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WATER STRESS MEDIATED CHANGES IN GROWTH, PHYSIOLOGY AND SECONDARY METABOLITES OF DESI AJWAIN (*TRACHYSPERMUM AMMI* L.)

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Abstract

Biotic and abiotic stresses exert a considerable influence on the production of several secondary metabolites in plants; water stress is one of the most important abiotic stress factors. This study was carried out to elucidate the effect of drought stress on growth, physiology and secondary metabolite production in desi ajwain (*Trachyspermum ammi* L.). Plants were grown in pots and three drought levels (100%, 80% and 60%) of field capacity were created. The experiment was laid out in complete randomized design (CRD) with three replicates. Data on growth, physiological and biochemical parameters were recorded and analyzed statistically. Physiological parameters like transpiration rate and stomatal conductance decreased significantly with increasing water stress levels, but internal CO_2 concentration increased. The photosynthetic rate showed non-significant reduction from 100% field capacity to 80% field capacity but increased at 60% field capacity. Growth parameters including plant height, herb fresh and dry weights were reduced significantly with increasing stress levels, while total phenolic contents and chlorophyll contents increased under water stress conditions. These results suggest that cultivation of medicinal plants like desi ajwain under drought stress could enhance the production of secondary metabolites.

Introduction

Desi ajwain (*Trachyspermum ammi* L.) is an aromatic herb and belongs to family Apiaceae (Umbelliferae). It originated in the Eastern Mediterranean region, probably in Egypt, and came to India with the Greeks, who were called Yavanas by South Indians.(Boskabady & Shaikhi, 2000) The name ajwain originated from the Sanskrit words yavanaka or ajomoda. Ajwain is very widely cultivated in black soil, particularly along the riverbanks in Egypt and many other countries like India, Iran and Afghanistan (Kiritikar & Basu, 1999; Boskabady & Shaikhi, 2000). Ajwain is highly esteemed as a remedial agent for flatulence, flatulent colic, atonic dyspepsia, diarrhoea - in short, as a digestive aid and also as an antiseptic (Bentely & Trimen, 1999; Kiritikar & Basu, 1999; Cragg & Newman, 2005). Oil of desi ajwain contains thymol and its specific gravity and odour resembles the volatile oil. The oil contains a liquid hydrocarbon, 1-methyl-4-isopropylbenzol, and another hydrocarbon which is isomeric with oil of turpentine (Bentely & Trimen, 1999).

Biotic and abiotic stresses exert a considerable influence on the production of several secondary metabolites in plants (Jaleel *et al.*, 2007). Drought is one of the most important abiotic stress factors (Dash & Mohanty, 2001), affecting plant growth and leaf photosynthesis (Flexas *et al*., 2004) and altering biochemical properties of plants (Zobayed *et al*., 2007). Drought stress is also known to increase the secondary metabolite production in a variety of medicinal plants, like artemisinin in leaves of *Artemisia annua* (Charles *et al*., 1993), hyperforin in *Hypericum perforatum* leaf tissues (Zobayed *et al*., 2007), and ajmalicin in *Catharanthus roseus* roots (Jaleel *et al*., 2008). Cultivation of a medicinal plant like desi ajwain (*Trachyspermum ammi*) in water-deficient areas would increase its defense system and the level of active compounds.

There is little information available on the effect of water stress on growth of *Trachyspermum ammi*, physiology and secondary metabolite production. This study elucidates the effects of drought stress on growth, physiology and bioactive compounds of *Trachyspermum ammi*, a commercially important crop.

Materials and Methods

The investigation was conducted at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Seeds of desi ajwain (*Trachyspermum ammi* L.) were obtained from a local market. A sand culture experiment was established in nine earthen pots, each containing 5 kg of washed river sand. Fifty healthy seeds were sown in each pot. All the pots were arranged in a completely randomized design. Fifteen days after emergence, seedlings were thinned and six plants in each pot were maintained for 67 days. Pots were regularly irrigated with 1/2 strength Hoagland's nutrient solution. Then three water stress levels (100, 80 and 60% field capacity) were maintained by weighing the pots on an electrical balance every day. Field capacity of sand was measured according to the guidelines in Handbook 60 (Anon., 1962).

Physiological parameters (photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*C*) internal CO_2 concentration, growth) and biochemical parameters (total phenols, chlorophyll content) were determined twenty days after the imposition of water stress.

The parmeters like photosynthetic rate or CO_2 assimilation rate (*Pn*), transpiration (*E)*, stomatal conductance (*C)* and internal CO_2 concentration were estimated through using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). All the measurements were taken from 9.00 to 11.00 a.m. with the following adjustments: molar flow of air per unit leaf area 403.3 mmol $m^{-2}s^{-1}$, atmospheric pressure 99.9 kPa, water vapor pressure into the chamber ranged from 6.0 to 8.9 mbar, photosynthetic Active Radiation (PAR) at leaf surface was maximum up to 1711 µmol m^{-2} s^{-1}, temperature of leaf ranged from 28.4 to 32.4°C, ambient temperature ranged from 22.4 to 27.9°C and ambient CO_2 concentration was 352 µmol mol^{-1} of air.

Chlorophyll contents were estimated using the methods of Arnon (1949) and Davies (1976). Fresh leaves (0.5 g) were chopped into segments of 0.5 cm and extracted overnight in 5 ml acetone (80%) at 0°C. The material was centrifuged at 15000 rpm for 5 minutes and the absorbance of the supernatant was measured at 480, 645 and 663 nm in a spectrophotometer-2800 (Hitachi, Japan). Chlorophyll *a*, *b* and total chlorophyll contents were calculated using the following formulae (Davies 1976):

Chl *a* = [12.7 (OD 663) - 2.69 (OD 645)] x V/1000 x W
Chl *b* = [22.9 (OD 645) - 4.68 (OD 663)] x V/1000 x W
Total Chl= [20.2 (OD 645) + 8.02 (OD 663)] x V/1000 x W

where: V= Volume of the extract; W=Weight of leaf segments

Total phenols were determined according to the method of Julkunen-Titto (1985). Oven-dried leaf material was ground and then 1 g was extracted in 10 ml 80% acetone solution in a 50 ml test tube that was shaken vigorously for 2 h. The solvent was changed 3 times (3 x 10) for complete extraction. Then whole extract was centrifuged at 10,000 rpm at 30°C for 10 min. The supernatant was cooled and brought to a volume of 100 ml. One ml of this crude extract was mixed with 2 ml water in a 10 ml test tube. One ml of Folin-Denis reagent was added and the test tube was vigorously shaken. Immediately, 5ml of 20% sodium carbonate solution was added, the volume of the mixture was brought up to 10 ml and shaken thoroughly. After 20 min, the absorbance of the mixture was read at 700 and 735 nm. The spectrophotometer was zeroed with a blank cuvette. Tannic acid was used for standard curve. Plant growth was measured by harvesting the plants and weighing the fresh weights of herbs. The plants were then dried in an oven at 65°C for 72 h and their dry weights were recorded.

Statistical analysis: Data were analyzed using analysis of variance (ANOVA) with the STATISTICA computer program. Graphs were plotted using Microsoft Excel. The least significant difference test at 5% probability level was used to assess the differences among means (Steel *et al.*, 1997).

Results and Discussions

Effect of drought stress on growth: With the increase in drought stress from 100 to 60% field capacity, plant height, herb fresh and dry weights were reduced from 60.33 to 44 cm, 2.24 to 1.09 g and 1.25 g to 0.796 g respectively (Fig. 1, 2 & 3). Restricted water supply is a major environmental limitation in the productivity of plants. Moisture deficiency induces various physiological and metabolic changes. A reduction in soil moisture may reduce the availability of nutrients to the plant and consequently reduce plant height, growth and yield (Razmjoo *et al.*, 2008). Mohamed & Abdu (2004) found that water stress, imposed by restricting the frequency of irrigation, significantly decreased plant height. In *Ocimum basilicum* L., total shoot fresh weights of plants decreased at injurious levels of drought (50%) as compared to well-watered plants; both plant growth and productivity were adversely affected (Khalid, 2006). Our experiment also found that fresh and dry weights of herbs were reduced by increasing drought stress levels (Fig. 2 & 3). This may be due to the low availability of moisture around the roots, restricted proliferation of root biomass or limited absorption of nutrients (Staniszewska *et al.*, 2003). But this reduction of plant height and biomass was less than 50%, suggesting that this herb has the potential to survive under drought stress conditions.

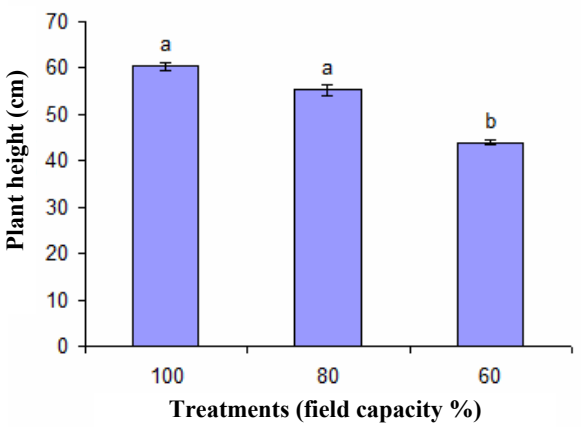

Fig. 1. Effect of water stress on plant height of desi ajwain.

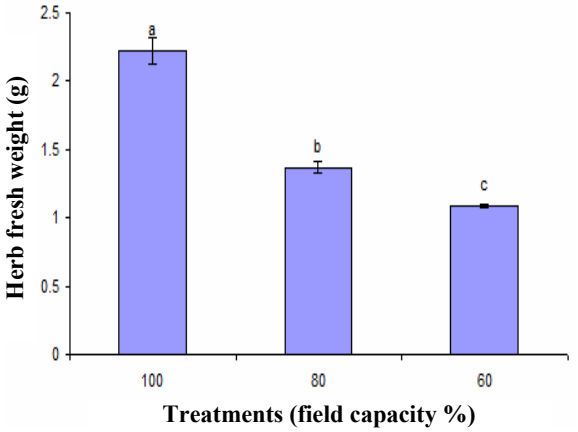

Fig. 2. Effect of water stress on fresh weight of desi ajwain.

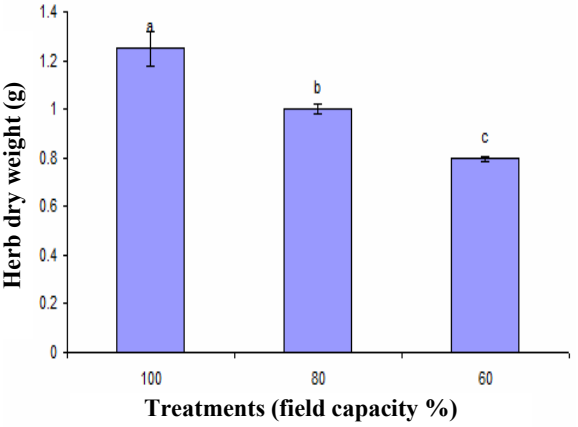

Fig. 3. Effect of water stress on dry weight of desi ajwain.

Effect of drought stress on physiological and biochemical aspects: Fig. 4 shows that treatment values did not differ significantly with regard to chlorophyll *a* contents. The highest chlorophyll *a* contents (0.57 mg/g) were found in plants growing at 60% field capacity, followed by plants at 100% (0.54 mg/g) and 80% field capacity (0.52 mg/g).

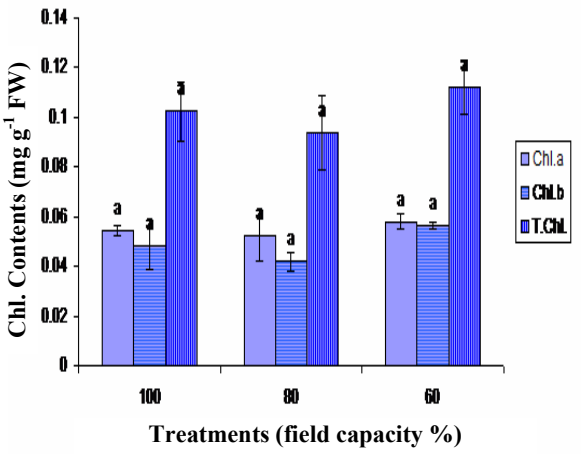

Fig. 4. Effect of water stress on chlorophyll *a*, *b* and total chlorophyll contents of desi ajwain.

The highest values for chlorophyll *b* and total chlorophyll contents were found at 60% field capacity (0.56 mg/g and 1.1 mg/g), with the lowest values at 80% field capacity (0.41 mg/g and 0.93 mg/g) (Fig. 4). These results are in agreement with findings that drought stress increased chlorophyll contents in *Withania somnifera* (Megdiche *et al*., 2008). In photosynthesis, chlorophyll plays a key role in trapping the sunlight and converting it into chemical energy, so any disturbance in chlorophyll contents may result in a reduction in photosynthesis. In the present study chlorophyll contents increased with increasing water deficit conditions. Moller (2001) also reported that in African nightshade (*Solanum scabrum* Mill.), chlorophyll contents increased in drought stressed plants. Although chlorophyll contents increased with drought stress, stomatal conductance and transpiration rates were reduced significantly with an increase in drought levels. Stomatal conductance was reduced from 15.4 to 9.98 mmol $m^{-2}s^{-1}$ and transpiration rate from 11.4 to 8.45 mmol m-2s-1 (Fig. 6 & 7). A similar reduction in transpiration rate with increased drought stress was also reported by Saefi *et al*., (2006) in rosemary (*Rosmarinus officinalis* L.). Water deficiency in plants may lead to physiological disorders, such as a reduction in photosynthesis and transpiration (Saccardy *et al*., 1998), because in order to prevent transpirational water loss, plants close their stomata (Ashraf & Ibram, 2005). This closure of stomata may result from direct evaporation of water from the guard cells (hydropassive closure).

In the present study there was no significant reduction in photosynthetic rate from 100% field capacity (0.211 µmol $m^{-2}s^{-1}$) to 80% field capacity (0.11 µmol $m^{-2}s^{-1}$); the photosynthetic rate of the 60% field capacity treatment was higher than that of the 80% treatment (Fig. 5). This shows that desi ajwain has the potential to maintain a high photosynthetic rate under water stress. It is a common observation that the photosynthetic rate in plants is reduced when they are subjected to drought. Valentovic *et al*. (2006) concluded that in olive trees the maximum value of net photosynthetic rate was observed in controlled plants as compared to drought treated plants. A decrease in photosynthetic rate under drought stress occurs through stomatal closure and reduction of protoplasm activity. A reduction in photosynthetic activity can be due to the reduction in stomatal conductance and uptake of water from roots but repetition of water stress cycles might cause photosynthetic adaptability (Matthews *et al*., 1990). In the present study the photosynthetic rate fell in plants from 100 to 80% field capacity but was maintained in plants from 80 to 60% field capacity. This shows that desi ajwain has ability to cope with drought stress conditions.

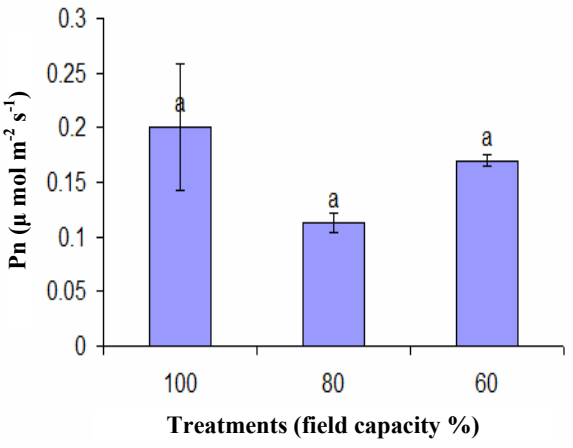

Fig. 5. Net CO_2 assimilation rate (*Pn*) of desi ajwain affected by water stress treatment.

The total phenolic contents increased significantly with increasing drought stress levels. The highest total phenolic contents (4.44 mg/g) were observed at 60% field capacity, followed by 3.95 mg/g at 80% field capacity and 2.23 mg/g at 100% field capacity (Fig. 9). Similar results were observed for *Prunella vulgaris* L. plants, where phenolic contents (rosmarinic acid, ursolic acid and oleanolic acid) increased under drought stress (Chen *et al*., 2011). Some nutrients, especially carbohydrate supplies, influence the phenolic composition. Stress factors like drought can increase the phenolic compounds (ferulic acid) in the leaves of triticale seedlings (Hura *et al*., 2009). Drought stress reduces growth, so the carbon fixed during photosynthesis could be used to form secondary metabolites (phenolics) (Hale *et al*., 2005). Our results confirmed that *Trachyspermum ammi* L. plants under dry conditions decreased vegetative biomass accumulation but enhanced their phenolic contents. This suggests that desi ajwain can be cultivated in areas prone to drought. The reduction in plant growth and yield was less than 50% and plant have the ability to cope with stress by generating secondary metabolites including phenols.

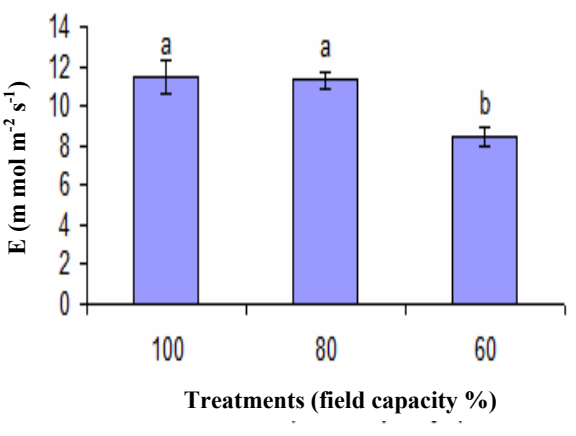

Fig. 6. Transpiration rate (*E*) of desi ajwain affected by water stress treatment.

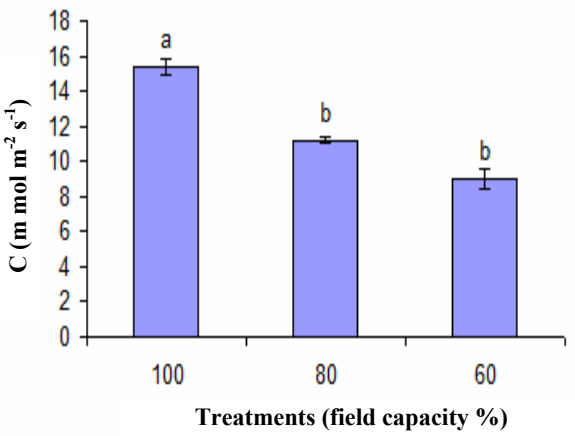

Fig. 7. Stomatal conductance (*C*) of desi ajwain affected by water stress treatment.

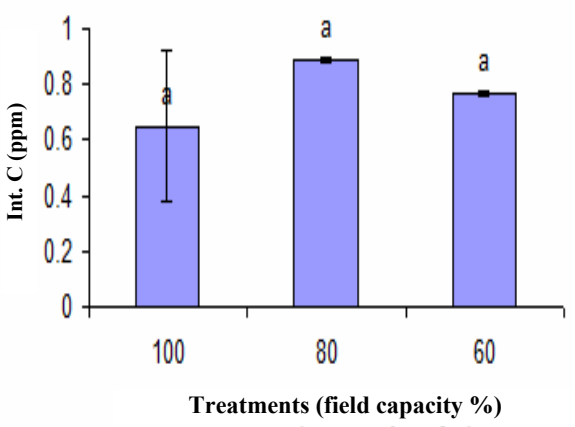

Fig. 8. Internal CO_2 concentration of desi ajwain affected by water stres treatment.

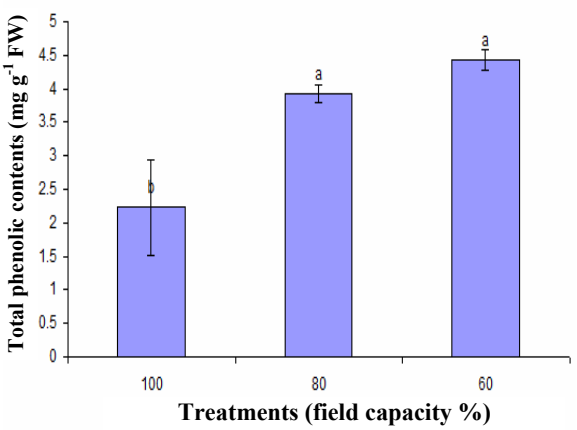

Fig. 9. Effect of water stress on total phenolic contents of desi ajwain.

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