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# Impact of PEG Induced Drought Stress on Growth and Physiology of Fenugreek (*Trigonella foenum graecum* L.)

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**ABSTRACT** Drought stress is one of the critical abiotic stress factors that limit the seed germination, growth, development and yield of plant. The objective of the present investigation was to explore the effects of application of drought stress, which is induced by different concentrations of polyethylene glycol (5, 10 and 15%) in fenugreek seedlings. Drought stress remarkably inhibited the seed germination and growth (shoot length, dry and fresh weight of seedling and seedling vigour index). Germination percentage decreased with increasing concentration of PEG. Seedlings shoot and root length and biomasses were inhibited with an increasing the concentration of PEG. The lengths of shoot and root were gradually inhibited by the PEG treatment. Fresh and dry weights by PEG treatment were inhibited with application of PEG. The content of protein and proline were gradually inhibited with increase in the amount of PEG.

## INTRODUCTION

Fenugreek (*Trigonella foenum graecum* L.) of the family Leguminosae is a yearly legume crop and was mostly cultivated in the Middle East region and Egypt. Fenugreek is a plant whose seeds and leaves may be used for different purposes such as spice in preparation of food, and powder of the seed mixed with flour for the preparation of bread. In India, new seedlings are used as a source of vegetables and for medicinal use, like for lowering cholesterol level and blood sugar, anti-diabetic, anti-microbial, anti-cancer, etc. (Basch et al. 2003). Fenugreek crop is more susceptible to adverse conditions like salinity, drought and chilling (Almansouri et al. 2001). Drought stress is the most prominent and severe water stress that limits yield and growth of the plant worldwide, particularly in semiarid and arid provinces (Jackson et al. 2000; Sadak 2016). In nature, drought conditions occur when the availability of soil water is decreased and environmental situations cause non-stop water loss by evaporation or transpiration (Khajeh-Hosseini et al. 2003). Polyethylene glycol (PEG) molecules are non-ionic and inert, producing drought stress by lowering water potential, which leads to reduce in growth of the plant (Zhu 2002; Kulkarni and Deshpande 2007). Gholamin et al. (2010) found that morphological parameters were decreased in wheat cultivars under drought stress by polyethylene glycol. Hammad and El-Gamal (2004) observed that total phenolics in the leaves of pepper were remarkably increased under drought stress. Phenolic compounds are the main part of non-enzymatic antioxidants in the plants (Fujita et al. 2006). Phenolic compounds has many functions and they fulfil several roles in plants, like cell wall components, regulating growth and developmental of plant, and its processes, play effective defence role mechanism against pathogen and diseases, and in addition, they are involvement in different responses of a plant, mainly in abiotic stress like drought and salinity (Cheynier et al. 2013).

Soil fertility is mainly regulated by the moisture level present in soil and it is deteriorated by drought condition.

In arid and semiarid areas, the length of days of drought as well as unsuitable passionate irrigation create an amount of solutes in soil peripherals so that fifteen to twenty percent of soils in these areas suffer difficulties of deficiency of soil moisture and one-third fertile lands in the world are the facing problem of drought (Hoffman et al. 1980; Jefferies 1981). Drought (less and unpredictable rainfall) is one of the important limitations to crop productivity

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globally (Sadeghzade et al. 2009). The germination stage is one of the crucial stages in the growth and development cycle of a plant, due to its male parts in final compression. However, it is well reported that plants exhibit relative tolerance to salinity and drought in various vegetative and reproductive growth phases (Javan et al. 1997). The aim of the present study was to evaluate the effect of drought stress induced by application of PEG in vitro on growth and developmental parameters of fenugreek.

## METHODOLOGY

### Experimental Material

The present investigation was performed in in vitro conditions at the Department of Biotechnology, Central University of South Bihar, Gaya in Bihar, India. Fresh seeds of fenugreek were collected and experiments were setup as a completely randomised design with three replications.

### Stress Treatment

Effect of drought stress was created by exposing various osmotic potential intensities [0 (control), 5, 10, 15, 20, 25 and 30%] to PEG 6000 treatments and germination was studied.

### Determination of Seed Germination

Seeds were sown in a test tube under hydroponics condition for determination of germination under in vitro condition. Seeds were considered germinated when radical length reached up to 2mm or more after 3 weeks. Germination percentage of seeds was evaluated by using the equation:

Percentage of Germination = Germinating seeds number/Total seeds number × 100

For a seed treated beyond fifteen percent PEG, germination was completely inhibited, and hence lower doses of five percent, ten percent and fifteen percent PEG were used for the study.

### Growth Measurements

Phonotypical characters, that is, root length and shoot length were measured after 3 weeks of germination. Shoot and root length per plant, dry mass and fresh mass per plant, were calculated. Root by shoot ratio was determined as length of root divided by length of shoot into 100. Dry mass was calculated after dehydrating at 70 to 80 °C for 72 hours. Relative content of water was determined as fresh mass minus dry mass divided by fresh mass into 100. Vigor index was obtained as $VI=S\times\Sigma$ (Gt/Dt), where S is the length of seedling on the $7^{th}$ day, Gt is the germinated seeds number on the $7^{th}$ day, Dt is days number from the $1^{st}$ day to the $7^{th}$ day.

### Extraction and Estimation of Proline

Total proline was calculated using the procedures developed by Bates et al. (1973). 100 mg of tissue was grinded in 4 ml of three percent aqueous sulphosalicylic acid and further centrifuged at 10,000 RPM for 12 minutes and debris was discarded. Further, 2 ml of the upper layer was taken and mixed with 2 ml glacial acetic acid and 2 ml of acid ninhydrin (10 ml of 6 M orthophosphoric acid and 625 mg ninhydrin in 15 ml glacial acetic acid) in a test tube and boiled for 60 minutes. The reaction was stopped by freezing the tubes in an ice bucket. The chromosphere, which was formed after the reaction, was pulled out with 6 ml of toluene and the absorbance was obtained at 520. Total content of proline was determined by using a standard curve prepared using L-prolin.

### Protein Extraction and Estimation

300 mg of green tissue was grinded at 4°C in 2 ml protein extraction buffer [(10 mM ethylene diamine tetra acetic acid (EDTA), 200 mM Tris-HCl, 2 mM phenyl methyl sulfonyl fluoride (PMSF), 1% insoluble polyvinyl pyrrolidone (PVP), and 0.5 M sucrose (pH8.5)]. The mixture was centrifuged at 10,000 rpm for 45 minutes and the upper layer was taken for estimation of protein content (Lowry et al. 1951).

## RESULTS

### Seed Germination

As compared to control, less percentage of germination was found in PEG treatment. As shown in Figure 1, the percentage of germination decreased with enhancing concentration of PEG. Highest percentage of germination (98.7%) was

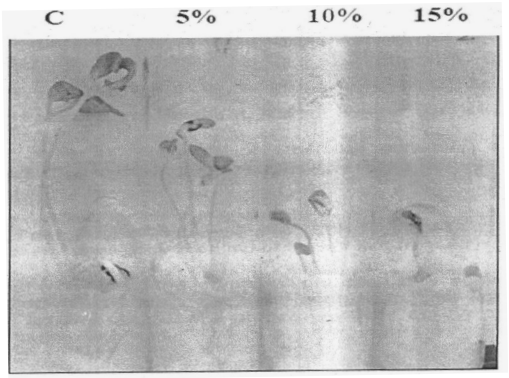


**Fig. 1. Effect of different concentration of PEG on seedling growth of Fenugreek**

found in control whereas, only 11.1 percent seed germination was found in fifteen percent after treatment of PEG (Fig. 1).

## Growth Measurements

Due to treatment of PEG the seedling growth was decreased at all concentrations. The reduction of seedling growth was directly proportional to concentration of PEG. There was gradual inhibition in shoot length and root length was observed with increasing concentration of PEG. When seedlings were growing in the presence of five percent PEG, a reduction of eighteen percent shoot length was found as compared to control. Further inhibition of twenty-three percent and twenty-six percent of shoot length occurred at ten and fifteen percent of PEG concentrations, respectively (Table 1). When seedlings grew in the presence of five percent PEG, a reduction of fifteen percent root length was found as compared to control. Inhibition of fifty-six percent and sixty-seven percent of root length occurred at ten and fifteen percent of PEG concentrations, respectively (Table 1). When seedlings grew in the presence of five percent PEG, an inhibition of fifteen percent fresh weight and twenty percent dry weight was found as compared to control. Inhibition of twenty-six and forty-four percent fresh weight, and twenty-three and forty-four percent dry mass occurred at five percent and fifteen percent PEG concentrations, respectively. However, no remarkable differences were observed for relative water content with PEG treatment. There was gradual reduction in seedling vigour index was observed with increasing concentration of PEG (Table 2).

**Table 1: Effect of different concentration of PEG on germination and plant length of fenugreek**

| *PEG concentration (%)* | *Germination (%)* | *Shoot length (CM)* | *Root length (CM)* | *Root: Shoot ratio* |
|---|---|---|---|---|
| 0 | 98.7 ± 1.73 | 4.89 ± 1.16 | 3.17 ± 1.73 | 0.64 ± 0.13 |
| 5 | 98.3 ± 2.71 | 3.98 ± 1.65 | 2.67 ± 1.50 | 0.67 ± 0.19 |
| 10 | 35.57 ± 1.63 | 3.72 ± 1.30 | 1.37 ± 0.87 | 0.36 ± 0.11 |
| 15 | 11.1 ± 2.03 | 3.59 ± 0.976 | 1.05 ± 0.86 | 0.29 ± 0.09 |

## Effect of PEG on Quantitative Differences in Accumulation of Proteins and Proline

Application of PEG caused a decrease in soluble protein accumulation. When seedlings were grown in the presence of five percent PEG, a reduction of eighteen percent protein content was found as compared to control. Inhibition of twenty-seven and thirty-nine percent protein content occurred at five percent and fifteen percent PEG concentrations, respectively (Table 3).

PEG stress resulted in increase in accumulation of proline. When seedlings were grown in the presence of five percent and ten percent PEG, an accumulation of fifty-one percent and sixty-five percent proline content was found as compared to control. Inhibition of eighty percent proline content occurred at fifteen percent PEG concentrations (Table 3).

**Table 2: Effect of different concentration of PEG on growth and vigor index of fenugreek**

| *PEG concentration (%)* | *Fresh weight (mg/plant)* | *Dry weight (mg/plant)* | *Relative water content (%)* | *Seedling vigor index* |
|---|---|---|---|---|
| 0 | 45.58 ± 2.61 | 4.79 ± 1.53 | 90.64 ± 5.53 | 17.46 ± 2.13 |
| 5 | 38.8 ± 4.11 | 3.84 ± 2.44 | 90.01 ± 6.33 | 14.21 ± 2.34 |
| 10 | 32.97 ± 5.34 | 3.69 ± 1.94 | 91.5 ± 5.83 | 13.28 ± 1.33 |
| 15 | 25.38 ± 3.18 | 2.70 ± 2.32 | 89.36 ± 6.54 | 12.82 ± 1.52 |

**Table 3: Effect of different concentration of PEG on protein and proline content of fenugreek**

| *PEG concentration (%)* | *Protein content (mg/gm of FW* | *Proline content (µg/gm of FW)* |
|---|---|---|
| 0 | 105.97 ± 6.56 | 1.02 ± 0.13 |
| 5 | 86.94 ± 5.23 | 1.554 ± 0.14 |
| 10 | 76 ± 6.16 | 1.69 ± 0.13 |
| 15 | 64 ± 5.26 | 0.19 ± 0.03 |

## DISCUSSION

Soil moisture level is a limiting factor for the optimum growth of a plant. Drought stress is one of the major factors, which inhibit the growth of a plant. It is well reported that injury in plants takes place due to drought stress at vegetative and reproductive stages of development (Daneshian and Zare 2005). Creation of drought stress by application of PEG on the growth of plant showed remarkable deviation from normal levels of moisture. PEG enhances the solute potential in plant, which causes drought stress (Zhang and Kirkham 1995). In the presented study it is observed that, germination of the seed, shoot length and root length, fresh mass and dry mass decreased with respect to control with PEG treatment (Tables 1 and 2). Similar observation was also observed that PEG decreased the overall growth of plant by inducing drought stress (Siahsar et al. 2010; Jamaati-e-Somarin and Zabihi-e-Mahmoodabad 2011). Drought stress was created in plants after application of PEG is may be due to the high viscosity of PEG. High viscosity may cause depletion of the boundary oxygen layer around the root of a plant, and due to this depletion of oxygen, this occurs around roots of a plant, that is, it creates a hypoxia condition around the root (Verslues et al. 1998). It is reported that the inhibition herbage yield in chamomile and oregano upon drought stress takes place due to decreasing the plant canopy structure and photosynthesis. In some plants it is also reported that plants exhibit drought stress adaptive mechanisms by inhibiting their growth and development while scarcity of water takes place around the rhizosphere. The inhibition of plant growth and development of the fenugreek plant might be measured as a major factor in inhibiting the ratio of root to shoot. RWC is one of the most appropriate and important measures of plant water status level in terms of the physiological consequence of cellular water shortage. Actually RWC showed that relative content of water exists in the plant cells and tissues. In the present investigation, RWC reduced remarkably under drought stress (Table 2). The inhibition of RWC indicates a loss of turgor pressure in plants that causes restricted availability of water for broadening development of cell and subsequently inhibition of growth and development of plants take place (Fig. 1). A research using microscopic study of dried plant cells showed the appearance of cleavage in cell membrane and its enhancing penetrability in drought situations. They cited that the amount of appropriate solutes that could protect membrane structure were not enough and the plant was not able to fit osmotically. The effect of increased osmotic pressure caused by drought stress resulted in a significant enhancement in endogenous proline level. It has been reported that the proline is an important compatible solute accumulated in higher plants under conditions of abiotic stress (Delauney and Verma 1993). Proline plays a crucial role in osmoregulation and osmotolerance (Szabados and Savouré 2010). It is considered as a biomarker of stress.

Various instigations proved that an enhancement if free proline concentration due to drought stress situations caused by application of PEG has been shown in various crops like bread wheat (Ji et al. 2014) and maize (Jain et al. 2013). It is observed by Handa et al. (1982) that the accumulation of proline content depends not only on the loss of turgor pressure or plant osmotic potential, but also on its ability of drought stress adaptation of the plant. The reduction in protein concentration was observed, as reduction is directly proportional to the PEG concentration in nutrient medium. The reduction in protein content was may be due to enhancement of hydrolysis of protein (Uprety and Sarin 1976) or low synthesis of protein (Hall and Flowers 1973).

## CONCLUSION

Drought stress had a major effect on all the measured characters at all PEG concentrations applied. Water stress delays the beginning of germination in relation to the control treatment. Seedlings shoot and root length and biomasses were inhibited with an increasing the concentration of PEG. The lengths of shoot and root were gradually inhibited by the PEG treatment. Fresh and dry

weights by PEG treatment were inhibited with application of PEG. The content of protein and proline were gradually inhibited with increase in the amount of PEG.

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## REFERENCES

Almansouri M, Kinet JM, Lutts S 2001. Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum Desf.). *Plant and Soil,* 231: 243-254.

Basch E, Ulbricht C, Kuo G, Szapary P, Smith M 2003. Therapeutic application of fenugreek. *Alt Med Rev,* 8: 20-27.

Bates LR, Waldren RP, Teare ID 1973. A rapid determination of free proline for water stress studies. *Plant Soil,* 39: 205–207.

Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S 2013. Plant phenolics: Recent advance son their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry,* 72: 1-20.

Daneshian J, Zare D 2005. Diversity for resistance drought on soybean. *Journal of Agricultural Science,* 1: 23–50.

Delauney AJ, Verma DPS 1993. Proline biosynthesis and osmoregulation in plant. *The Plant Journal,* 4: 215-223.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K 2006. Crosstalk between abiotic and biotic stress responses: A current view from the points of convergence in the stress signaling networks. *Curr Opin Plant Biol,* 9: 436-442.

Gholamin R, Zaeifizadeh M, Khayatnezhad M 2010. Study of drought tolerance in Durum wheat genotypes. *American-Eurasian J Agric Environ Sci,* 9: 465-469.

Hall JL, Flowers TJ 1973. The effect of salt on protein synthesis in the halophytes Suaeda maritime. *Planta,* 110: 361–368.

Hammad SAR, El-Gamal SM 2004. Response of pepper plants grown under water stress condition to biofertilizers (Halex 2) and mineral nitrogen Minufiya. *J Agric Res,* 29(1): 1-27.

Handa AK, Bressan RA, Handa S, Hasegawa PM 1982. Characteristics of cultured tomato cells after prolonged exposure to medium containing poly-ethylenem glycol. *Plant Physiol,* 69: 514-552.

Hoffman GJ, Ayers RS, Doering EJ, McNeal BL 1980. Salinity in irrigated agriculture. In: Design and operation of farm irrigation systems. *Am Soc Agric Eng,* 145-185.

Jackson RB, Sperry JS, Dawson TE 2000. Root water uptake and transport: Using physiological processes in global predictions. *Trends Plant Sci,* 5: 482–488.

Ji H, Liu L, Li K, Xie Q, Wang Z, Zhao X, Li X 2014. PEG-mediated osmotic stress induces premature differentiation of the root apical meristem and outgrowth of lateral roots in wheat. *J Exp Bot,* 65(17): 4863–4872.

Jamaati-e-Somarin S, Zabihi-e-Mahmoodabad R 2011. Evaluation of drought tolerance indices of lentil varieties. *Adv Environ Biol,* 5: 581–584.

Javan M, Ahmadiani A, Semnanian S, Kamalinejad M 1997. Antinociceptive effects of Trigonella foenum-graecum leaves extract. *J Ethnofarmacol,* 53: 149-155.

Jefferies RL 1981. Osmotic adjustment and response of halophilic plants to salinity. *Bioscience,* 31: 42-46.

Khajeh-Hosseini M, Powell AA, Bingham IJ 2003. The interaction between salinity stress and seed vigor during germination of soyabean seeds. *Seed Sci Technol,* 31: 715-725.

Kulkarni M, Deshpande U 2007. In vitro screening of tomato genotypes for drought resistance using polyethyleneglycol. *African Journal of Biotechnology,* 6: 691-696.

Lowry OH, Rosebrough NJ, Farr AL, Randall RI 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193(1): 265-275.

Jain M, Mittal M, Gadre R 2013. Effect of PEG-6000 imposed water deficit on chlorophyll metabolism in maize leaves. *J Stress Physiol Biochem,* 9(3): 262-271.

Sadak MS 2016. Mitigation of drought stress on fenugreek plant by foliar application of trehalose. *Inter J of Chem Tech Res,* 9(2): 147–155.

Sadeghzade AD, Kashi AK, Hassandokht MR, Amri A, Alizade K 2009. Assessment of drought tolerance in Iranian fenugreek landraces. *Food Agric Environ,* 7(3-4): 414-441.

Siahsar BA, Ganjali S, Allahdoo M 2010. Evaluation of drought tolerance indices and their relationship with grain yield of lentil lines in drought-stressed and irrigated environments. *Aust J Basic Appl Sci,* 4: 4336–4346.

Szabados L, Savouré A 2010. Proline: A multifunctional amino acid. *Trends Plant Sci,* 15(2): 89-97.

Uprety DC, Sarin MN 1976. Physiological studies on salt tolerance in Pisum sativum L. IV, Ionic composition and nitrogen metabolism. *Acta Agron Hung,* 25: 455–460.

Verslues PE, Ober ES, Sharp RE 1998. Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. *Plant Physiology,* 116(4): 1403–1412.

Zhang J, Kirkham MB 1995. Water relations of water-stressed, split-root C4 (Sorghum bicolor; Poaceae) and C3 (Helianthus annuus; Asteraceae) plants. *American Journal of Botany,* 82(10): 1220–1229.

Zhu JK 2002. Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol,* 53: 247–273.

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