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Response of Ajowan to water stress induced by polyethylene glycol (PEG) 6000 during seed germination and seedling growth

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Abstract

Seed germination and subsequent metabolic changes in Ajowan (*Trachyspermum ammi* L.) (NRCSS AA-2) seedlings was studied under water limiting conditions, imposed by increasing concentrations of polyethylene glycol (PEG 6000). Five water stress conditions (0, -0.05, -0.1, -0.15 and -0.2 MPa) were created in the laboratory in a completely randomized design. The results revealed that water stress (-0.2 MPa) significantly reduced seed germination components like final germination percent (80%) radical (64%) and plumule (63%) length, fresh (63%) and dry (74%) weight of seedlings and vigor index (SVI) by 92% over control. Decrease in osmotic potential resulted in decreased protein content (56%) with concomitant increase in total sugars (55%) at -0.2 MPa as compared to control. Significant increase in free proline and glycine betaine content by 1.5 to 2 folds was observed at the highest water stress condition. The seedlings exhibited increased activity of superoxide dismutase and peroxidase under stressed condition. In the present study, it was found that Ajowan was a moderately drought tolerant species at laboratory level.

Key words

Ajowan, Antioxidants, Organic constituents, Polyethylene glycol, Seed germination

Introduction

Water availability is a major limiting factor for the establishment of arid and semi-arid plant species. Moisture conditions, immediately prior to and during germination, play a dominant role in regulating germination in dry environments (Adams, 1999). Drought or water deficit is one of the most important environmental stress that influence plant production (Abdalla and Elkhoshiban, 2007). Seed germination and early seedling growth is considered as the most critical phase for establishment of any crop (Pratap and Sharma, 2010).

Water potential is one of the most effective parameter in water absorption and seed swelling, as water stress reduces water absorption. Seed germination needs a minimum water to be absorbed, swelled and that is why osmotic potential should not be less than a certain amount. Decreasing osmotic potential results in decreased water absorption by seeds and consequently, germination is reduced. Osmotic adjustment plays an important role in sustaining growth under stressed condition (Alizadeh, 1990). The response of plants differ significantly at various organizational levels, depending upon intensity and duration of stress as well as plant species and its developmental stage (Chaves *et al.*,2003).

Polyethylene glycol molecules with a molecular weight ≥ 6000 (PEG 6000) are inert, non-ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Lu and Neumann, 1998). The molecules are small enough to influence the osmotic potential, but large enough as not to be absorbed by plants (Krizek, 1985).

Ajowan is native to India, but is also cultivated in arid and semi-arid regions of world like Iran, Egypt, Pakistan and Afghanistan. Among traditional potential herbs, Ajowan (*Trachyspermum ammi* L.) belonging to family Apiaceae, is widely used for curing various diseases in both humans as well as animals (Ashraf and Orooj, 2006). Ajowan is also known by other names in literature as owa, omum, ajwain, Bishop's weed, carom, or Ethiopian cumin. A number of medicinal and therapeutic properties have been ascribed to various parts of this plant.

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Aqueous extract of Ajwan-ka-arak is popular remedy for diarrhea, juice of leaves is anthelmintic; root: carminative, diuretic, febrifuge; useful in stomach troubles (Asima Chatterjee, 1995). Fruit or seed possesses stimulant and carminative properties and is regarded as antispasmodic. It is an important remedial agent for flatulence, atonic dyspepsia, and diarrhea (Bentely and Trimen, 1999). The seed of ajwain is bitter, pungent, and acts as anthelmintic, carminative, laxative, and stomachic. It also cures abdominal tumors, abdominal pains and piles (Krishnamoorthy and Madalageri, 1999). Ajowan seeds contain an essential oil with 40-50% thymol which is a strong germicide, anti-spasmodic and fungicide. Thymol is used as an ingredient of deodorants, mouthwashes, toothpastes and many pharmaceutical preparations (Malhotra and Vijay, 2004).

Global climate change, in the form of rising temperature, has altered soil moisture and water deficiency has been projected to decrease the yield of food crops over the next 50 years (Thomson *et al.,*2005). The current study was planned to determine the effects of different levels of water stress induced by polyethylene glycol (PEG 6000) on seed germination, growth and physiology of Ajowan seedlings.

Materials and Methods

The authentic seeds of Ajowan, *Trachyspermum ammi* L. var. AA-2 were procured from National Research Centre on Seed Spices, Tabiji Farm, Ajmer (Rajasthan). Research work was carried out in laboratory of Plant Physiology of Department of Botany, University of Pune (M.S.) India. Laboratory water stress was created by using Polyethylene Glycol 6000. Five water stress conditions (Control, -0.05, -0.1, -0.15 and -0.2 Mega Pascal (MPa) were used in a completely randomized design. The osmotic potential of the aqueous solutions of PEG 6000 was calculated according to the empirical equation developed by Michel and Kaufmann (1973). PEG 6000 was dissolved in water and placed in a shaker bed (25 °C) for 16 hr.

Seed germination bioassay was carried out in sterilized Petri plates (9 cm inner diameter) lined with a germination paper. The seeds were surface sterilized using 0.1% $HgCl_2$ for 5 min, followed by thorough rinsing with distilled water. About 20 healthy and uniform seeds were used for germination and 5 ml solution of respective concentration of PEG 6000 was added in each Petri plate. This was maintained on every second day. Seed germination bioassay was carried out in seed germinating chamber at constant temperature 20±3 °C, 80% relative humidity and uniform light conditions.Seeds were considered to be germinated with radical emergence of 1mm.

Seed germination and seedling growth was investigated and recorded on 12 DAS. Total number of germinated seeds in each Petri dish on 14th day was regarded as the final germination percentage (FGP). 10 seedlings from each treatment were selected randomly and the average plumule and radicle length were measured. Then, they were oven-dried at 65 °C for 24 hrs to acquire their mean dry weight. Seedling vigour index (SVI) was recorded by the formula given by Abdul Baki and Anderson (1973).

The seedlings were allowed to grow for 20 DAS in the same medium, washed with distilled water, blotted and 100mg were used for the estimation of organic constituents, antioxidants and antioxidant enzymes.

Organic constituents : Protein content of the seedlings was determined by following the method of Lowry *et al.* (1951) and total sugars content by the method described by Hedge and Hofreiter (1962).

Free proline content : Proline content was determined by the method of Bates *et al*. (1973). Fresh seedlings were homogenized in 3% sulphosalicylic acid. The reaction mixture containing filtrate, GAA and ninhydrin was kept in boiling water bath for 1 hr. The reaction was terminated by placing the tubes in ice bath and the red colour developed by addition of toulene was measured at 520 nm by spectrophotometer (Shimadzu- 1601).

Glycine betaine content : Glycine betaine content was determined according to Ishitani *et al.* (1993). For this seedlings were incubated in 1 N H_2SO_4 and then centrifuged (2000 rpm) for 10 min. The reaction mixture contained supernatant, H_2SO_4 and cold I_2KI reagent. The tubes were then cooled to 0°C and centrifuged again. Ethylene dichloride was added and absorbance was recorded at 365 nm.

Enzyme activity : Superoxide dismutase activity was assayed by monitoring photochemical inhibition of nitroblue tetrazolium (NBT) following the method of Dhindsa *et al.* (1981). The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.8), 13 mM Methionine, 0.1 mM EDTA, 75 µM nitroblue tetrazolium chloride (NBT), and 50 µl enzyme extract. The reaction was initiated by adding 2 µM riboflavin and by placing the tubes below 15 W fluorescent lamp for 15 min in dark and others were illuminated for 15 min. Total SOD activity was defined as the amount enzyme required to causes 50% inhibition of NBT at 560 nm.

Peroxidase activity was estimated following the method Vidyasekharan and Durairaj (1973). 1 gm fresh sample of seedlings was homogenized in chilled mortar, in 5 ml of 1 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 15,000 rpm for 20 min in refrigerated centrifuge and the supernatant was used as enzyme source. The assay mixture of 3 ml contained 1.8 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml freshly prepared 10 mM Guiacol, 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H_{22}.Initial optical density was read at 430 nm and then increase in optical density was noted at the interval of 30 sec on UV-visible spectrophotometer. The enzyme activity was expressed as Δ OD min^{-1} mg^{-1} protein.

Statistical analysis : The data was analyzed by analysis of variance (ANOVA) using SPSS (version 20.0), post-hoc for comparison of means and the significance of differences was determined according to Duncan's multiple range test (DMRT). P values < 0.05 are considered to be significant.

Results and Discussion

Water stress induced by polyethylene glycol (PEG 6000) caused a significant reduction in all the measured traits of seed germination and seedling growth, including seed germination percentage (SGP), plumule and radicle length, fresh and dry weight and seedling vigor index (SVI). Ajowan seeds showed minimum germination at an osmotic potential of – 0.2 MPa. Beyond this level there was complete inhibition (100%) of germination (not shown in table). Although, the seeds did not germinate at an osmotic potential less than – 0.2 MPa, this might indicate an important survival mechanism that ensures dormancy until sufficient moisture is available for optimal seed germination and seedling establishment.

Germination of Ajowan seeds as shown in Table 1 was highest (67%) in control and there was a significant decline in final germination percentage with decrease in osmotic potential. There was about 12%, 26%, 42% and 80% inhibition in seed germination at -0.05, -0.1, -0.15 and -0.2 MPa respectively as compared to control. The plumule and radical length, as well as, the fresh and dry weights also decreased significantly with increase in PEG concentration. The roots were adversely affected as compared to shoots. The seedling vigor index was greatly hampered at -0.15 (111.35) and -0.20 (21.66) MPa levels over control (308.85). Similar inhibition in seed germination was earlier reported by Pratap and Sharma (2010); Bagheri and Yeganeh, (2011) and Baghizadeh and Hajmohammadrezaei (2011). This may be an adaptive strategy of seeds to prevent germination under stressful environment for ensuring proper establishment of the seedlings. It was claimed that decrease in water potential gradient between seeds and their surrounding media may be adversely affecting the seed germination and subsequent metabolic events in seedling growth and development. High concentration of PEG might hamper the process of water uptake by seeds and thereby inhibiting the process of seed germination (Afzali *et al.*, 2006). The plumule and radicle length reduction during water stress may be due to induction of metabolic disorders by both the stress conditions. Water stress might affect the mobilization of reserve foods, suspended cell division and injury to hypocotyls that resulted in reduced plumule and radicle length. The reduction in seedling growth may also be due to reduced water absorption and nutritional deficiency created due to osmotic imbalance and decrease in metabolic activities under water stress.

PEG induced osmotic stress caused a significant reduction in protein content (Fig.1) of treated seedlings as compared to control. There was about 39% and 56% reduction in protein content at osmotic potential of -0.15 and -0.2 MPa as compared to control. The decrease in protein content under drought stress might be due to proteolysis and protein degradation or inhibition of protein synthesis. It may also be due to decreased translocation of reserves from the cotyledons to growing parts of the seedlings (Panneerselvam *et al.,* 1998) or due to damage of the protein synthesis machinery which blocks the transcription of RNA from DNA with decreased translation into protein as recommended by Datta *et al.* (1997). There was a significant accumulation of total sugars in the seedlings subjected to stress (Fig. 1). There was about 38% and 55% increase in total sugars at -0.15 and -0.2 MPa as compared to control. The results of present investigation are in conformity with the earlier reports of Saffan (2008) and Grzesiak *et al.* (2012).

Experimental findings on antioxidant system indicate that ajowan seedlings responded differently under normal and water stress conditions. Decrease in osmotic potential showed significant increase in antioxidant contents like proline and glycine betaine (Fig. 2) and antioxidant enzymes like SOD and POD (Fig. 3) as compared to control. Proline and glycine betaine was significantly increased by 1.5 to 2 folds with decreasing osmotic potential. The increased accumulation of proline and glycine betaine is in accordance with previous reports of Manivannan *et al.* (2007) and Mohammadkhani and Heidary (2008), which was suggested to be associated with drought tolerance. The glycine betaine content increased under drought stress in barley (Nakamura *et al.,* 2001). During stress conditions, protein degrades and consequently the proline and glycine betaine increases (Roy *et al.,* 2009). High level of proline enabled

Table 1 : Effect of different levels of osmotic stress on seed germination and seedling growth of Ajowan.

Treatment (Mpa)	Final germination (%)	Plumule length(cm)	Radicle length(cm)	Fresh weight per 10 seedlings (mg)	Dry weight per 10 seedlings (mg)	Seedling vigor index (SVI)
Control	67.14±1.87a	3.02±0.20a	1.39±0.09a	62.6±2.77a	13.2±o.35a	308.85±19.8a
-0.05	58.41±2.96b	3.12±0.20a	1.15±0.13ab	51.73±3.02b	11.33±1.15ab	249.49±28.31a
-0.10	49.04±3.52c	3.04±0.07a	1.09±0.17b	50.6±1.04b	10.73±0.64ab	203.17±24.19b
-0.15	38.58±3.12d	2.09±0.15b	0.79±0.16c	41.67±2.89c	9.8±0.72b	111.35±18.76c
-0.2	13.38±2.93e	1.11±0.18c	0.49±0.08d	22.97±5.14d	3.4±0.42c	21.66±7.28d

Means (± SD) in same column represented by different letters indicate statistically significant differences (P <0.05).

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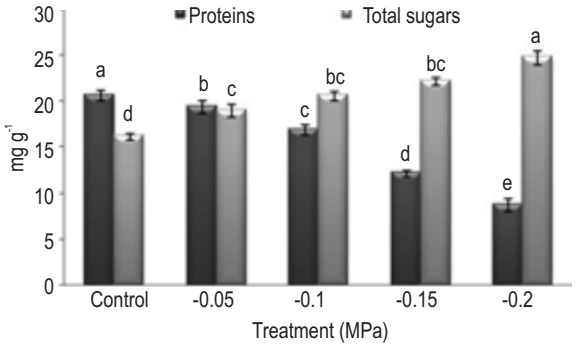

Fig. 1 : Effect of different levels of osmotic stress on organic constituents of Ajowan seedlings. Figures represent means± SD. Bars with different letters indicate statistically significant differences at P<0.05. Vertical bars represent standard deviation.

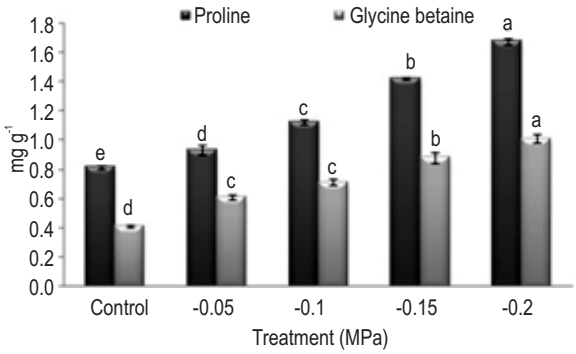

Fig. 2 : Effect of different levels of osmotic stress on proline and glycine betaine content of Ajowan seedlings. Figures represent means± SD. Bars with different letters indicate statistically significant differences at P <0.05. Vertical bars represent standard deviation.

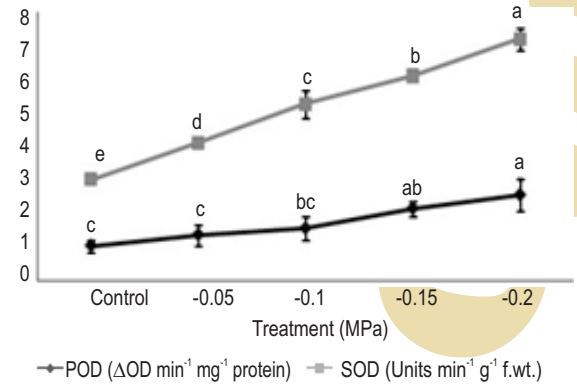

Fig. 3 : Effect of different levels of osmotic stress on antioxidant enzyme activities in Ajowan seedlings. Figures represent means± SD. Bars with different letters indicate statistically significant differences at P <0.05. Vertical bars represent standard deviation.

the plant to maintain low water potentials. By lowering water potentials, the accumulation of compatible osmolytes, involved in osmoregulation allowed additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism (Kumar *et al.*,2003).

Superoxide dismutase and Peroxidase (POD) activity was enhanced by 2 to 2.5 folds at -0.15 and -0.2 MPa water stress. It is known that plants have a well-organized defense system against ROS under stress conditions and SOD constitutes the first line of defense via detoxification of superoxide radicals (Sairam *et al.*,2000). Peroxidase, another important OH^- scavenging enzyme, also followed the similar trend (Fig. 3). Enhanced POX activity under various stress conditions has been linked with protection from oxidative damage, lignifications and cross-linking of cell wall to prevent from such adverse conditions (Dalal and Khanna-Chopra, 2001). SOD catalyses the conversions of superoxide anions to hydrogen peroxide and water. Several reports have shown that over-expression of SOD leads to increased tolerance to abiotic stress such at low temperature and water stress as reviewed in Bohnert and Sheveleva, (1998). The results of present investigation are in conformity with the reports of Candan and Tarhan (2003), Fazeli *et al.* (2007) and Pan *et al.* (2006). The enhanced SOD and POD activities in ajowan seedlings under stress condition showed a well organized defense system against ROS under stress condition.

In the present study, it was found that Ajowan was a moderately drought tolerant species under laboratory conditions. Also, the response of Ajowan to water stress under pot and field conditions is in progress.

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