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RESPONSE AND RECOVERY OF *CORIANDRUM SATIVUM* L. VARIETY INDOORI EXPOSED TO SOIL MOISTURE STRESS

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SUMMARY

Influence of soil moisture stress was investigated in *Coriandrum sativum* L. var. Indoori subjected to soil moisture stress (SMS) for four and eight days. The study revealed a decline in relative water content (RWC) with an increase in leaf water potential (LWP) upto 2.1 MPa. A decrease in total chlorophylls upto 34% and a minor reduction in chlorophyll stability index (CSI) was visible. Significant accumulation of free proline, free amino acids, ascorbic acid and total flavanoids was evident in plants facing eight days SMS. Lipid peroxidation and superoxide dismutase activity enhanced, but proline oxidase activity declined due to water deficit. Upon rewatering, RWC increased and amount of proline and ascorbic acid decreased. Lipid peroxidation decreased and enzyme activities recovered upon rewatering. The variety is susceptible to SMS but can be recovered from stress within five days after rewatering. Accumulation of antioxidants such as ascorbic acid and flavanoids may help the plant to face oxidative stress.

Key words: Antioxidants, free amino acids, leaf water potential, lipid peroxidation, proline, proline oxidase.

INTRODUCTION

Coriander (*Coriandrum sativum* L.) known as 'Dhania' belongs to the family Apiaceae and is cultivated extensively in India for fruits and green leaves. It is sown in both kharif and rabi seasons in Maharashtra. The crop is sensitive to temperature, rainfall and weather conditions at different growth stages (Chatterjee *et al.* 2001). When plants are exposed to soil moisture stress a low osmotic pressure, developed in the roots and leaves affects plant metabolism and ultimately the growth and yield are reduced. Water stress induces generation of active oxygen species such as superoxide radical, hydrogen peroxide and hydroxyl radical causing membrane damage, enzyme inactivation, protein degradation, pigment bleaching etc. Antioxidative functioning of certain metabolites such as ascorbic acid and oxidative enzymes like superoxide dismutase are known to provide better protection against oxidative stress (Reddy *et al.* 2005, Agarwal and Pandey 2003). In the present investigation coriander plants exposed to soil moisture stress were analyzed for water status, pigments, few enzymes and antioxidants. Response of water stressed plants after rewatering was also investigated.

MATERIALS AND METHODS

Experiments were conducted in the botanical garden of Botany department, Shivaji University, Kolhapur. Seeds of *Coriandrum sativum* L. var. Indoori were procured from Hemani Ex-IMP Corporation, Mumbai. Healthy fruits (Schizocarpic) were selected and split into two halves (mericarps) and sown in earthen pots filled with soil mixture (Soil: FYM 3:1). Soil moisture stress for four and eight days was given to the plants after 30

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days of growth by withholding water supply. Plants maintained at a normal water supply served as the control. Leaves of control and treated plants were collected on the next day after treatment to estimate different parameters and then plants were watered normally. Leaf samples were analyzed again five days after rewatering. For determination of RWC preweighed leaf discs were floated on distilled water for four hours and then turgid and dry weights were recorded as per standard procedure (Slatyer and Mcllory 1961). Proline oxidase activity was measured according to the method of Anthony *et al.* (1979). Fresh plant leaves were homogenized in Tricine-KOH buffer (pH 7.5) containing 0.6 M sucrose. All the intact and broken chloroplasts in the homogenate were removed by centrifugation at 5000 g and supernatant was recentrifuged at 25,000 g in a cooling centrifuge. The pellet was dissolved in 50 mM Tricine-KOH buffer (pH 7.5) containing 0.6 M sucrose. The reaction mixture contained 0.15 M Tris-HCL (pH 8.5), 75 mM $MgCl_2$, 7.5 mM NAD, 15 mM KCN, 15 mM phenazine methosulfate, 0.9 mM DCPIP, 0.6 M proline and fresh mitochondrial preparation. The reaction was initiated using proline and the absorbance during 60 seconds was recorded at 600 nm. Total flavonoid content was determined by the method of Luximon-Ramma *et al.* (2002). Plant material was extracted in 80 % acetone and then filtered through Buchners funnel using Whatman No.1 paper. The filtrate was reacted with 2 % methanolic aluminum chloride and absorbance was read at 368 nm against blank (2% $AlCl_3$). Amount of total flavonoids was calculated from the standard curve of Rutin (0.03 mg/ml in alcohol). Lipid peroxidation was determined by the method of Carkmak and Hort (1991). Fresh leaves were homogenized in 0.5 % thiobarbituric acid (TBA) prepared in 20 % Trichloroacetic acid (TCA). The extract was heated for 30 minutes in a boiling water bath. The reaction was then stopped by placing the tubes in ice-water. The flocculent precipitate was removed by centrifugation at 10,000 g and absorbance was measured at 535 nm. Non-specific absorbance measured at 660 nm was subtracted. Osmotic potential of cell sap was calculated as per the method of Janardhan and Krishnamoorthy (1975). Total chlorophylls were determined following the method of Arnon (1949). Free proline, amino acids and ascorbic acid content were estimated by the methods described by Bates *et al.* (1973) and Sadasivam and Manickam, (1992) respectively. Superoxide dismutase was measured by the method of Giannopolitis and Ries (1977). Plant material was extracted in K-phosphate buffer (pH7.8) and then centrifuged at 4^0 C. The enzyme assay consisted of K-phosphate buffer, methionine, Nitroblue tetrazolium chloride, EDTA and riboflavin. The absorbance was read at 560 nm before and after exposing to full sunlight for 30 minutes. Data were analysed on the basis of "Randomised Block Design" following the method of Goon *et al.* (1979).

RESULTS AND DISCUSSION

Relative water content in the leaves during soil moisture stress declined by about 20% and 40% in *C. sativum* exposed to four and eight days stress, respectively, accompanied by an increase in leaf water potential (Table 1). Total chlorophylls declined with a marginal change in chlorophyll stability index. A

Table 1. Effect of soil moisture stress (SMS) on physiological and biochemical parameters in *Coriandrum sativum* L. var. Indoori

Treatment	RWC%	LWP(MPa)	Total* chlorophylls	CSI	Proline@	Ascorbic acid@	Free amino acids *	Total flavonoids #
Control	82.31	00.8	1.23	0.86	008.90	150.17	0.82	2.89
4 days (SMS)	66.87 (-18.76)	1.30 (+62.5)	1.11 (-9.76)	0.84 (-2.33)	108.67 (+1121.01)	236.02 (+57.17)	1.92 (+134.5)	3.32 (+14.88)
8 days (SMS)	50.18 (-39.04)	2.10 (+162.25)	0.81 (+34.15)	0.80 (-7.00)	213.92 (+2303.60)	409.94 (+172.98)	4.28 (+421.95)	3.53 (+22.15)
C. D. at 5 %	7.673	0.039	0.100	0.009	0.807	0.166	0.045	0.020

@ mg/100 g fw, * g/100 g dw, # mg rutin equiv./g fw, values in parentheses indicate per cent increase or decrease over control.

conspicuous enhancement in the free proline was noticed in the stressed plants. The level of ascorbic acid increased by 57% and 173%, after four and eight days stress respectively. Accumulation of free amino acids and flavanoids was also evident in stressed plants (Table 1).

Lipid peroxidation declined significantly after eight days compared to the plants exposed to moisture stress for four days. Significant decline in proline oxidase activity was clearly observed in the stressed plants, whereas the activity of superoxide dismutase increased in stressed plants (Fig.1).

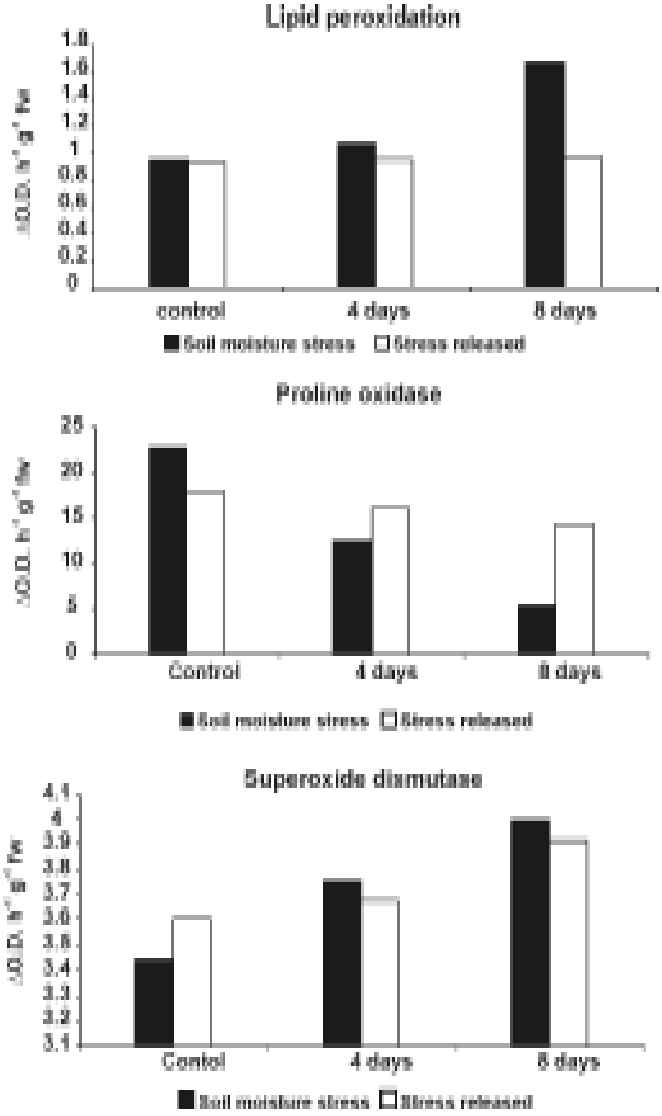

Fig.1. Effect of soil moisture stress on lipid peroxidation, proline oxidase and superoxide dismutase activities in *Coriandrum sativum* L. var. Indoori

After rewatering, four days stressed plants were found to recover faster than eight days stressed plants. Relative water content increased while proline and ascorbic acid content dropped in these plants (Table 2). The study of lipid peroxidation revealed a value similar to control. Proline oxidase enhanced rapidly in the eight days stressed plants after recovery. The activity of superoxide dismutase declined in recovered plants and level was comparable to that of control.

Table 2. Recovery of *Coriandrum sativum* L. var. Indoori from soil moisture stress

Treatment	RWC%	Total* chlorophylls	Proline@	Ascorbic acid@
Control	84.04	1.00	8.67	164.00
4 days (SMS)	83.10 (-1.12)	0.93 (-7.00)	8.81 (+1.61)	177.02 (+7.94)
8 days (SMS)	81.13 (-3.46)	0.79 (-21.00)	9.04 (+4.27)	192.55 (+17.41)
C. D. at 5 %	0.209	0.119	0.008	0.006

@ mg 100^{-1}g fw, *g100^{-1}g dw, Values in parentheses indicate percent increase or decrease over control.

A reduction in relative water content and leaf water potential during drought stress has been reported in crops such as chickpea and pigeonpea (Moinuddin and Chopra 2004, Jain *et al.* 2006). Damage to the photosynthetic apparatus and reduction in chlorophyll content on exposure to water stress is also seen in *Cyamopsis tetragonoloba* (Burman *et al*. 2004). Synthesis and accumulation of stress metabolites is of common occurrence in plants exposed to soil moisture stress because of altered metabolic processes. Increased proline concentration in plant tissues might help in osmotic adjustment under water stress condition. Proline accumulation during soil moisture stress is a common response and reported in several studies (Phutela *et al.* 2000, Garg *et al.* 2001, Ashraf and Iram 2005). A parallel decrease in proline oxidase enzyme which converts proline to glutamate is observed in the present investigation. Accumulation of free amino acids is also significant under water stress and may be due to induced hydrolysis of proteins as reported in crops like *Arachis hypogeae, Vicia faba* (Purushotham *et al.* 1998, EI-Tayab 2006).

Under stress condition certain secondary metabolites are found to function as antioxidants. An appreciable enhancement in non enzymatic antioxidants such as ascorbic acid and flavanoids is a characteristic of plants growing under stress. In *Cassia angustifolia* leaves under water stress, ascorbate might be functioning as an antioxidant and help in non-enzymatic scavenging of superoxide radicals and hydrogen peroxide to protect *Cassia* from the reactive oxygen species (Agarwal and Pandey, 2003). Similar observations have been made in crops like *Brassica parachinensis*, *Cicer arietinum* etc. (Zhang *et al.* 2000, Nayyar and Chander 2004).

Increased lipid peroxidation due to water stress is an indication of damage to the membrane system as reported by several workers in various crops (Thankamani *et al*. 2003, Zlatev *et al.* 2006, EI-Tayab 2006). Superoxide dismutase scavenges superoxide radicals and converts them to O_2 and H_2O_2. H_2O_2 is then detoxified by catalase. Enhancement of superoxide dismutase during stress and its decline after removal of stress, indicates the recovery of plants from stress condition. Zhang and Kirkham (1994) found that SOD activity enhanced in wheat in the early phase of drought and then decreased with further increase in water stress. In drought stressed leaves of *Arabidopsis thaliana* SOD increased and plants recovered rapidly after rewatering, but activity remained high (Jung 2004). In the present study also similar results were obtained.

The study of different physiological and biochemical parameters in *Coriandrum sativum* during SMS revealed that the plants are susceptible to drought. A water stress for more than four days alters the metabolic activities of plant. However, after rewatering, four days stressed plants recovered fast and exhibited normal behaviour.

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