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RESEARCH ARTICLE

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Metabolic and Enzymatic Evaluation of Lentil (*Lens culinaris* M.) Seedlings under Drought Stress

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ABSTRACT

Background: In the agricultural context, drought is a period of deficient water conditions, resulting in negative effects on crop growth and yield. A continuous increase in global temperature, leading to warmer climatic conditions, is expected to further increase the severity and frequency of drought.

Methods: The experiments were carried out during 2018-2019 under laboratory conditions to study the effects of PEG-6000 on seedling growth in eight genotypes of lentil (*Lens culinaris* Medik.).

Result: Our research work evaluated the effect of PEG-6000 induced drought stress on metabolite and enzymatic activity of eight genotypes of lentil (*Lens culinaris* Medik.) (PL-4, L-4147, L-4594, L-4596 belonging to *microsperma* subspecies and K-75, L-4076, DPL-15, DPL-62 belonging to *macrosperma* subspecies). Drought stress significantly increased metabolite content *i.e.*, protein, proline and carbohydrate and enzyme activity *i.e.*, acid phosphatase, invertase in drought tolerant genotypes (PL-4, L-4594, DPL-15 and DPL-62) and reduced amount of these metabolites and enzymes was observed in drought susceptible genotypes (L-4147, L-4596, K-75, L-4076).

Key words: Drought, Macrosperma, Microsperma, Polyethylene glycol (PEG-6000).

INTRODUCTION

Drought stress decreases both germination percentage and seedling growth in plants (Kaya *et al*. 2006). It is also dependent on the growth of plant and stress conditions. Short-term drought stress affects the plants, which ultimately leads to the decline in growth and yield (Muscolo *et al*., 2014). Drought stress induced crop yield loss is generally more prominent then other biotic and abiotic stresses, because severity and duration of drought stress are critical (Gul *et al*., 2020). Drought causes changes in nutrient acquisition and assimilation, water relations, photosynthesis, assimilates partitioning and enzyme functioning (Ayyaz *et al*., 2021; Xiong *et al*., 2020; Bano *et al*., 2021).

Lentil (*Lens culinaris Medik*) is amongst the oldest domesticated plants in the world. It is traditionally cultivated in the Mediterranean basin and originated from the near East and central Asia (Zohary, 1972). Lentil is grown in semiarid environment where unfavourable soil moisture at sowing conditions leads to an irregular seedling emergence, it negatively affects the establishment of a crop stand and a reduction in crop yield (Okcu *et al*., 2005). Lentil is economically important because its seeds are an important source of protein for the human beings and the entire plant is a valuable animal feed. Lentil yield can be increased with regular irrigation (Salehi *et al*., 2008). This can improve seed yield, seed size, biomass yield and harvest index (Khourgami *et al*., 2012). In annual plant species successful plant establishment largely depends on proper seed germination and seedling emergence (Forcella *et al*., 2000). Rapid and uniform seed germination ultimately leads to a successful crop establishment in semiarid areas and this is associated to the ability of seeds to germinate under low water availability. In such cases drought tolerance studies at the germination stage has specific importance (IPCC 2007). Drought can reduce the germination percentage (GP) and germination rate (GR) which ultimately leads to negative seedling growth (Abdellaoui *et al*., 2019). Polyethylene glycol (PEG) is a natural polymer molecular. Molecular weight of PEG is 6000. It is water-soluble and nonionic (Ranjbarfordoei *et al*., 2000). PEG 6000 mimics drought stress causing osmotic stress resulting in lowering of plant's water potential (Muscolo *et al*., 2014). Effect of PEG can be seen during seed germination and emergence of seedlings is also affected by PEG that directly affects development, root and shoot growth and even flowering and pod formation.

Water plays an important role in the hydrolytic breakdown of proteins, lipids and carbohydrates in the storage tissues of germinating seeds. Water is also involved

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in solubilisation and transportation of metabolites and in many enzymatic reactions (Biaecka and Ke²pczyn´ ski, 2010). However, drought stress studies can be done under natural field conditions but sometimes it is difficult as rainfall can eliminate natural drought stress conditions. Now-a-days *in vitro* drought-screening methods are in progress to understand drought-resistance traits. These are also helpful in selection of drought-tolerant genotypes. Germination and seedling establishment stages are most sensitive stages to drought stress (Desclaux *et al*., 2000). Polyethylene glycol (PEG-6000) solutions have been effectively used to develop drought stress in lab conditions (Hohl and Schopfer, 1991).

The aim of research work is to evaluate the drought tolerant genotypes amongst the eight genotypes of *L. culinaris* Medik. under drought stress conditions by analysing some biochemical parameters.

MATERIAS AND 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 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 Indian Agricultural Research Institute, Pusa, New Delhi. Preparation of PEG-6000 solution was done by dissolving 15 gm of PEG-6000 in 100 ml of water.

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 till seven days. Then the seeds were treated with PEG-6000 solution (15%) up to 20 days, leaves from the seedlings were collected for the evaluation of drought tolerance based on various parameters. The experimental sets for the eight genotypes were replicated three times.

The biochemical responses (protein content, proline content and carbohydrate content) were observed in leaves of 20 days old seedling. Some of the enzyme (acid phosphatase, invertase and nitrate reductase enzymes) activities were also measured. Thus, the main objective of this research was to understand the biochemical mechanisms of adaptation to drought stress by lentil during the early seedling stages.

Biochemical parameters: Metabolites

Following metabolic parameters were studied in all eight lentil genotypes.

Protein content

Bradford's method (1976) was used for the estimation of soluble proteins.

Total carbohydrate content

Total carbohydrate content measurement was done by the method of Dubois *et al*. (1956).

Proline content

Free proline content was measured by the method given by Bates *et al*. (1973). Proline contents were calculated in terms of mg proline/ gm fresh weight.

Biochemical parameters: Enzymes

Extraction of enzymes

All extractions were carried out at 4°C. The leaves were excised, washed with water and blotted dry. The leaf samples (1 g) were homogenized with 10 ml of appropriate phosphate buffer like for *acid phosphatase* phosphate buffer (0.1M, pH-7.0) was used, for *invertase* acetate buffer (0.2 M, pH-4.8) and for *nitrate reductase* 0.1 M phosphate buffer having pH-7.5 was used. The homogenate was extracted at 10,000xg for 20 minutes. The supernatant thus collected was used for *Acid phosphatase* (Zink and Veliky, 1979), *Invertase* (*Ha*rris and Jeffcoat, 1974), *Nitrate reductase* (Jaworski, 1971) assay.

Statistical analysis

The statistical analysis are the mean values of data of three independent experiments which are performed under same laboratory conditions. One-way analysis of variance (ANOVA) was performed to determine significant differences between 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 physiological and biochemical parameters in eight lentil (*Lens culinaris* Medik.) genotypes were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Biochemical parameters: metabolites

Changes in certain metabolites were quantitatively determined in the lentil seedlings of different genotypes in response to simulated moisture stress, which lead to the identification of genotypes that are susceptible and tolerant to drought stress. Salient features of such changes in certain metabolites are described below.

Protein content

The data were analyzed for protein content of lentil Table 1. All the results were found to be significant ($P<0.05$) at genotypic level. Significant results observed between the genotypes under stress conditions. PL-4 showed significantly higher protein content to the other lentil genotypes. The content of protein was highest (11.75 mg/g f. wt.) in PL-4 seedlings followed by L-4147 (9.68 mg/g f. wt.), L-4594 (9.53 mg/g f. wt.) and L-4596 (9.04 mg/g f. wt.). Amongst macrosperma genotypes it was highest (11.91 mg/g f. wt.) in L-4076 followed by K-75 (10.16 mg/g f. wt.), DPL-15 (7.58 mg/g f. wt.) and DPL-62 (7.41 mg/g f. wt.). A general

Table 1: Effects of osmopriming on metabolites in eight genotypes of lentil (*Lens culinaris* Medik.).

Genotype × stress	Protein content			Carbohydrate			Proline content		
	NS	WS	Genotype mean	NS	WS	Genotype mean	NS	WS	Genotype mean
PL-4	11.750	10.583	11.167a	9.267	21.867	15.567a	0.017	0.327	0.172c
L-4147	9.675	6.167	7.921b	8.667	9.200	8.933d	0.023	0.128	0.075d
L-4594	9.525	8.750	9.138b	5.333	18.000	11.667c	0.017	0.497	0.257b
L-4596	9.042	7.583	8.313b	7.867	13.467	10.667c	0.028	0.160	0.094d
K-75	10.167	5.792	7.979b	5.200	6.533	5.867d	0.044	0.288	0.166c
L-4076	11.917	8.500	10.208a	8.933	12.400	10.667c	0.084	0.233	0.159c
DPL-15	7.583	6.917	7.250c	10.533	23.200	16.867a	0.023	0.768	0.396a
DPL-62	7.417	5.958	6.688c	9.067	19.200	14.133b	0.051	0.373	0.212b
Stress mean	9.634	7.531	8.583	8.108	15.483	11.796	0.036	0.347	0.191

Showing the values at the 0.05 probability, NS= Non stress, WS = Water stress.

Table 2: Effects of osmopriming on enzymes in eight genotypes of lentil (*L. culinaris* Medik.).

Genotype × stress	Acid phosphatase			Invertase			Nitrate reductase (NR)		
	NS	WS	Genotype mean	NS	WS	Genotype mean	NS	WS	Genotype mean
PL-4	5.527	4.953	5.240 a	0.067	2.313	1.460 b	48.000	46.667	47. 333a
L-4147	4.537	4.977	4.757 a	0.669	1.490	1.080 b	56.667	31.000	43.833a
L-4594	4.457	4.427	4.442 a	0.632	2.510	1.571 b	42.333	41.333	41.833a
L-4595	3.560	5.017	4.288 a	0.713	1.093	0.903 b	41.333	21.000	31.167b
K-75	2.083	2.683	2.383 b	0.777	1.587	1.182 b	26.667	15.000	20.833b
L-4076	2.767	3.280	3.023 b	0.715	1.353	1.034 b	52.333	29.000	40.667a
DPL-15	4.417	4.623	4.520 a	1.238	3.697	2.468 a	26.333	23.666	25.000b
DPL-62	4.630	3.553	4.092 a	0.776	3.937	2.357 a	42.333	40.667	41.500a
Stress mean	3.997	4.189	4.093	0.766	2.248	1.507	42.000	31.042	36.521

Showing the values at the 0.05 probability, NS= Non stress, WS = Water stress.

decrease in protein content was recorded in all the genotypes by water stress, the reduction was less in drought tolerant and it was more in drought susceptible. Drought stress negatively affects the accumulation of various seed components such as proteins (Asthir *et al*., 2012; Farooq *et al*., 2017a,b) by inhibiting the enzymatic processes of synthesis of proteins (Triboï *et al*., 2003).

Total carbohydrate content

In general, the level of carbohydrate increased in seedlings subjected to drought stress. Data in Table 1 shows the results and were found to be significant between genotypes and significant variations are also observed between stressed and non-stressed seedlings of genotypes. Drought stress led to significant (P<0.05) increase in carbohydrate content in PL-4 and DPL-15 and increment was significantly more in DPL-15. In response to water stress PL-4 had highest level (21.86 mg/g f. wt.) of carbohydrate followed by L-4594 (18.00 mg/g f. wt.), L-4596 (13.46 mg/g f. wt.) and L-4147 (9.20 mg/g f. wt.). Under water stress seedlings of DPL-15 accumulated highest content (23.2 mg/g f. wt.) of carbohydrate followed by DPL-62 (19.2 mg/g f. wt.), L-4076 (12.4 mg/g f. wt.) and K-75 (6.53 mg/g f. wt.). Amongst all genotypes DPL-15 showed significantly highest carbohydrate content. *Vigna radiata* nodules showed a significant increase in sugar content under drought conditions (Hooda *et al*., 1999). Alfalfa plants also showed an increment in total soluble sugars in leaves and nodules under drought stress conditions (Irigoyen *et al*., 1992). Soyabean genotypes shown a considerable variation in the sucrose level under drought conditions for example, myo-inositol and sucrose levels in the leaves decreased drastically in the sensitive soyabean genotype, but no significant changes were observed in the tolerant genotype (Silvente *et al*., 2012).

Proline content

The free proline accumulation showed a common trend of increase in the seedlings under drought stress as depicted in Table 1. A Significant (P<0.05) increment in free proline content was observed in seeds of all genotypes under drought stress. DPL-15 showed significantly highest proline content under drought conditions. The proline content in microsperma subspecies was highest in L-4594 (0.49 mg/g f. wt.) followed by PL-4 (0.327 mg/g f. wt.), L-4596 (0.160 mg/g f. wt.) and L-4147 (0.128 mg/g f. wt.). Among macrosperma group the proline accumulation was highest (0.768 mg/g f. wt.) in DPL-15 followed by DPL-62 (0.373

mg/g f. wt.), K-75 (0.288 mg/g f. wt.) and L-4076 (0.233 mg/g f. wt.). *Vigna radiata* nodules showed a significant increase in proline content under drought conditions (Hooda *et al*., 1999). Increase in proline content was reported in alfalfa leaves and nodules under drought stress (Irigoyen *et al*., 1992). Proline accumulation has been reported under drought stress in different plant species (Ashraf, 2004; Kumar *et al*., 2017; Awana *et al*., 2019). PEG induced drought stress in tomato showed 10-fold increase in proline content (Zgalli *et al*., 2005).

Biochemical parameters: Enzymes

Acid phosphatase

Activity of acid phosphatase was enhanced in response to drought stress in PL-4 (5.52 mM P-nitrophenol) followed by L-4147 (4.53 mM P-nitrophenol), L-4594 (4.45 mM P-nitrophenol) and lowest (3.56 mM P-nitrophenol) in L-4596 genotype of microsperma lentil. The comparative data analysis is shown in Table 2. Contrary to microsperma group the activity of acid phosphatase in macrosperma group was lowered under drought stress and the reduction was highest in L-4076 where acid phosphatase activity was lowest (2.76 mM P-nitrophenol) as compared to control seedlings (3.28 mM P-nitrophenol). Acid phosphatase activity was increased by salt and drought stress in both cultivars of *Medicago sativa* explants under *in vitro* culture and the difference in activities between two genotypes of *Medicago sativa* indicated that the acid phosphatase activity is highly genotype dependent (Ehsanpour and Amini, 2003). Increased acid phosphatase activity has also been reported in different crop plants under different stresses (Kumar *et al*., 2021; Sheteiwy *et al.,* 2021).

Invertase

Simulated water stress also significantly increased invertase activity in seedlings under ambient laboratory conditions shown in Table 2. PEG-6000 treated seedlings of L-4594 recorded the highest (2.50 mg/g f. wt.) invertase activity under water stress followed by PL-4 (2.31 mg/g f. wt.), L-4147 (1.49 mg/g f. wt.) and L-4596 (1.09 mg/g f. wt.). Amongst macrosperma genotypes DPL-62 showed highest (3.93 mg/g f. wt.) invertase activity in the seedlings followed by DPL-15 (3.69 mg/g f. wt.), K-75 (1.58 mg/g f. wt.) and L-4076 (1.35 mg/g f. wt.). The invertase activity was observed to be increased under various biotic and abiotic stress (Roitsch *et al.,* 2003) in maize.

Nitrate reductase (NR)

There was a general reduction in the activity of nitrate reductase under simulated water stress in seedlings of lentil genotype seedlings, accordingly shown in Table 2. PEG-6000 treated seedlings of L-4594 showed the minimum reduction (2.79%) it is followed by PL-4 (2.36%), L-4147 (44.64%) and L-4596 (49.19%). Amongst macrosperma genotypes DPL-62 showed minimum reduction (3.94%) in nitrate reductase activity under simulated water stress followed by DPL-15 (10.14%), K-75 (43.73%) and L-4076 (44.58%). Our results are in accordance with previous results of water-stress-induced losses in nitrate reductase activity in other species (Wellburn *et al*.,1996). In tomato also drought observed to decrease the Nitrate Reductase activity (Brewitz *et al*., 1996). Similar results were observed in leaves of maize (Foyer *et al*., 1998).

CONCLUSION

This study found that the seedling growth of PL-4, L-4594, DPL-15, DPL-62 genotypes are highly tolerant to increased level of drought stress. In the present study, seedlings of *microsperma* and *macrosperma* sub species showed altered levels of various metabolites and enzymes due to the application of PEG-6000 Solution. PEG-6000 has a high molecular weight. In most of the seed germination experiments it is used to regulate water potential and PEG causes osmotic stress. Earlier studies also found that potentials between -0.4 and -0.8/ MPa are the best condition for studying seed germination related parameters of different genotypes of plants under drought stress (Khazayi *et al*., 2008). PEG also used as drought simulator, non-penetrating solution and inert osmotic in germination tests. Osmotic stress obstructs seed germination by reducing water absorption. Drought stress not only inhibited seedling growth in L-4147, L-4596, K-75, L-4076 but also significantly inhibited various metabolites and enzymes. This information can provide a scientific basis for understanding the occurrence of drought resistance in crop plants and provide key information for the management of resistant genotypes amongst all. So, these data are valuable for increasing agricultural production and farmer incomes in drought prone areas. Such kind of studies will be helpful in those areas where most of the time drought conditions prevail in a year. In Rajasthan such drought prone areas are large in numbers and in such areas drought tolerant genotypes may prove better than other drought susceptible genotypes of lentil.

Such kind of studies may lead to an analysis of the drought tolerance potential of seedlings of various crops, that ultimately gives the knowledge about various morphological and biochemical levels that may be treated as drought tolerant trait in the field conditions. In conclusion by observing changes in the metabolites and enzymatic activities drought tolerant genotypes can be obtained in lentil genotypes.

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