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Polyethylene Glycol Induced Morphological Changes in *Fagopyrum esculentum* Moench of Indian Himalayan Region

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ABSTRACT: Water deficit stress is one of the most adverse factors of plant growth and productivity. The objective of present work was to determine the impact of water deficit stress on seed germination and seedling growth in *Fagopyrum esculentum* Moench. Water deficit stress was induced by using PEG-6000 (Polyethylene glycol 6000) solution. Different concentrations of PEG-6000 (15%, 20%, 25%, 30%, 35% and 40% PEG) were used for seed treatment. The seeds were kept for 15 days in B.O.D. incubator at 20±2°C. The results indicated that drought stress had significant effect on germination percentage and seedling growth parameters. The various osmotic potential generated showed significant decrease in germination percentage except at 15% PEG. All the seedling growth parameters (root length, shoot length, fresh and dry weight) decrease with increase in PEG concentration except the root length at 15% PEG.

Keywords: Adverse factor; Germination percentage; Osmotic potential; Polyethylene glycol 6000; Seedling growth; Water deficit.

INTRODUCTION: The Indian Himalayan Region (IHR) is a mega hot spot of biological diversity[1]. Medicinal and Aromatic Plants (MAPs) of IHR form one of the important components for the socio-economic development of native communities. It has a large altitudinal range (300-8000 m) and supports about 18, 440 species of plants (25.3% species endemic), 1748 species of medicinal plants and 675 species of wild edibles[2]. Thus IHR is rich repository of plant diversity that are used for a variety of purpose i.e. food, fodder, medicine etc[3]. Since in the present century the use of medicinal plants by drug industry has increased, there is also need to know the existing medicinal plants being cultivated for their growth potential under diverse stressful growth factors so that their geographical ranges are identified for cultivation.

Fagopyrum esculentum Moench, commonly known as common buckwheat, belong to family Polygonaceae, is cultivated and produced in China, Russia, Ukraine and Kazakhstan, but in recent years, it has been cultivated all over the world and in particular in Asia, Europe and America[4].

In India, buckwheat is present in the upper regions of the Himalayas and has been an integral part of daily life of communities of high altitude areas[5]. Buckwheat is mainly used for the treatment of celiac disease as it is gluten free and eaten in place of wheat[6]. The primary antioxidants in buckwheat are rutin, quercetin, hyperin and catechins[7]. Rutin is recognized as anti-inflammatory and anticarcinogenic[8].

Plants, because of their sessile nature, depend on environment for their growth and development and face several environmental stresses such as extreme temperature, water deficit, water logging, salinity and heavy metals etc. Among the different environmental stresses, water deficit stress is one of the most adverse factors to plant growth and productivity. In water deficit situations water potential and turgor are reduced enough to interfere with normal functions of the plant[9]. Plants resist drought stress by their morphological, physiological and metabolic changes reflected in all their organs[10].

MATERIAL AND METHODS:

Seed Source: The seeds of *Fagopyrum esculentum* Moench were obtained from Himachal Pradesh Krishi Vishvavidyalaya, Research Station, Sangla, Kinnaur (H.P). Experiments were conducted in the botany laboratory of Shoolini University, Solan.

Seed germination: Seeds of *F. esculentum* selected for uniformity (on the basis of colour and size), were surface sterilized with 0.1% $HgCl_2$ for 5 min. followed by thorough washing under tap water. Then, the seeds were soaked in distilled water (control) or in solutions of stated concentrations of the effector substance namely, i) PEG 15% ii) PEG 20% iii) PEG 25% iv) PEG 30% v) PEG 35% and, vi) PEG 40% for 24 h at 20±2ºC. Thereafter, the seeds were transferred to petriplates (100 mm×15 mm) lined with two layers of filter paper moistened with distilled water (control) and PEG solution of same concentrations. PEG solution was applied initially (one time) at a rate of 5 ml/plate. Subsequently, appropriate volume of distilled water was added, when required, to maintain the substratum wet. The seeds were allowed to germinate in an incubator at 20±2ºC under continuous illumination provided by fluorescent white light (PAR: 40 µmol m^{-2} s^{-1}). The seeds of *Fagopyrum ecsulentum* Moench are non-dormant and start germinating within a day of incubation. Emergence of 2-5 mm radicle was taken as seed germination[11]. Germination was recorded regularly until the final count. Experiments were performed in 15 replicates; each replicate comprised 20 seeds.

Seedling growth: After 15 days of germination, seedling growth was measured in terms of root length, shoot length, seedling fresh and dry weight. At the end of experiment, data was subjected to analysis of variance (ANOVA). The statistical analyses were done using Graph Pad Prism® 5.2.

RESULTS AND DISCUSSION:

Effect of water deficit on germination percentage: The seeds of *F. esculentum* Moench were non-dormant and germinated within a day of incubation. Seeds responded differently to PEG concentration (water deficit). The germination percentage in response to different treatments of PEG and control (distilled water treatment) is presented in Figures 1. Seed germination in control treatment initiated on 1st day of incubation and thereafter progressed gradually. After 15 days of incubation seed germination was 67.23% in control. In PEG treated seeds germination also initiated on 1st day of incubation and progressed gradually. After 15 days of incubation seed germination in 15%, 20%, 25%, 30%, 35% and 40% PEG was 81.25%, 52.45%, 40.83%, 23.33%, 13.75% and 8.33%, respectively. It is evident from Figure 1 that PEG at higher concentration i.e. 20%, 25%, 30%, 35% and 40% decreased germination gradually. At lower water deficit (15% PEG concentration) the seed germination increased by 17.25% as compared to control. Reduction in seed germination was 21.98%, 39.27%, 65.30%, 79.55% and 87.61% than control at 20%, 25%, 30%, 35% and 40% PEG, respectively.

These results are in agreement with prior findings for *Lycopersicon esculentum* cultivar[12], for *Cassia angustifolia*[13], for *Artemisia vulgaris*[14] and for *Zea mays* varieties[15]. Slow and poor germination under water stress is due to decreased water potential of the germination medium, which restricts the water availability to the seeds[16-17].

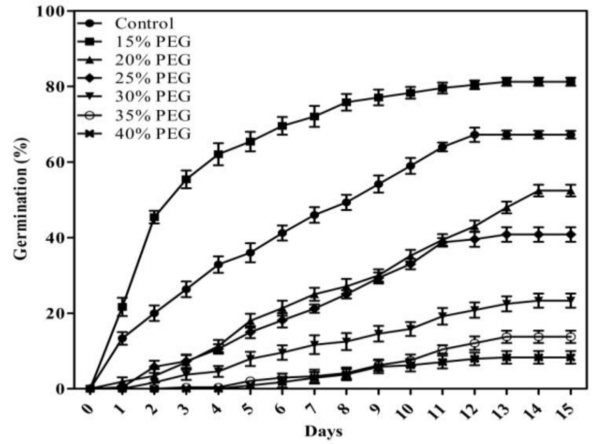
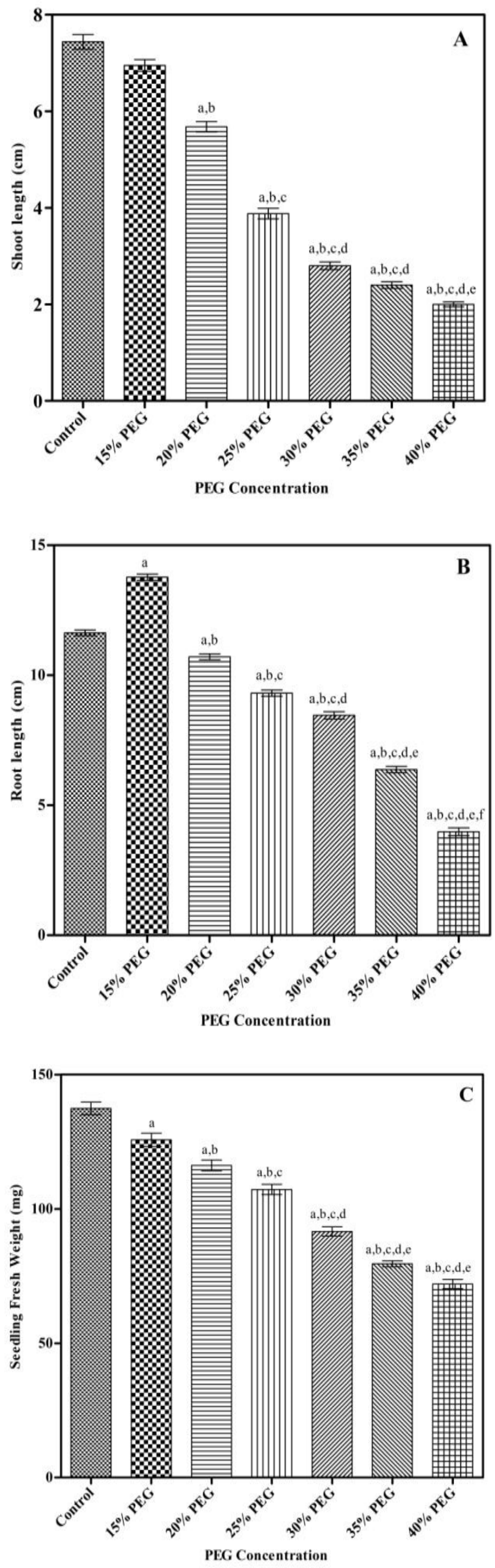


Figure 1: Time-course of germination of *Fagopyrum esculentum* seeds as affected by PEG. Values are mean ± S.E. n=15.

Effect of water deficit on seedling growth: Elevated PEG concentrations decreased the shoot length in concentration dependent manner (Figure 2A). Decrease in shoot length was 6.5%, 23.7%, 47.8%, 62.4%, 67.7% and 73.1% than control at15%, 20%, 25%, 30%, 35% and 40% PEG treatments, respectively. After 15 days of incubation the degree of decrease in shoot length was minimum in 15% PEG (6.95 cm) and maximum in 40% PEG (2.00 cm) as against 7.44 cm stem length in control. In water-deficit seedlings, the degree of reduction in shoot length increased with an increase in PEG concentration. Similar results were obtained in *Catharanthus roseus*[18] and *Jatropha curcas*[19-20].

Root length increased at lower concentration of PEG (15%) but as the concentration of PEG increased (20%, 25%, 30%, 35%, 40%) root length decreased gradually (Figure 2B). It increased by 18.4% at 15% PEG when compared with control. Decrease in root length was 7.91%, 19.95%, 27.26%, 45.23% and 65.79% than control at 20%, 25%, 30%, 35% and 40% PEG, respectively. The degree of inhibition in root length was minimum in 20% PEG (10.71cm) and maximum in 40 % PEG (3.98 cm). The root length was 11.63 cm after 15 days of incubation in control. The importance of root system in acquiring water from soil is a known fact. Similar results were obtained by researchers for *Sesamum indicum*[21] and *Eucalyptus microthec*[22]. Interestingly in the present study, under water deficit stress, root length increased at lower concentration (15% PEG) but decreased at higher concentrations (20%, 25%, 30%, 35% and 40%). Our results are consistent with previous study that attributed increased root length due to drought stress treatment in *Setaria italica*[23].



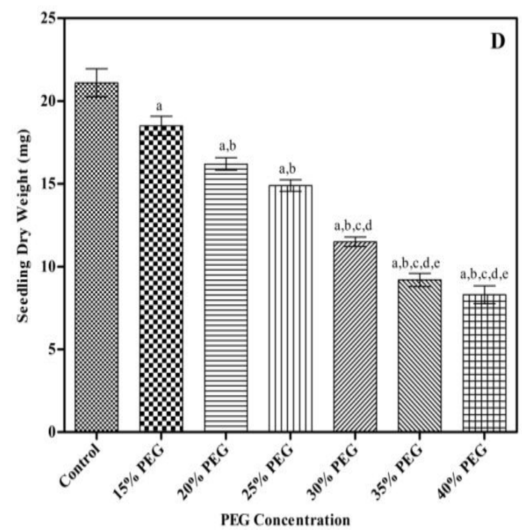


Figure 2: Effect of PEG on shoot length (A), root length (B), fresh weight (C) and dry weight (D) of seedling of *Fagopyrum esculentum.* Values are mean ± SE; n=9. [a]$p<0.05$ vs control, [b]$p<0.05$ vs 15%PEG, [c]$p<0.05$ vs 20% PEG, [d]$p<0.05$ vs 25% PEG, [e]$p<0.05$ vs 30% PEG, [f]$p<0.05$ vs 35% PEG, [g]$p<0.05$ vs 40% PEG; analysed by One-way ANOVA followed by Tukey's multiple comparison test.

Elevated PEG concentrations decreased the weight of seedling in concentration dependent manner, i.e., by 8.5% in 15% PEG; by 15.4% in 20% PEG; by 22.0% in 25% PEG; by 33.4% in 30% PEG; by 42.1% in 35% PEG and by 47.5% in 40% PEG as compared to control (Figure 2C). After 15 days of incubation the degree of decrease in seedling fresh weight was minimum in 15% PEG (125.67 mg) and maximum in 40% PEG (72.07mg) as observed against 137.40 mg in control. In *F. esculentum* water-deficit treatments caused reduction in seedlings fresh weight which is in agreement to several earlier reports[14,24].

Similar to seedling fresh weight, a decline in dry weight was found in *F. esculentum* seedling due to induced water stress in the present study. Elevated PEG concentrations decreased the weight of seedling in concentration dependent manner, i.e., by 12.3% in 15% PEG, by 23.2% in 20% PEG, by 29.4% in 25% PEG, by 45.5% in 30% PEG, by 56.4% PEG in 35% PEG and by 60.7% in 40% PEG, as compared to control (Figure 2D). After 15 days of incubation the degree of decrease in seedling dry weight was minimum in 15% PEG (18.5 mg) and maximum in 40% PEG (8.3 mg) as observed against 21.1 mg in control. These results are in agreement with study of *Trachyspermum ammi*[25], *Arachis hypogeae*[26] and *Ricinus communis*[27] under water deficit stress. It is attributed that due to decrease in growth of plant in water deficit conditions had its impact on biomass (fresh and dry weight).

An early morphological response to water-deficit stress was reduction in shoot length, root length, total fresh and dry matter in *F. esculentum*. Our results are consistent with many previous studies who attributed reduction in growth to avoidance mechanism in plants[28 & 29]. It is reported that suppression of plant growth under stress conditions might be due to decreased availability of water[30]. Other researchers suggested that the reduction in growth parameters would be primarily due to the reduction of the water absorption and nutrients deficiencies[31].

CONCLUSION: Plants have inbuilt ability to adjust to seasonal environmental variables. Apart from the environmental variables, there may be certain other rapid and predictable environmental disturbances resulting in stressful conditions[32]. However, plants differ in ability to tolerate such stressful situations. This study shows that water deficit stress restricts the seed germination and seedling growth of *F. esculentum* Moench, decreasing shoot length, root length, fresh weight and dry weight at higher stress regimes. However, plant can grow well under mild stress conditions. The finding presented demonstrate that, in terms of the parameters being investigated and in comparison of previous reports available in the literature, *F. esculentum* Moench would be classified as a species tolerant to water deficit stress at lower level of stress.

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