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Effect of Polyethylene Glycol (PEG) on Seed Germination of Important *kharif* Pulse Crops of Kashmir Valley

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ABSTRACT

Pulses form an important part of human diet as they are a potent source of proteins. Drought is one of the most important abiotic factors which adversely affect pulse production. Seed germination is considered as the first and foremost feature which directly affect the growth and yield of crop plants. To study the effect of PEG (polyethylene glycol) induced drought on germination of *kharif* pulse crops of Kashmir including cowpea, common bean and mung bean, an experiment was laid out under laboratory conditions at SKUAST-K Shalimar. Different parameters including, length of radical, number of laterals, root biomass and total number of germinating seeds were measured under different concentrations of PEG-6000 viz., 0% (Control), 5%, 10% and 20%. All the parameters estimated across the genotypes showed progressive decline with the increase in PEG concentration from 0 to 20%. In common bean, French Yellow exhibited lesser reduction in all the germination parameters with an increase in concentration of PEG-6000. For cowpea, genotypes UDC-46 and C-37 showed lesser reduction in the traits recorded. Similarly in case of mung bean, KM-1046 and KM-1854 exhibited lesser reduction in different parameters with the increase in concentration of PEG-6000. These genotypes after further evaluation can be used in breeding programs for the development of drought tolerant varieties.

Keywords Drought, Abiotic factor, PEG, *Kharif* pulses, Cowpea.

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INTRODUCTION

The major food requirement of human beings is being fulfilled by cereals and legumes. Legumes rank second after cereals in terms of food production and contribute about 33% of the protein needs. They have been recognized as a major source of vegetable protein rich in minerals and vitamins. Legumes are grown in all types of climatic conditions with wide range of soil types (Graham and Vance 2003). They play a vital role in maintaining soil fertility by fixing atmospheric nitrogen. According to the projections of Intergovernmental Panel on Climate Change (IPCC), reduction in yield as a result of climate change related abiotic stresses will be more pronounced for pulse crops. Among the abiotic stresses, drought is one of the most important environmental factors that reduce growth, development and yield of crop plants (Verslues *et al*. 2006, Xiong *et al*. 2006, Trenberth *et al*. 2014, Zhao and Dai 2015). Therefore, drought serves as the most important constraint for pulse productivity and the current challenge is to reduce the yield gaps between drought and non-drought conditions (Waza *et al*. 2013a, Dharbale *et al*. 2019, Majid *et al*. 2020a). Transfer of drought tolerance from tolerant to susceptible genotypes serves as the most coherent approach to develop the drought tolerant varieties. Drought stress tolerance being a complex phenomenon involves clusters of gene networks. As such, despite of various efforts during the past few decades, identifying plant types for use in breeding programs to transfer drought resistance traits into high yielding cultivars has become a challenge for plant breeders (Serraj and Buhariwalla 2004, Waza *et al*. 2013b, Dar *et al*. 2021). To achieve this objective, the screening for sources of drought resilient is indispensable.

The first and foremost effect of drought stress is on germination of seeds (Harris *et al*. 2002, Duman 2006, Waza *et al*. 2014). For research purpose, the creation and maintenance of pure water potential in the environment of soil is almost a difficult job. In this regard, establishing conditions of dryness stress using different osmotic materials to create the osmotic potential is considered as one of the best methods to study the effects of dryness stress on germination. Among these substances, due to the simulation of natural environmental conditions, polyethylene glycol (PEG) has many applications and is widely used *in vitro* (Ibrahim *et al*. 2001, Umesh *et al*. 2015). Because this compound has a high molecular weight, it cannot pass through the cell wall and therefore it is used to regulate water potential in germination tests. The principal aim of this study is to investigate the effects of osmotic stress generated by PEG on germination characteristics of some elite pulse crops grown under Kashmir conditions. This is to screen the germplasm for drought stress at an early stage of plant growth.

MATERIALS AND METHODS

Experimental details

This experiment was carried out during 2020 under laboratory conditions at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K). Twenty two pulse germplasm lines consisting of eleven common bean (*Phaseolus vulgaris*) genotypes viz., WB-9716, WB-1446, WB-934, WB-112, WB-22, DARS-10, R-15, French Yellow, Shopian local, Budgam local , Ganderbal local; six cowpea (*Vigna unguiculata*) genotypes viz., SKUASTC-407, UDC-45, UDC-46, C-37, C-46, C-5 and five mung bean (*Vigna radiata*) genotypes viz., SKUAST M-1047, Mung Local Budgam, KM-2241, KM-1046 and KM-1854 were evaluated in the present study. The design followed was Completely Randomized Design (CRD) with three replications.

Induction of water stress using PEG-6000

Water stress was induced using different osmotic potential levels [0 (control), 5, 10 and 20%] to evaluate the effect of PEG-6000 treatments on seed germination. For each level of stress, four seeds of each cultivar were selected and sterilized in sodium hypochlorite (1%) and then washed twice in distilled water. The seeds of pulse crops were germinated in petri dishes on 2 layers of filter paper in a germinator maintained at 25 °C and 75% humidity in darkness. These petri plates were visualized on daily basis, filter papers replaced if needed and PEG solution was added as per the requirement.

Recording of data

After seven days, the length of radicle (cm), number of laterals, radicle weight (g) and total number of seeds germinated was estimated. Length of radicle was estimated with the help of scale and graph paper. Number of laterals was visually counted with the help of magnifying glasses. Radicle weight was estimated with the help of digital weighing machine. Percentage of germinating seeds was determined by counting the number of seeds germinated out of total number of seeds.

Table 1. Effect of osmotic potential induced by PEG - 6000 on length of radicle (cm), number of laterals and root biomass (g) of common bean genotypes.

Genotypes	Radical length				Number of laterals				Root biomass			
	0%	5%	10%	20%	0%	5%	10%	20%	0%	5%	10%	20%
WB-9716	4	3	2.5	1	7	5	4	3	0.08	0.06	0.04	0.01
WB-1446	22	12	7	5	58	21	5	4	0.94	0.54	0.14	0.06
WB-934	20.5	7	7	7	16	7	6	4	0.54	0.07	0.06	0.06
WB-112	15	15	10	1	26	25	3	1	0.62	0.41	0.31	0.02
WB-22	7	4	3	1	4	4	2	1	0.09	0.07	0.05	0.02
DARS-10	13.5	10.5	9	2	19	19	7	1	0.58	0.12	0.11	0.04
R-15	10	6.5	5	1	13	4	2	1	0.14	0.08	0.5	0
French Yellow	14	12	7.5	4	18	16	14	13	0.43	0.28	0.19	0.17
Shopian local	11	6	4	1	9	5	4	2	0.36	0.06	0.05	0.02
Budgam local	11	5	4.5	4	31	6	5	5	0.18	0.06	0.05	0.04
Ganderbal local	7	5	4	1	8	6	3	3	0.09	0.08	0.05	0.01
Mean	12.27	7.82	5.77	2.18	19.00	10.73	5.00	3.00	0.37	0.17	0.14	0.04
CD (p ≤0.05)	Genotype=0.625 PEG levels=0.267 Genotype x PEG levels=1.23				Genotype = 0.87 PEG levels =0.32 Genotype x PEG levels=1.78				Genotype=0.012 PEG levels=0.004 Genotype x PEG levels=0.02			

RESULTS AND DISCUSSION

Genotypic variation for radical length, number of laterals and root biomass under different stress levels

Comparison of the data revealed that length of radical, number of laterals and root biomass shows different performance under different stress levels (Tables 1 - 3).

Under controlled conditions the length of radical was found to be highest in WB–1446 (22cm) in common bean, C-407 (15cm) in cowpea and KM-1046 and KM-1854 (20cm) in case of mung bean. Under 5% PEG, the length of radical was found to be highest in WB-112 (15cm) in common bean, C-37 (12cm) in cowpea and KM-1046 (16cm) in case of mung bean. For 10% PEG, the length of radical was found to be highest in WB-112 (10cm) in common bean, C-37 (7.5cm) in cowpea and KM-1046 and KM-1854 (12cm) in case of mung bean. Using 20% PEG, the length of radical was found to be highest in WB-934 (7cm) in case of common bean, C-37 (6.5cm) in cowpea and KM-2241 (10cm) in case of mung bean.

Under controlled conditions number of laterals was observed to be highest in WB-1446 (58) in common bean, SKUAST C-407(16) in cowpea and KM-1046 (28) in case of mung bean. For 5% PEG,

Table 2. Effect of osmotic potential induced by PEG - 6000 on length of radicle (cm), number of laterals and root biomass (g) of cowpea genotypes.

Genotypes	Radical length				Number of laterals				Root biomass			
	0%	5%	10%	20%	0%	5%	10%	20%	0%	5%	10%	20%
SKUAST C-407	15	8	4.5	3.5	16	6	3	1	0.31	0.28	0.24	0.21
UDC-45	10	8	7	1	4	3	2	1	0.24	0.22	0.11	0.01
UDC-46	9	8	7	5	4	3	3	2	0.24	0.12	0.09	0.06
C-37	14	12	7.5	6.5	9	7	6	4	0.36	0.26	0.13	0.09
C-46	13	7.5	4	2	6	4	2	2	0.35	0.05	0.03	0.02
C-5	8	7	5	1	5	3	1	1	0.16	0.09	0.04	0.01
Mean	11.50	8.42	5.83	3.17	7.33	4.33	2.83	1.67	0.28	0.17	0.11	0.07
CD (p≤ 0.05)	Genotypes=0.312 PEG levels=0.179 Genotype x PEG levels=1.46				Genotypes=0.210 PEG levels=0.10 Genotype x PEG levels=1.12				Genotype=0.018 PEG levels=0.06 Genotype x PEG levels=0.08			

Table 3. Effect of osmotic potential induced by PEG - 6000 on length of radicle (cm), number of laterals and root biomass (g) of mung bean genotypes.

Genotypes	Radical length				Number of laterals				Root biomass			
	0%	5%	10%	20%	0%	5%	10%	20%	0%	5%	10%	20%
SKUAST M-1047	16	10	9	7	10	9	8	3	0.36	0.14	0.1	0.09
Mung Local Budgam	15	12	6	0	16	9	6	0	0.38	0.33	0.06	0
KM-2241	18	12	10	10	15	13	12	10	0.28	0.14	0.12	0.09
KM-1046	20	16	12	8	28	25	16	14	0.39	0.25	0.16	0.12
KM-1854	20	15	12	9	16	14	10	10	0.36	0.23	0.19	0.14
Mean	17.8	13.0	9.80	6.80	17.0	14.0	10.4	7.40	0.35	0.22	0.13	0.09
CD ($p \leq 0.05$)	Genotype=0.14 PEG levels=0.54 Genotype x PEG levels=1.24				Genotype=0.35 PEG levels=0.8 Genotype x PEG levels=1.41				Genotype=0.016 PEG levels=0.03 Genotype x PEG levels=0.06			

the number of laterals was found to be highest in WB-112 (25) in common bean, C-37 (7) in cowpea and KM-1046 (25) in case of mung bean. Using 10% PEG, the number of laterals was found to be highest in French Yellow (14) in common bean, C-37 (6) in cowpea and KM-1046 (16) in mung bean. Using 20% PEG, the number of laterals was found to be highest in French Yellow (13) in case of common bean, C-37 (4) in cowpea and KM-1046 (14) in case of mung bean.

Under controlled conditions, the root biomass was found to be highest in WB-1446 (0.94g) in common bean, C-37 (0.36g) in cowpea and KM-1046 (0.39g) in case of mung bean. For 5% PEG, the root biomass was found to be highest in WB-1446 (0.54g) in common bean, SKUAST C-407 (0.28g) in cowpea and KM-1046 (0.25g) in mung bean. Using 10% PEG, the root biomass was found to be highest in WB-112 (0.31g) in common bean, SKUAST C-407 (0.24g) in cowpea and KM-1854 (0.19g) in case of mung bean. On application of 20% PEG, the root biomass was found to be highest in French Yellow (0.17g) in common bean, SKUAST C-407(0.21g) in cowpea and KM-1854 (0.14g) in case of mung bean.

The effect of PEG in physiological processes has been reported in cowpea (Badiane *et al*. 2004, Jain and Saxena 2016), mung bean (Afzal *et al*. 2005, Jain *et al*. 2015), moth bean (Soni *et al*. 2011), black gram (Priyanka *et al*. 2013, Yadav *et al*. 2013) and common bean (Nunes *et al*. 2008, Silva *et al*. 2016, Majid *et al*. 2016). The *in vitro* culture techniques minimize confounding effects on account of lack of control in environmental variables due to defined nutrient media, controlled conditions and homogeneity of stress application. Polyethylene glycols (PEG) of high molecular weights have been used to simulate water stress in plants as non-penetrating osmotic agents, lowering the water potential in a way similar to soil drying (Larher *et al*. 1993, Majid *et al*. 2017).

Germination percentage of genotypes under different concentration of PEG-6000

In common bean, germination percentage of genotypes under different concentration of PEG-6000 is shown in Table 4. Under controlled conditions germination percentage was found to be highest in

Table 4. Germination percentage of genotypes under different concentration of PEG–6000.

Genotypes	Germination percentage (%)			
	0% PEG	5% PEG	10% PEG	20% PEG
	Common bean			
WB-9716	75	50	25	25
WB-1446	75	75	75	50
WB-934	75	75	50	50
WB-112	75	75	75	0
WB-22	75	75	25	0
DARS-10	75	75	75	50
R-15	75	75	75	0
French Yellow	100	100	75	50
Shopian local	75	75	75	25
Budgam local	75	75	75	50
Ganderbal local	75	50	25	25

Table 4. Continued.

Genotypes	Germination percentage (%)			
	0% PEG	5% PEG	10% PEG	20% PEG
		Common bean		
		Cowpea		
SKUAST C-407	100	100	75	50
UDC-45	75	75	75	50
UDC-46	100	100	100	75
C-37	100	100	100	100
C-46	75	75	50	25
C-5	75	50	50	25
		Mung bean		
SKUAST M-1047	100	100	100	100
Mung Local Budgam	100	100	100	0
KM-2241	100	75	75	75
KM-1046	100	100	75	75
KM-1854	100	100	100	100

French Yellow (100%) in case of common bean. While as SKUAST C-407, UDC-46 and C-37 (100 %) showed highest germination percentage in case of cowpea. Further genotypes viz., SKUAST M-1047, Mung Local Budgam, KM-2241, KM-1046 and KM-1854 (100%) recorded highest and equal germination percentage in case of mung bean. Under 5% PEG, germination percentage was found to be highest in French Yellow (100%) in common bean, SKUAST C-407, UDC-46 and C-37 (100 %) in cowpea and SKUAST M-1047, Mung Local Budgam, KM-1046 and KM-1854 (100%) in case of mung bean. For 10% PEG, germination percentage was found to be highest in WB-1446, WB-112, DARS-10, R-15, French Yellow, Shopian local and Budgam local (75%) in common bean; UDC-46 and C-37 (100 %) in cowpea and SKUAST M-1047, Mung Local Budgam and KM-1854 (100%) in case of mung bean. On application of 20% PEG, germination percentage was found to be highest in WB-1446, WB-934, DARS-10, French Yellow and Budgam local (50%) in common bean, C-37 (100%) for cowpea and SKUAST M-1047 and KM-1854 (100%) in case of mung bean. According

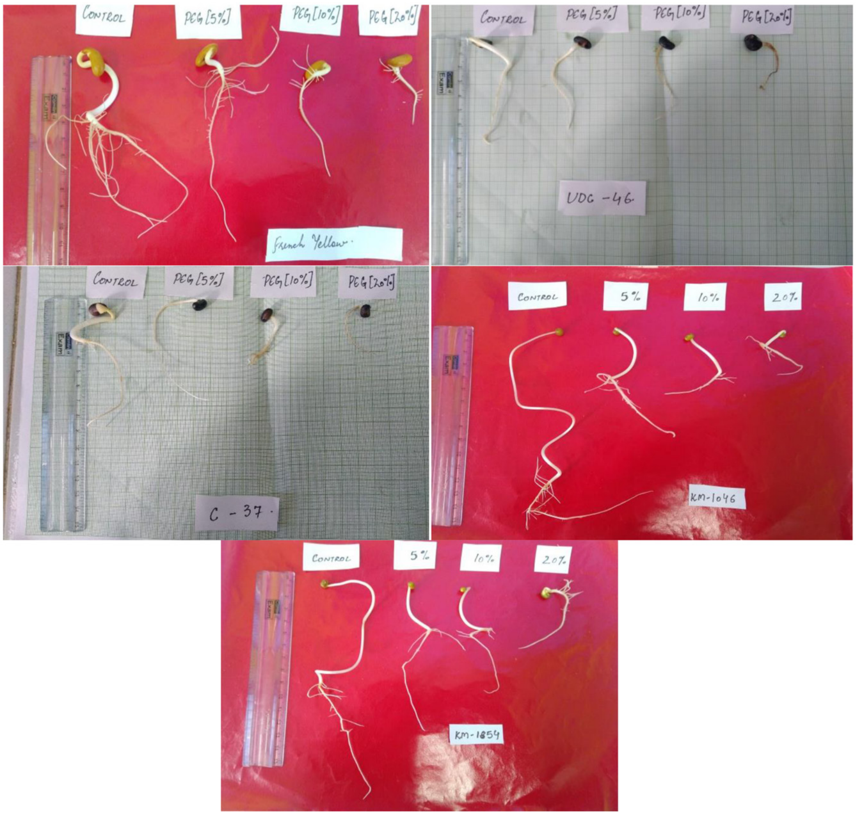

Fig. 1. Promising genotypes of cowpea, common bean and mung bean under different PEG concentrations.

to Ayaz *et al*. (2000), decrease in seed germination under stress conditions is due to the occurrence of some metabolic disorders. Water deficit shows negative effect on germination of seed and the growth of seedlings. The adverse effect of water shortage on germination and seedling growth has been well reported in different crops (Mostafavi *et al*. 2011).

Genotypes exhibiting least reduction with increase in concentration of PEG–6000

In common bean, French Yellow exhibited lesser reduction in different growth parameters with the increase in concentration of PEG-6000 from 0% to 20%. Similarly in case of cowpea, genotypes UDC-46 and C-37 showed lesser reduction in the different growth traits. For mung bean, KM-1046 and KM-1854 exhibited lesser reduction in all the growth parameters with the increase in concentration of PEG-6000 (Fig. 1). The genotypes need to be further evaluated under green house and field conditions. According to the previous studies, induced water deficit by polyethylene glycol shows similar values to that observed under the field conditions (Thill *et al*. 1979, Majid *et al*. 2020b).

CONCLUSION

Keeping in view the above stated research findings, it can be concluded that some of the genotypes exhibited lesser reduction in different growth parameters with the increase in concentration of PEG-6000 from 0% to 20%. The genotypes include French Yellow in common bean, UDC-46 and C-37 in cowpea and KM-1046 and KM-1854 in mung bean. These genotypes after further evaluation can be used in breeding programs for the development of drought tolerant varieties.

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