



Effect of PEG-6000 Osmoticum on Seed Germination and Seedling Growth of Lentil (*Lens culinaris* Medik.) Genotypes

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ABSTRACT

Background: Drought, extreme temperatures, salinity, chemical toxicity and oxidative stress are such environmental stresses, that are serious threats to agriculture globally and result in the deterioration of the environment and crops. To study the effect of drought stress on seed germination and early establishment in different crop varieties, PEG (Polyethylene Glycol) induced drought stress is the most common screening method. The species and its variety, its growing conditions and the developmental stage of the plant, as well as osmotic concentration responded differently to osmotic stress. The current study aimed to study the effect of different concentrations of PEG-6000 in eight genotypes of lentil in order to understand the management and control of drought stress-related problems in crop plants.

Methods: The experimental work was conducted during 2018-2019 at the Laboratory of Botany, S.S. Jain Subodh P.G. (Autonomous) College, Jaipur to study the effect of drought stress during early growth of genotypes of Lentil (*Lens culinaris* Medik.) under osmotic stress. The plant material consisted of eight genotypes belonging to the *macrosperma* and *microsperma* subspecies of lentil (*Lens culinaris* M.). PL-4, L-4147, L-4594 and L-4596 belong to the *microsperma* subspecies and K-75, L-4076, DPL-15 and DPL-62 belong to the *macrosperma* subspecies.

Result: In conclusion, due to their better values of germination percentage (GP%), germination relative index (GRI), seedling vigor index (SVI) and seedling growth under simulated drought conditions PL-4, L-4594, DPL-15 and DPL-62 stand out as the most tolerant lentil genotypes among eight species studied in this work. These genotypes showed a significant enhancement in germination percentage (%), germination relative index, seedling vigor index and seedling length under mild moisture stress. Our results confirmed that PEG-induced drought stress can be used to study the drought-tolerant species by studying a few parameters viz. germination percentage, germination relative index and, seedling vigour index, that will result in increased crop production.

Key words: Drought stress, Lentil, *Macrosperma*, *Microsperma*.

INTRODUCTION

Drought, extreme temperatures, salinity, chemical toxicity and oxidative stress are such environmental stresses, that are serious threats to agriculture globally and result in the deterioration of the environment (Odabas *et al.*, 2014; Temizel *et al.*, 2014; Toosi *et al.*, 2014). Drought stress is considered one of the main abiotic stresses that negatively affect seed germination and other germination-related parameters. Germination is adversely affected by conditions due to a lack of irrigation and rainfall as reported by Mwale *et al.* (2003). Bouaziz and Hicks. (1990) reported that drought and salt stress cause the prevention or reduction of the mobilization of stored reserves and the structural organization of seeds. In germinating embryos synthesis of proteins is also restricted under stress reported by Ramagopal. (1990). Such abiotic stresses as drought and salt stresses have resulted in the inhibition of the germination of seeds (Abid *et al.*, 2011; Li *et al.*, 2011; He *et al.*, 2011).

Numerous reports are there that describe the effects of drought and salt stress on seed germination (Abbasi *et al.*, 2010; Bajehbai, 2011; Erdal *et al.*, 2011; Fethi *et al.*, 2011; Saeedipour, 2011). Black gram (*Phaseolus mungo*) has been shown to inhibit the germination percentage and all the seedling growth parameters when treated with a solution of increased osmotic potentials generated by polyethylene

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glycol (PEG-6000) as reported by Pratap and Sharma. (2010). Farooq *et al.* (2012) also reported that seed priming is a method that is used to increase quick and uniform seed emergence, better yields and high vigor in angiosperms. To study the effect of drought stress on seed germination and early establishment in different crop varieties, PEG (Polyethylene Glycol) induced drought stress is the most common screening method (Awan *et al.*, 2021). The species

and its variety, its growing conditions and the developmental stage of the plant, as well as osmotic concentration all, respond differently to osmotic stress (Yadav *et al.*, 2019; Lokhande *et al.*, 2010). The seedling vigor index (SVI) can be used as an indicator of resistance to various temperatures and water potentials in seeds and plants (Khaeim *et al.*, 2022; Khan *et al.*, 2022; Shah *et al.*, 2021).

Low water negatively affects the seed germination parameters such as germination percentage and the length of the radicle and plumule (Duman, 2006). To establish conditions of dryness stress, different osmotic materials are used to create the osmotic potential and it is considered the best method to study the effects of drought stress on germination. Many chemicals such as PEG, CaCl_2 , KCl, KNO_3 , K_3PO_4 , mannitol and NaCl have been examined as potential osmopriming materials (Jisha *et al.* 2013; Zhang *et al.* 2015). Seed priming is a promising technique used in modern crop production management. It is mainly used in biotic and abiotic stress management. Seed priming is utilized in improving seed germination and studying the efficacy of stress responses (Lal *et al.*, 2018). Recent reports on Egyptian clover (*Trifolium alexandrinum* L.) found that antioxidants play a key role in improving tolerance to low-temperature stress when primed with salicylic acid (Kaur and Goyal, 2019). The best-studied material is Poly Ethylene Glycol-6000 (PEG-6000) as it has a high molecular weight, is not able to pass through the cell wall and is most commonly used to regulate water potential in seed germination tests.

Polyethylene glycol (PEG) is the most commonly used chemical for drought stress simulation. PEG is used as a drought simulator and it causes osmotic stress (Toosi *et al.*, 2014). Due to PEG treatment water absorption is reduced leading to drought stress conditions in plants (Channaoui *et al.*, 2017). In the seeds, hydrolysis of nutrient reserves decreases due to PEG, leading to a reduction in seed germination percent (Fischer *et al.*, 1978).

MATERIALS AND METHODS

The plant material consisted of eight genotypes belonging to *macrosperma* and *microsperma* subspecies of lentil (*Lens culinaris* M.). PL-4, L-4147, L-4594 and L-4596 belong to the *microsperma* subspecies and K-75, L-4076, DPL-15 and DPL-62 belong to the *macrosperma* subspecies.

Drought stress treatments and experimental designs

Germination potential and certain related parameters are considered important indices of tolerance to environmental stresses. Therefore, these parameters were studied under ambient laboratory conditions using PEG-6000 as the stress-inducing agent. Preliminary trials had indicated that osmotic potential lower than -5.0 bars are deteriorative for the germination process, therefore four levels of abiotic stress were simulated (namely -0.5, -2.0, -3.5 and -5.0 bars) using PEG-6000 osmoticum. The experimental work was conducted during 2018-2019 at the Laboratory of Botany, S.S. Jain Subodh P.G. (Autonomous) College, Jaipur to study

the effect of drought stress during early growth of genotypes of Lentil (*Lens culinaris* Medik.) under osmotic stress. The work involved eight genotypes of lentil (*Lens culinaris* Medik.) i.e., PL-4, L-4594, L-4147, L-4596, belonging to *microsperma* subspecies of lentil and K-75, L-4076, DPL-15, DPL-62 belonging to *macrosperma* subspecies. Genotypes used were procured from the Indian Agricultural Research Institute, Pusa, New Delhi. For each genotype, the seeds were decontaminated with sodium hypochlorite solution (2%) for 2 min. The seeds were then rinsed with distilled water several times to remove any impurities on the seeds. The seeds were then germinated in petri dishes containing a layer of Wattman paper soaked with distilled water and the seeds were germinated for seven days. Then the seeds were treated with PEG-6000 solution (namely -0.5, -2.0, -3.5 and -5.0 bars) using PEG-6000 osmoticum up to 20 days seedlings were collected for the evaluation of drought tolerance based on various parameters. The experimental sets for the eight genotypes were replicated three times.

Seed germination percentage

This investigation was originally based on the method given by ISTA (1985). Seed germination potential was calculated on the basis of germinated seeds. Seeds were checked daily. A seed was counted as germinated if it produced a 2 mm long radicle. The number of germinated seeds at the final count for each treatment was recorded and the germination percentage was calculated by the following formula:

Germination percentage (%) =

$$\frac{\text{No. of seeds germinated at final count}}{\text{Total number of seeds sown}} \times 100$$

Germination relative index (GRI)

Numbers of germinated seeds were recorded at every 24 hours interval and the germination relative index was calculated by the formula as given by Srivastava and Sareen, (1972):

$$\text{GRI} = \sum X_n (h-n)$$

Where

X_n = Number of seeds germinated at the n^{th} count.

h = Total number of counts and n is the count number.

Seedling vigor index (SVI)

Seedling vigor was calculated by the formula given by Abdul-Baki and Anderson, (1973) and modified by Singh and Singh, (1983).

$$\text{SVI} = \text{Germination (\%)} \times \text{Seedling length}$$

Seedling length (Shoot length and root length)

It was measured directly with the help of scale. Shoot length was considered from the root-shoot transition point to the shoot apex in Petri plates.

It was also measured in the same manner as the shoot length. Root length was also measured from the root-shoot transition point to the root tip of the main root in Petri plates.

Statistical analysis

The statistical analysis is the mean values of data from three independent experiments which are performed under the same laboratory conditions. One-way analysis of variance (ANOVA) was performed to determine significant differences between the mean values of control and each drought-stressed seedling genotype in lentil (*Lens culinaris* Medik.). The variations in the effects of PEG-induced osmotic stress on various germination-related parameters in eight lentil (*Lens culinaris* Medik.) genotypes were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Seed Germination percentage (%)

The most reliable way of assessing seed viability is the germination test (Davies *et al.*, 2015). A germination test is an appropriate way to know if the seeds will grow or not when being planted. It was observed that the germination percentage was above the Indian minimum seed certification standards (*i.e.*, above 75.00%) in all the eight genotypes of lentil under laboratory conditions in the seeds which did not experience the abiotic stress (Table 1). Osmopriming with a mild concentration of PEG-6000 led to a significant ($P < 0.05$)

increase in seed germination percentage. In the *microsperma* subspecies, the germination percentage was enhanced by moisture stress in genotypes PL-4 and L-4594. The beneficial effect of mild moisture stress was also evident in DPL-15 and DPL-62 genotypes of *macrosperma* lentil, however, the effect was more prominent in DPL-62. Except for 2.0 MPa, other levels (3.5 MPa and 5.0 MPa) of water stress progressively reduced germination percentage in *microsperma* as well as *macrosperma* genotypes of lentils. Seeds treated with PEG-6000 generally show an improved and uniform germination rate and early emergence of seedlings as reported by Bai *et al.* (2018) and Zhang *et al.* (2015). Priming was found to significantly improve the germination rate of lentil (Horbach *et al.*, 2018). However, Prolonged drought stress dramatically decreased the growth of three alfalfa varieties, when treated with 1.2 MPa polyethylene glycol (PEG-6000) (Zhang *et al.*, 2019).

Germination relative index (GRI)

A general enhancement in the speed of germination was observed in PL-4, L-41 and 47, L-4594. In the case of *macrosperma* genotypes, DPL-62 responded most favorably by osmopriming followed by DPL-15 and K-75. All results were found significant at $P < 0.05$. Primed seeds

Table 1: Germination percentage (%) in seedlings treated with PEG-6000 (0 to 5 MPa Bars).

Germination percentage (%) in 0 to 5 MPa bars seedlings					
Table of Interaction means					
Geno × Stress	0	2	3.5	5	Geno Mean
PL-4	86.667	76.667	68.000	56.000	71.83 a
L-4147	83.333	77.333	62.667	32.667	64.00 b
L-4594	86.667	68.667	58.667	36.000	62.50 b
L-4596	90.000	66.000	52.667	35.333	61.00 b
K-75	76.667	69.333	57.333	34.000	59.33 c
L-4076	96.667	62.667	48.000	36.000	60.83 b
DPL-15	80.000	73.333	63.333	48.000	66.17 b
DPL-62	86.667	78.000	71.333	59.333	73.83 a
Conc. Mean	85.833	71.500	60.250	42.167	64.938

Table 2: Germination relative index (GRI) in seedlings treated with PEG-6000 (0 to 5 MPa bars).

Germination relative index (GRI) in 0 to 5 MPa bars seedlings						
Table of interaction means						
Geno × Stress	0	0.5	2	3.5	5	Geno Mean
PL-4	33.000	42.000	29.000	20.000	18.000	28.40a
L-4147	38.667	51.667	23.333	14.333	10.667	27.73a
L-4594	28.333	43.000	33.667	19.667	17.000	28.33a
L-4596	39.000	31.000	23.333	17.667	7.667	23.73b
K-75	34.000	55.667	23.667	12.667	1.667	25.53a
L-4076	26.667	44.333	26.667	16.333	4.000	23.60b
DPL-15	33.333	37.667	34.333	20.667	17.333	28.67a
DPL-62	35.000	37.333	30.000	27.000	17.000	29.27a
Conc. mean	33.500	42.833	28.000	18.542	11.667	26.908

proved better with increased germination percentage, increased germination index and reduced mean germination time (Table 2).

The seedling vigor index (SVI)

It is a good criterion for evidencing the crop establishment potential of the plant genotypes. The seedling vigor index is a good selection criterion for the classification of genotypes as tolerant, moderately tolerant, moderately susceptible and susceptible on the basis of drought tolerance (Koskosidis *et al.*, 2020). In the present study, it was computed on the basis of germination percentage multiplied by seedling length. It was recorded that the seedling vigor index was highest in the DPL-62 genotype followed by PL-4, DPL-15 and L-4594 under controlled conditions. It was enhanced by mild moisture stress in genotypes PL-4, L-4147, L-4594 and L-4596 (Table 3). Other tested levels of moisture stress significantly reduced the seedling vigor in all the genotypes of *microsperma* as well as *macrosperma* lentils. Amongst *microsperma* genotypes, L-4147 was adversely affected to the maximum extent and in the *macrosperma*, subspecies group genotype L-4076 showed the lowest seedling vigor index (Table 3). The seedling vigor index (SVI) was significantly enhanced by priming with PEG as observed in soybean as reported by Sadeghi *et al.* (2011) and chickpeas as reported by Kumar *et al.* (2016).

Seedling length (SL)

Under controlled conditions seedlings of PL-4 had the highest seedling length followed by L-4594, L-4147 and L-4596. Amongst the *macrosperma* genotypes seedlings of DPL-15 elongated to the maximum extent under controlled conditions. Mild moisture stress exhibited a promotory effect on seedling length in L-4596, L-4594, L-4147 and PL-4 genotypes (Table 4). The adverse effect of higher levels of osmotic stress was reflected in the seedling length of all eight lentil genotypes. In two genotypes (L-4594 and L-4596) of *microsperma* subspecies, the root length was higher than the shoot. On the contrary, in the case of *macrosperma* genotypes, the root length was generally lower than the shoot lengths (Table 4). Polyethylene glycol was found to enhance the root length in maize hybrids (Anjum *et al.*, 2017). Polyethylene glycol (PEG) is responsible to induce water scarcity in alfalfa (*Medicago sativa*), such kind of water scarcity decreased dry and fresh weights of shoots and roots, hypocotyl elongation and ability to seed germination (Afzal *et al.*, 2016; Fathi *et al.*, 2016; Begum *et al.*, 2019). Four bread wheat varieties have been evaluated to see the impact of drought stress on seed germination and seedling growth and a general reduction was observed in these traits with significant differences among tested varieties (Farooq *et al.*, 2019; Yassin *et al.*,

Table 3: Seedling vigour index (SVI) in seedlings treated with PEG- 6000 (0 to 5 MPa Bars).

Seedling vigour index (SVI) in 0 to 5 MPa bars seedlings						
Table of interaction means						
Geno × stress	0	0.5	2	3.5	5	Geno mean
PL-4	1214.333	1892.333	1039.333	591.067	229.067	993.23 a
L-4147	954.667	1012.667	647.067	283.000	79.467	595.37 d
L-4594	1181.667	1483.333	795.067	406.467	122.533	797.81 b
L-4596	1075.333	1364.333	664.600	226.667	73.533	680.89 c
K-75	1090.333	1330.000	694.867	431.333	75.200	724.34 c
L-4076	1500.000	1447.333	720.667	271.133	61.267	800.08 b
DPL-15	1333.333	1567.333	1136.667	596.333	206.067	967.95 a
DPL-62	1293.333	1856.333	962.467	606.800	299.000	1003.59 a
Conc. mean	1205.375	1494.208	832.592	426.600	143.267	820.408

Table 4: Seedling length (SL) in seedlings treated with PEG- 6000 (0 to 5 MPa bars).

Seedling length (SL) in 0 to 5 MPa bars seedlings						
Table of interaction means						
Geno × stress	0	0.5	2	3.5	5	Geno mean
PL-4	14.033	19.600	13.533	8.700	4.133	12.00 b
L-4147	11.467	12.567	8.400	4.500	2.500	7.88 d
L-4594	13.700	16.433	11.667	6.967	3.400	10.43 b
L-4596	11.967	15.100	10.067	4.233	2.100	8.69 c
K-75	14.233	16.833	10.000	7.533	2.167	10.15 b
L-4076	15.500	17.333	11.500	5.633	1.700	10.33 b
DPL-15	16.667	17.433	15.467	9.400	4.367	12.67 a
DPL-62	14.900	19.867	12.367	8.633	5.067	12.17 a
Conc. mean	14.058	16.896	11.625	6.950	3.179	10.542

2019). The density and length of the root hairs were found to be increased substantially in lateral roots in Binadhan-11 when treated with PEG 5% compared to the control ones (Hannan *et al.*, 2020). Moderate drought stress caused more root length in rapeseed as observed by Channaoui *et al.* (2017).

CONCLUSION

A study carried out to assess the drought tolerance of the selected genotypes amongst all eight genotypes of lentil clearly indicated that of eight genotypes four genotypes are drought tolerant. All eight genotypes of lentil differed in their response to drought stress, these responses showed a direct link between seed germination capacity and initial seedling growth and development.

In conclusion, due to their better values of germination percentage (GP%), germination relative index (GRI), seedling vigor index (SVI) and seedling growth under simulated drought conditions PL-4, L-4594, DPL-15 and DPL-62 stand out as the most drought tolerant lentil genotypes. These genotypes showed a significant enhancement in germination percentage, germination relative index, seedling vigor index and seedling length under mild moisture stress. However, further research is required for the assessment of the physiological and biochemical responses of these tolerant genotypes to drought stress.

Conflict of interest: None.

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