1. Increased/ decreased seed germination percentage of blackgram at various concentration of PEG over its control

IV. CONCLUSION

The present experiment results revealed that during the germination, the blackgram genotypes IC426766 and IC382811 shown higher germination percentage at higher concentration of PEG (>50% germination percentage). The chosen genotypes can be used for more field condition in research because they have been shown to have tolerance traits to withstand water stress.

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