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Comparative effect of polyethylene glycol and mannitol induced drought on growth (*in vitro*) of canola (*Brassica napus*), cauliflower (*Brassica oleracea*) and tomato (*Lycopersicon esculentum*) seedlings

Fazal Hadi*, **Muhammad Ayaz, Sartaj Ali, Muhammad Shafiq, Rizwan Ullah, Amin Ullah Jan**

Department of Biotechnology, Faculty of Biological Sciences, University of Malakand, Chakdara 18800, Khyber Pakhtunkhwa, Pakistan

Key words: Canola, Cauliflower, drought stress, Polyethylene glycol, tomato.

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Abstract

Investigation was carried out to evaluate and compare the drought tolerance in seedlings of important crops (Canola, Cauliflower and Tomato). Polyethylene glycol (PEG6000) and Mannitol were used for induction of drought stress and compared their effects on seed germination, seedling growth and biomass and water contents. Five different treatments of PEG (1%, 3%, 6%, 9% and 12%) and Mannitol (0.5%, 1%, 1.5%, 2%, 2.5%) solutions were added to growth medium denoted as T1, T2, T3, T4, T5, and Control C (without PEG or Mannitol). Seed germination reduced significantly with increasing concentration of Mannitol and PEG, and minimum germination was found at T5. Similarly shoot and root length and biomass decreased with increasing concentration of PEG or Mannitol. The growth parameters highly reduced with Mannitol treatments as compared to PEG even the mannitol concentration was lower than PEG, shows toxic effect of mannitol. These findings suggest that polyethylene glycol is comparatively better than mannitol for assessment of drought tolerance potential of plants at early growth stage and canola had higher drought tolerance in respect of seed germination, shoot and root length and biomass and cauliflower was moderate and tomato was found very sensitive to drought stress.

* **Corresponding Author:** Dr Fazal Hadi ✉ dr.fhadi@uom.edu.pk

Abbreviation: PEG (Polyethylene glycol), MS (Murashig and Skoog), ANOVA (Analysis of Variance), LSD (Least significance differences).

Introduction

Plants are frequently exposed to many stresses such as drought, low temperature, salt, flooding, heat, oxidative stress and heavy metal toxicity while growing in nature. Drought is one of the major limiting factors and causes severe reduction in agriculture crop production. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought stress tolerance is seen in almost all plants but its extent varies from species to species and even within species. Water deficit is a global issue to ensure survival of agricultural crops and sustainable food production. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel *et al.,* 2008). Water accessibility is one of the most significant environmental factors manipulating the seed germination and early seedling growth directly or indirectly (Ravi *et al.,* 2011; Gamze *et al.,* 2005; Kaydan and Yagmur, 2008; Silva *et al.,* 2000). The germination of seeds and early seedling growth are considered the most crucial phases for seed establishment, determining successful crop production (Uniyal *et al*., 1998).

Three highly important crops were selected for the present investigation i.e. Canola, Cauliflower and tomato. Canola is a very important oil seed crop in the world. Its oil is of premium quality with low erusic acid and glucosinolates contents. Canola is generally considered to be more susceptible to drought. The yield is mainly affected by water shortages (Din *et al.*, 2011). Water stress induced a significant decrease in metabolic factors such as the decrease in chlorophyll contents in canola plants (Sakova *et al*., 1995). Cauliflower is one of the most popular vegetable crops globally. It is grown for its white curd, a rich source of vitamins C, K, and A (beta-carotene), folic acid, fibber, and flavonoids which give the cauliflower anti-inflammatory and antioxidant proprieties (Tossaint, 1994). Cauliflower is a potentially cancer preventative vegetable (Christoper, 1994). Similarly, tomato is the second most important vegetable crop in the world after potato. It is a valuable source of health promoting compounds such as the antioxidant lycopene whose consumption is known to reduce the incidence of many types of cancer (Rao and Agarwal, 2000).

Polyethylene glycol and Mannitol has been used to stimulate osmotic stress and these neutral polymers are being widely used to impose water stress in plants (Zgallai *et al.,* 2005). The non toxic PEG solution is used because of high molecular weight, which cannot enter into cell through plant cell wall (Emmerich *et al.,* 1990; Kaydan *et al*., 2008) as compared Mannitol, having low molecular weight to enter into cell and causes toxicity The objective of the present study was (1) to compare the effect of PEG and Mannitol on germination and early seedling growth (2) to evaluate and compare the induced drought stress resistance level among different crop species as canola, cauliflower and tomato.

Materials and methods

Experimental design

Experiments were carried out in the Department of Biotechnology, University of Malakand during the year 2011-12. Seeds of Canola (*B. napus*), Cauliflower (*B. oleracea*) and Tomato (*L. esculentum*) were surface sterilized by treating with Ethanol 70% for five minutes. Then properly washed with distilled water (sterilized) and placed in Petri plates (autoclaved) with the help of forceps (sterilized) in laminar air flow. For each treatment and control, three replicate Petri plats were used. Ten seeds in each Petri plate were inoculated containing filter-papers and cotton autoclaved prior to seed inoculation. Total of eighteen Petri plates were used for each plant.

Polyethylene glycol and Mannitol treatments

Polyethylene glycol (PEG6000) and Mannitol solutions of different concentrations were prepared with addition of basal media (Murashig and Skoog, 1962). Five different solutions of PEG (1%, 3%, 6%, 9%, and 12%) and Mannitol (0.5%, 1%, 1.5%, 2%, and 2.5%) were prepared for treatments denoted as T1, T2, T3, T4, and T5. Control (C) was treated only with distilled water and MS basal media as nutrients. Six ml of each solution either of PEG or Mannitol (treatments) added to each Petri plate before seed inoculation. Similarly, 6 ml of distilled water plus MS basal media was added to Petri plates of control (C) before seed inoculation.

Seeds germination and seedlings growth measurement

Subsequent to seeds inoculation the Petri Plates were placed in growth cabinet (21^{o} C, dark, and 53% relative humidity) for germination. In order to prevent dehydration effect, 1ml of sterilized distilled water was added to each Petri Plate after each two days and after each week, 1ml solution of distilled water plus MS basal media was added as a source of nutrients by using micropipette. After one week of seeds inoculation, the percent (%) germination rate was noted and five healthy seedlings were selected for growth (at 21^{o} C, 15 h photoperiod 210 µmol $m^{-1}s^{-1}$ light intensity and 50 % relative humidity). The seedlings were removed from Petri plates after two weeks treatments. Fresh biomass was measured promptly after removal to avoid water loss from seedlings. Then root and shoot length of each seedling was measured with centimetre rule (Model: DK-436, Danking Enterprise Ltd., Taiwan) from root and shoot joint to apices. Seedlings were then dried in oven at 70^{o} C for 24 h and dry biomass was measured.

Statistical analysis

Data was statistically analyzed using Minitab 15 statistical software for Analysis of variance (ANOVA) and mean values were compared by LSD for the significance of values.

Results

Effect of induced drought on seed germination

Seed germination data of canola, cauliflower and tomato are presented respectively in (Fig 1A, 2A and 3A). Polyethylene glycol and mannitol have a significant effect on % germination. Increase in polyethylene glycol and mannitol concentration linearly decreased the percent germination of canola (Fig 1A), cauliflower (Fig 2A) and tomato (Fig 3A). The minimum germination was observed at highest concentration of polyethylene glycol or mannitol T5. The mannitol highly reduced the germination rate compared to the PEG effect.

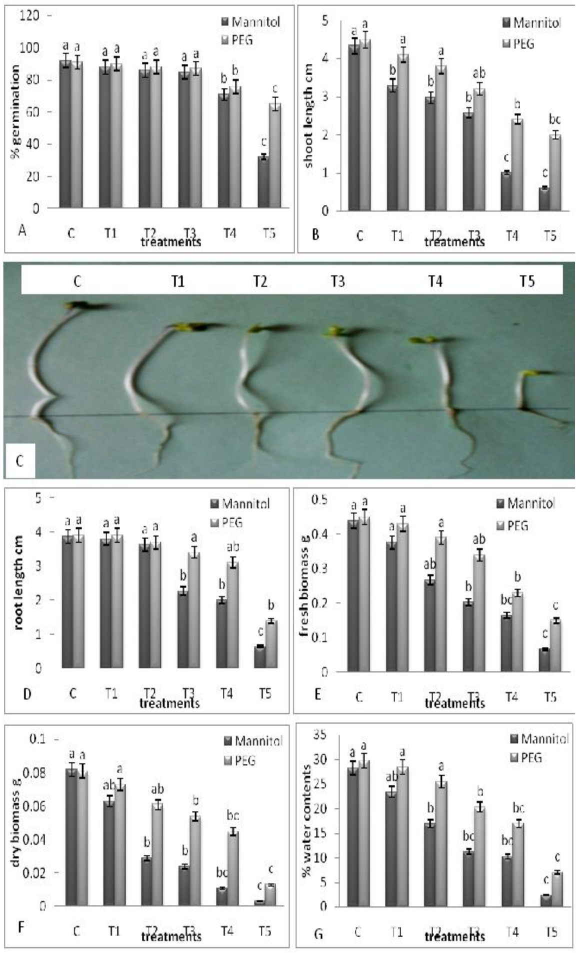

Fig. 1 (A-G). Effect of Manninto l and PEG on (A) %germination(B, C) shoot length (D) root length (E) fresh biomass (F) dry biomass (G) % water contents of Canola seedlings. Treatments T1 (1%), T2 (3 %), T3 (6%), T4 (9 %) and T5 (12 %) each of Mannitol and PEG solutions.

Effects of induced drought on shoot and root length (cm)

Polyethylene glycol and mannitol showed significant effect on shoot length during early seedling growth of canola (Fig 1B and 1C), cauliflower (Fig 2B and 2C) and tomato (Fig 3B and 3C) when compared with control (without PEG or Mannitol). The highest shoot length was found in control while lowest shoot length was observed in T5 (maximum PEG or Mannitol) for all of the three plant species. These findings demonstrate that Mannitol highly reduced the shoot length of canola, cauliflower and tomato as compared to polyethylene glycol treatments. Data on root length are presented in (Fig1D, 2D and 3D) respectively for canola, cauliflower and tomato. Polyethylene glycol and mannitol showed a significant effect on root length and the highest root length was noted in control C while lowest was found in T5. The data on root length showed reduction with increasing level of Polyethylene glycol and mannitol and Mannitol greatly decreased the root length as compare to poly ethylene glycol.

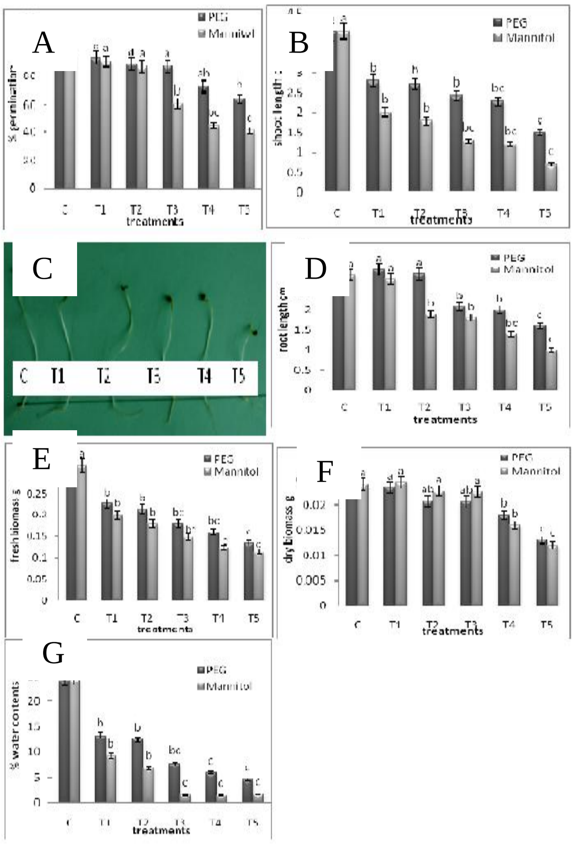

Fig. 2 (A-G). Effect of Mannintol and PEG on (A) %germination(B, C) shoot length (D) root length (E) fresh biomass (F) dry biomass (G) % water contents of Cauliflower seedlings

Effect of induced drought on fresh and dry biomass

Both PEG and mannitol significantly reduced the fresh biomass of canola (Fig 1E), cauliflower (Fig 2E) and tomato (Fig 3E). Fresh biomasses were adversely affected with increasing PEG and mannitol concentration and the maximum fresh biomass was found in control C while lowest was found in T5. Mannitol strongly reduced the canola seedling dry biomass (Fig 1F) compared to PEG treatments. The highest dry biomass of canola was found in control C while lowest dry biomass was found in T5 (Fig 1F). PEG showed slight effect on dry biomass of cauliflower (Fig 2F) and tomato (Fig 3F). Mannitol strongly decreased the dry biomass of three plant species as compared to PEG treatments.

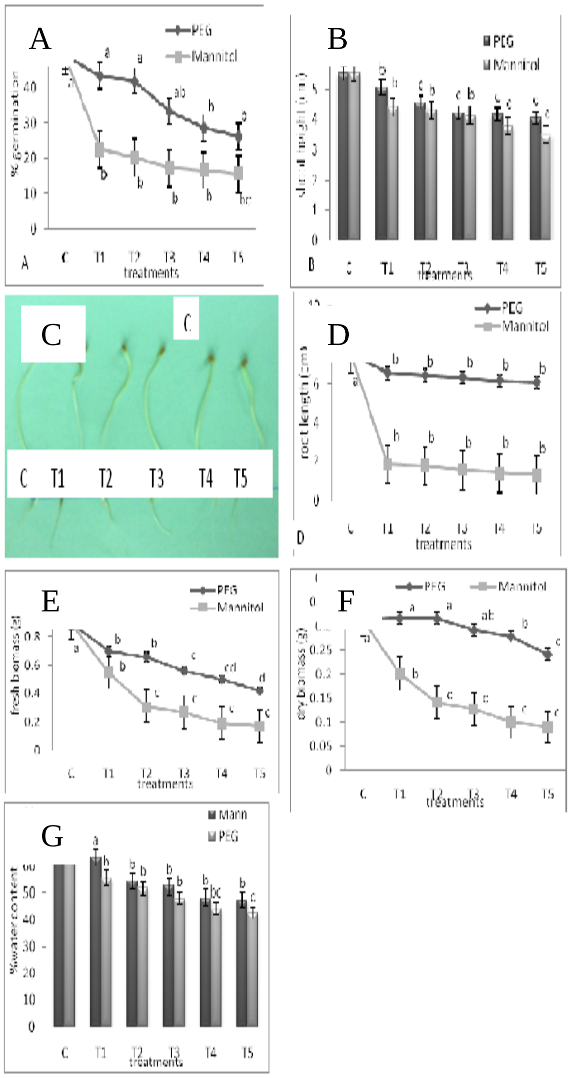

Fig. 3 (A-G). Effect of Mannintol and PEG on (A) %germination(B, C) shoot length (D) root length (E) fresh biomass (F) dry biomass (G) % water contents of Tomato seedlings

Percent (%) water content and overall response of seedlings to drought

Water content in seedling was significantly decreased with increasing concentration of Polyethylene glycol and mannitol. There was a negative relationship between water content in different tissues and drought induces solution concentration. Maximum water content was found in control C while lower water content was found in T5. Water content was greatly reduced in cauliflower (Fig 1G) followed by canola (Fig 2G) while in tomato (Fig 3G) water content was moderate. Overall canola seedlings showed higher better response to different treatments of PEG and mannitol compared to cauliflower and tomato (Fig 4A-F).

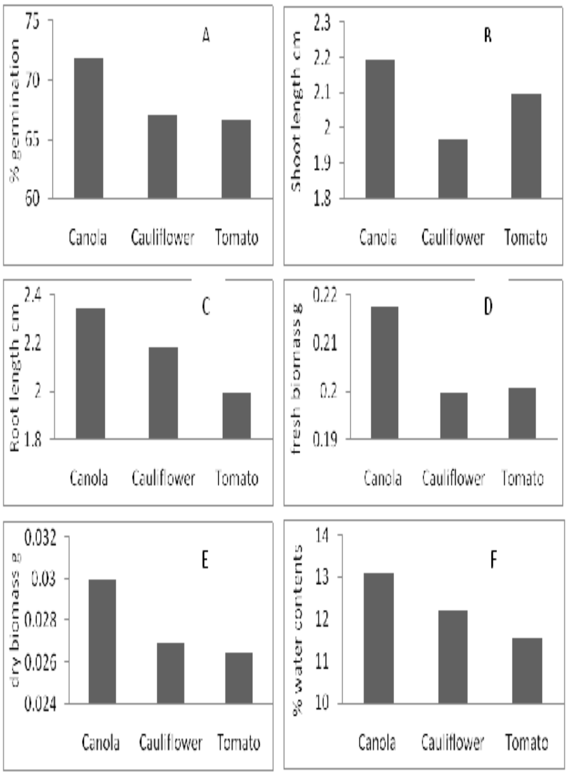

Fig. 4 (A-F). Overall effect (Mannitol and PEG mean value of all treatments) on germination (A), Shoot length (B), Root Length (C), fresh biomass (D), dry biomass (E), and % water content (F).

Discussion

Drought affects all stages of plant growth and development including germination (Hadas, 1976). Drought is a huge threat for the future agriculture production globally, that is why this problem has been studied by numerous researchers in a large number of important crops such as tomato (Taylor *et al.*, 1982), rice (Singh and Singh, 1983), wheat (Kerepesi and Galiba, 2000) and grass (Emmerich and Hardegree, 1990, 1991). A decline in seed germination rate stimulated by PEG has reported by several researchers (Gamze *et al.,* 2005, Ghazi *et al.*, 2007; Kaydan and Yagmur, 2008). Several methods for induction of water stress in plants have been used and it has been rationally well recognized that polyethylene glycol solutions are more appropriate to control water potential in seed germination (Emmerich and Hardegree, 1990). From the current findings it is suggested that the rate of seed germination is reduced by using different concentration of polyethylene glycol and mannitol solution. These finding confirm the previous results many scientists (Kaydan and Yagmur, 2008; Sosa *et al.,* 2005; Turk *et al.,* 2004; Amador *et al.,* 2002; Demir and Venter, 1999; Katembe *et al.,* 1998). Root and shoot length reduced by PEG and mannitol induced drought and similar findings have been reported by many scientists (El-Midaoui *et al.*, 2003; Parmar and Moore, 1965). Significant decrease in fresh and dry biomasses was observed in present findings, which confirming the previous findings of several researchers (Saeed *et al.,* 1997; Gill and Sing, 2002; Ravi *et al.*, 2011). It has been reasonably well established that polyethylene glycol and mannitol induced water stress that causes by withdrawal of water from plants (Emmerich and Hardegree, 1990).

Relatively higher water content was noted in progressive mild stress than severe stress indicating that plants have the ability to sustain their water content under mild stress, whereas this ability lost under severe stress treatment. Decrease in the water content by drought was reported in many plants (Hsu and Kao, 2003; Zgallai *et al.,* 2005). Similar observations of decreases in water level under stress conditions were found in sorghum (Gill *et al.,* 2002), chenopodium (Prado *et al.,* 2000), and alfalfa (Pennypacker *et al.,* 1990). In view of the fact that the response of plants to drought stress is very intricate and diverse, it is impractical to consider that there is a single gene responsible for drought tolerance and hence understanding of plants response under water stress is needed (Mundree *et a.,;* 2002). Present study indicates that different plant species responding to drought in different way as three different crops i.e. canola, cauliflower and tomato in this study proved it by having different drought resistance level in each plant. Conclusively, these findings suggest that polyethylene glycol is comparatively better than mannitol for assessment of drought tolerance potential of plants at early growth stage and canola had higher drought tolerance in respect of seed germination, shoot and root length and biomass and cauliflower was moderate and tomato was found very sensitive to drought stress. Further investigations are needed to improve the understanding of the effect of drought stress during early seedling growth.

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