*Full Length Research Paper*

# The effect of drought stress and exogenous abscisic acid on growth, protein content and antioxidative enzyme activity in saffron (*Crocus sativus* L.)

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This study was carried out to investigate the effect of drought and abscisic acid (ABA) on growth, protein content and antioxidative enzyme activity in leaves, roots and corms of *Crocus sativus* L. A comparative study of sample and control showed that leaf relative water content (RWC), length and number of leaves decreased due to drought and ABA when compared to control. Drought and ABA were effective in increasing root length in comparison to control. Though drought and ABA improved the protein content in corms, leaves and roots, maximum protein content was detected in corms. Drought and ABA increased superoxide dismutase (SOD) and peroxidase (POX) activitiy in roots, leaves and corms when compared to that of the control. The highest SOD activity was observed in the roots, followed by leaves and corms, respectively. Furthermore, highest POX activity was observed in roots.

**Key words:** Drought, ABA-treatment, *Crocus sativus*, peroxidase, superoxide dismutase.

## INTRODUCTION

Saffron crocus is a perennial herb cultivated in several countries of mild and dry climate. Saffron is mostly used as spice and food colorant. However, due to its analgesic and sedative properties, folk herbal medicines have used it for the treatment of numerous illnesses for centuries (Basker and Negbi, 1983). Among the abiotic environmental stresses, drought is known to adversely affect plant growth and productivity (Reddy et al., 2004). Early responses of plants to drought stress usually help the plant to survive for some time, while the acclimation of the plant subjected to drought is indicated by the accumulation of certain new metabolites associated with structural capabilities to improve plant functioning under drought stress (Pinhero et al., 2001). Higher plants have active oxygen-scavenging systems consisting of several antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and monodehydro ascorbate reductase (MDAR) (Bowler et al., 1992). These systems protect membranes from the deleterious effects of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide ($H_2O_2$), hydroxyl radicals and single oxygen, which are produced at elevated rates when plants are exposed to abiotic stress conditions (Bowler et al., 1992; Noctor and Foyer, 1998). The superoxide radicals are dismutated to $H_2O_2$ by SOD and POX metabolizes $H_2O_2$. SOD is present in all aerobic organisms and most subcellular compartments that generate activated oxygen. The three known types of SOD are classified by their metal cofactors: the copper/zinc, manganese and iron forms (Reddy et al.,

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**Abbreviations: RWC,** Relative water content; **SOD,** superoxide dismutase; **POX,** peroxidase; **ABA,** abscisic acid; **CAT,** catalase; **APX,** ascorbate peroxidase; **GR,** glutathione reductase; **MDAR,** monodehydro ascorbate reductase; **ROS,** reactive oxygen species; **EDTA,** ethylenediaminetetraacetic acid.

2004). The plant hormone, abscisic acid (ABA) is produced de novo under water deficit conditions and plays a major role in response and tolerance to dehydration (Shinozaki and Yamaguchi-Shinozaki, 1999).

Mutants with ABA deficiency were used to analyze drought-inducible genes and the results indicate that several genes are induced by exogenous ABA-treatment (Qin and Zeevart, 2002). In maize, the ABA content was further increased by ABA-pretreatment (Pospisilova et al., 2005). Exogenous application of ABA has been reported to significantly increase activities of SOD and POX (Jiang and Zhang, 2001). The objective of this work is to investigate the effect of drought stress and ABA treatment on growth, protein content and activities of anti-oxidant enzymes in leaves, roots and corms of *Crocus sativus* L.

## MATERIALS AND METHODS

### Plant material and treatment

Corms of *C. sativus* L. Gaenat were sown in plastic pots filled with 2 kg of garden soil (clay-sand) with pH 7-8. Pots of each plant were divided into 3 groups in 3 replicates. Plant growth was carried out in a controlled condition of 16 h photoperiod, 30/22°C day/night temperature and 55% air humidity. Plants were irrigated with complete Hoagland solution. One set of pots was kept as control (well watered) and two other sets were used for drought (drought stress) and ABA (drought and ABA-treatment together) assessment. Stress was induced by cessation of watering for a period of 1, 4, 7, 12, 22, 34 and 46 days. This experiment was focused only on vegetative growth in *C. sativus*. ABA (1 mM) was sprayed on whole leaves.

### Study of growth parameters

Relative water content (RWC) of leaves was calculated according to the formula: 100 [(fresh weight – dry weight) / (saturated weight – dry weight)] (Wheatherley, 1950). Saturated mass of the plant was determined by keeping them in water at 4°C in the dark for 24 h, followed by their drying in hot air oven (60°C for 48 h) till constant weight was achieved.

### Determination of total soluble protein content and enzyme activity

For determination of total soluble protein content and enzyme activity, 1 g of plant material was homogenized in a chilled (4°C) mortar using a 50 mM Tris-HCl buffer (pH 7.0) containing 10 mM ethylenediaminetetraacetic acid (EDTA), 2 mM MgSO4, 20 mM cystein, 10% (v/v) glycerol and 2% (w/v) PVPP ( Jasska, 1996). The homogenate was centrifuged in a refrigerated centrifuge at 13,000g for 45 min, and supernatant obtained was used for protein determination and enzyme assay. Protein content of the extracts was determined according to the method of Bradford (1976), using bovine serum albumin as the standard.

Total superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of thiazolyl blue (MTT) as described by Beauchamp and Fridovich (1971). The reaction mixture consisted of 0.1 cm3 enzyme extract, 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM MTT, 0.1 mM EDTA and 75 µM riboflavin.

Test tubes were shaken and placed 30 cm from three 30 W fluorescent lamps. The reduction in MTT was measured by reading absorbance at 565 nm. Blanks and controls were run in the same manner but without illumination and enzyme, respectively. One unit of SOD was defined as the amount of enzyme that produced a 50% inhibition of MTT reduction under the assay conditions (Giannopolitis and Ries, 1977).

Peroxidase (POX; EC 1.11.1.7) activity was assayed as described by Abeles and Biles (1991). The reaction mixture consisted of 2 cm3 of 0.2 M acetate buffer (pH 4.8), 0.2 cm3 H2O2 (3 %), 0.1 cm3 of 20 mM benzidine and 0.03 cm3 enzyme extract. The rate of benzidine oxidation was measured at 530 nm. Native gel electrophoresis (non-denaturing conditions) for isoenzyme assay was carried out according to the modified method of Davis (1964) with a 10% acrylamide gel at 4°C and 100 µg of protein per lane. A vertical electrophoresis apparatus (model LKB, Bromma, Stockholm, Sweden) was used. The electrophoresis run was carried out with 120 mV (30 mA) per plate towards the cathode. To determine SOD isoforms pattern, gels were incubated for 30 – 45 min at room temperature in the dark in 0.2 M Tris-HCl buffer (pH 8.0) containing 4 mg riboflavin, 2 mg Na-EDTA and 20 mg MTT. Then, the bands were apparent in 5 min at light (Wendel and Weeden, 1989). To determine Mn-SOD, Fe-SOD and Zn/Cu-SOD isoforms pattern gels were incubated by KCN and H2O2. Isoforms of POX were visualized by staining the gel in 0.2 M acetate buffer (pH 4.8) containing 3% H2O2 and 0.04 M benzidine in 50% methanol in the dark at room temperature till the brown color appeared.

Analysis of variance (ANOVA) using the statistical package for the social sciences (SPSS) and the Duncan's multiple range test (DMRT) at $P \leq 0.05$ determined the significance of the variations on the groups under the different treatments.

## RESULTS AND DISCUSSION

The present treatise embodies the work carried out on drought stress and exogenous abscisic acid on growth, protein content and antioxidative enzyme activity in saffron. Leaf RWC and length and number of leaves decreased by drought and ABA (Figure 1). Drought and ABA increased root length when compared to that of the control but thickness and number of root was decreased (no data). Leaf RWC decreased during drought stress, but RWC of ABA-treated plants were a little bit higher than untreated drought plants. Similar observations have been reported. Drought stress reduces plant growth by affecting various physiological and biochemical processes such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Farooq et al., 2008). It has been reported that the restriction of water supply from the soil reduced plant biomass. Our result showed that drought decreased fresh and dry mass in leaves and there was a significant difference in crocus growth during drought with control. Water deficits reduced the number of leaves per plant and individual leaf size (Chung et al., 1997). Keleo and Unyayar (2004) showed that drought stress cause significant declines in shoot length in Helianthus annus. The foliar photosynthetic rate of higher plants is known to decrease as the RWC decreases (Lawlor and Cornic, 2002).

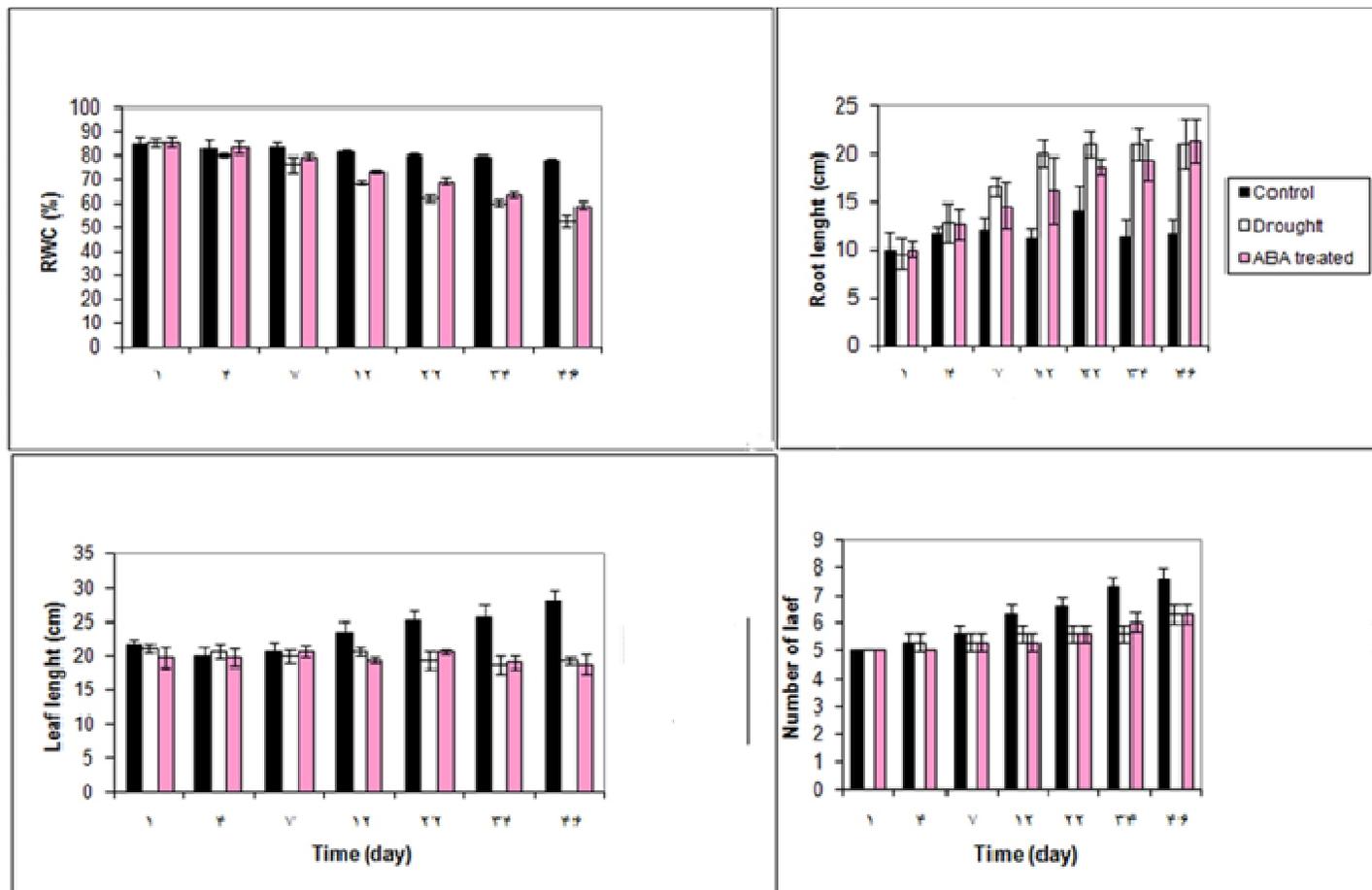According to Lenka and Mishra (1973) and Narasimham

**Figure 1.** Effect of drought and ABA on leaf RWC (A); highest leaf length (B); highest root length (C) and number of leaves (D). Vertical bars represent SE of the means; n = 3 and values carrying different letters are significantly different at P ≤ 0.05.

et al. (1977), water stress stimulates the growth of ground-nut roots into deeper soil. During water stress, roots in lower depths continue to grow deeper even though vegetative growth appears to stop (Allen et al., 1976).

ABA spray could elevate the RWC during drought stress but it was not more beneficial (Figure 1). According to Agarwal et al. (2005), a lower concentration of ABA was comparatively more beneficial in increasing RWC than higher concentration of ABA. Creelman et al. (1990) showed that ABA is a regulator of shoot growth and development under water stress. Shoot growth can be inhibited when xylem ABA is increased as a result of soil drying (Gowing et al., 1990). ABA application significantly decreases the shoot dry biomass and it significantly increase root/shoot ratio under water deficit conditions (Duan et al., 2007).

Drought and ABA increased the protein content of corms, leaves and roots in comparison to control (Figure 2). In this research, increase of protein content in roots by ABA was more than by drought. ABA pretreatment decr-eased the drying rate of protocorms and increased

dehydration tolerance. ABA pretreatment also promoted dry matter accumulation such as soluble proteins in pro-tocorms (Wang et al., 2002).

SOD activities in corms, leaves and roots increased significantly under ABA and drought at 7 to 46 days (Figure 3). Highest activity of SOD was found in roots than in leaves. SOD and POX constitute the first line of defense against ROS, and changes in their activities have been identified as an indicator of a redox status change under drought (Moran et al., 1994; Schwanz and Polle, 2001). In the last decade, the role of ABA in the induction of antioxidant defense has been the subject of extensive research. It has been documented that ABA can cause the increased generation of ROS (Synkova and Pospísilová, 2002), induce the expression of antioxidant genes encoding SOD and CAT and enhance the activities of antioxidant enzymes such as SOD, CAT and APX (Jiang and Zhang 2002). According to Keleo and Unyayar (2004), the specific activity of SOD increased under drought stress in leaves of H. annus. Moreover, ABA treatment led to increase of SOD activity

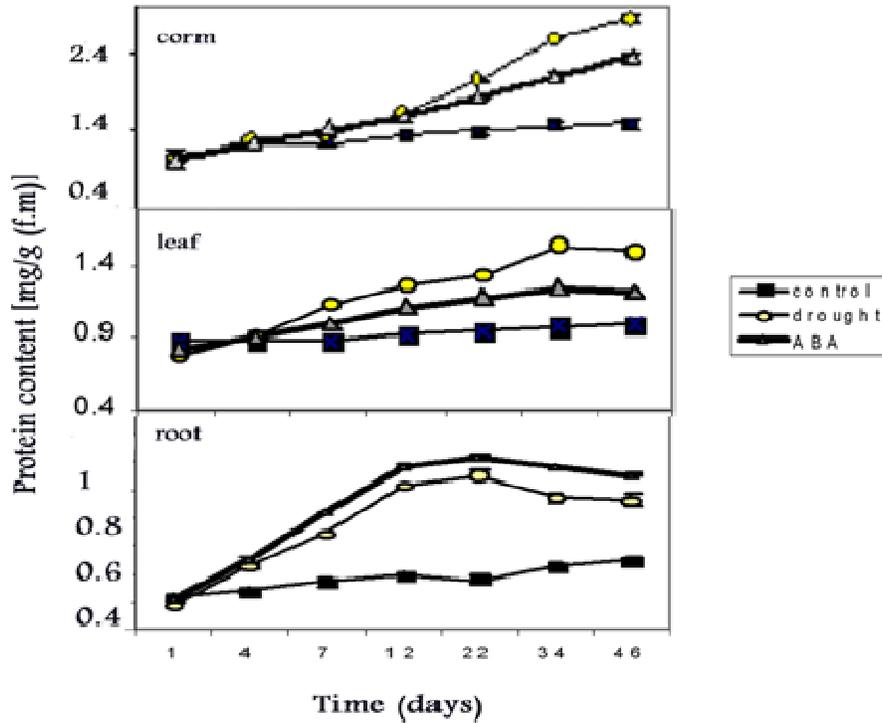**Figure 2.** Protein content [mg g$^{-1}$(f.m.)] in corms, leaves and roots of *C. sativus* L. under drought and ABA treatment. Vertical bars represent SE of the means; n = 3 and all were significant (P ≤ 0.05).
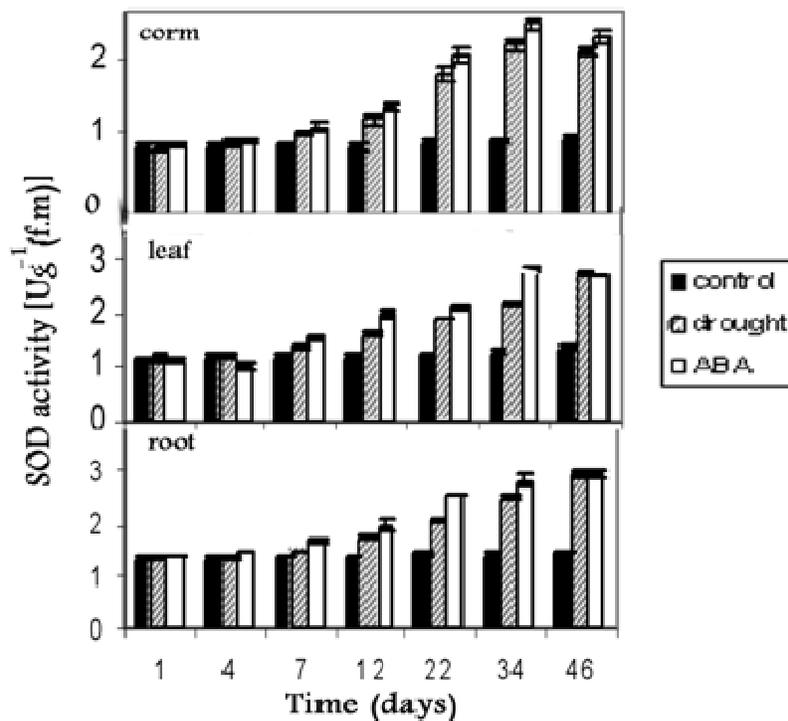


**Figure 3.** Activity of SOD [U mg$^{-1}$(protein)] in corms, leaves and roots of *C. sativus* L. under drought and ABA. Vertical bars represent SE of the means; n = 3 and all were significant (P ≤ 0.05).
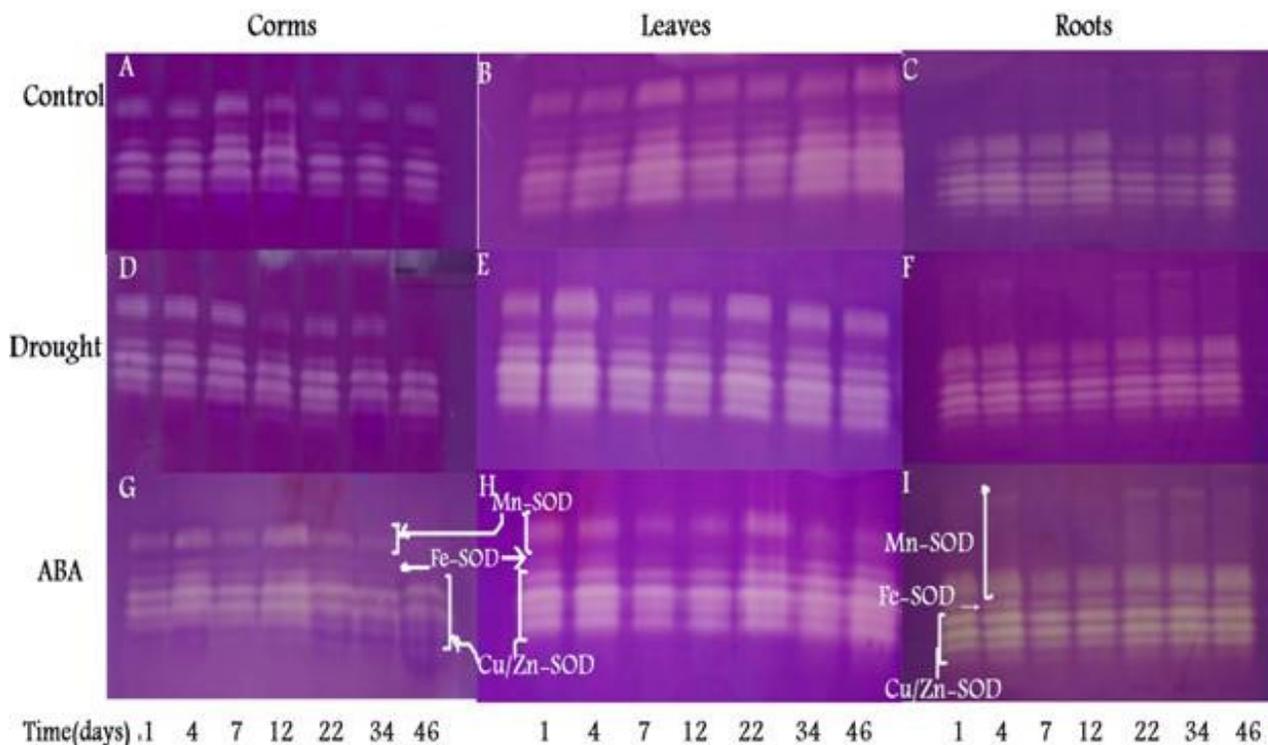
**Figure 4.** Activity staining for SOD in corms, leaves and roots of *C. sativus* L. at different days of drought and ABA.

in drought stress group. The analyses on gels revealed ten SOD isoforms in corm, leaf and root of *C. sativus* including 4 Mn-SOD, 2 Fe-SOD and 4 Cu/Zn-SOD.

According to Duan et al. (2007), ABA increased the activities of SOD, APX, GR, and CAT in comparison to unsprayed control plants. ABA application significantly increased ABA content and SOD activities under water-deficit conditions (Duan et al., 2007). SOD activity increased significantly at 0.5 mM ABA at 30 and 45 days. At 1.0 mM ABA, though the SOD activity increased over control plants, the increase was less than at 0.5 mM ABA (Agarwal et al., 2005). Increased MnSOD and FeSOD activity and concentration were shown to be induced by water stress in two cowpea cultivars (Brou et al., 2007).

Treatment with ABA increased antioxidant enzymes activities as well as drought stress in *C. sativus.* ABA plays a role in the enhancement of tolerance to oxidative stress by increasing the activity of antioxidant enzymes (Jiang and Zhang, 2001). It is clear that ABA increased the activity of antioxidant enzymes and ABA treatment decreased oxidative stress but in this plant or at this concentration, ABA did not increase antioxidant enzymes when compared to the drought plants.

Climatic conditions favorable for high yields of saffron are rainfall in the autumn, warm summer and mild winters. Water requirement of saffron is low and is cultivated under irrigated or rain fed conditions (Fernandez, 2004). The involvement of ABA in the osmotic signal trans-

duction process is well established (Trewavas and Jones, 1991) and it has recently been demonstrated that ABA influences the expression of genes encoding Cu/Zn-SOD and Mn-SOD (Zhu et al., 1994; Williamson and Scandalios, 1992; Sakamoto et al., 1995). According to polyacry-lamide gel electrophoresis (PAGE) analysis of SOD in corms (Figure 4), ABA induced much stronger bands intensity than drought. Under severe stress in corms in 46 days in both treatment drought and ABA, Mn-SOD bands were weaker than that of other days. Also, in leaves under drought and ABA, Fe-SOD bands were weaker than control. Interesting point was the appearance of new Mn-SOD band in roots when compared to that of leaves and corms. Although ABA seems to play a predominant role in the conversion of environmental signals into changes in the gene expression in plants (Zhu, 2002), all responses are not mediated by it (Gibson et al., 1991). ABA has also been shown to be involved in promoting drought tolerance when applied exogenously (Wang et al., 2003; Li et al., 2004).

POX activity of corm, leave and root increased signifi-cantly at 7 to 46 days under ABA and drought when compared to the control (Figure 5). Highest POX activity was determined in controlled and treated roots.POX activity in plants treated with ABA was more than that of drought alone. Stronger bands and new isoforms were detected under drought and ABA in corms and leaves (Figure 6). The increase in the intensities of isoforms in
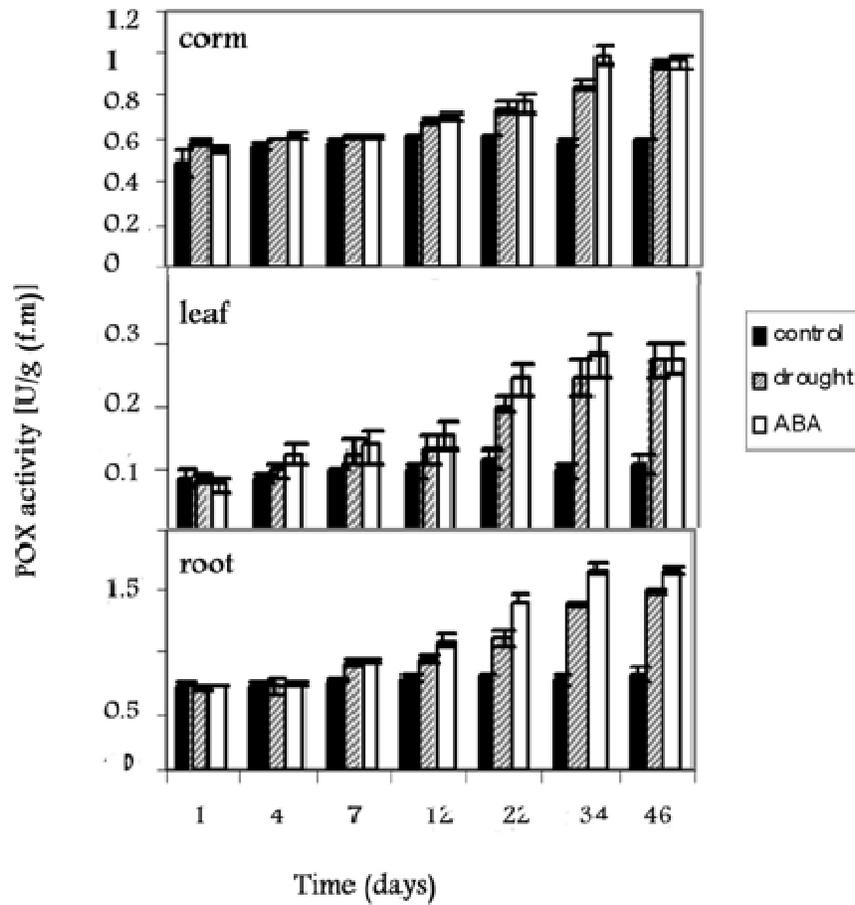
**Figure 5.** Activity of POX [U g$^{-1}$ (f.m)] in corms, leaves and roots of *C. sativus* L. under drought and ABA. Vertical bars represent SE of the means; n = 3 and all were significant at P ≤ 0.05.
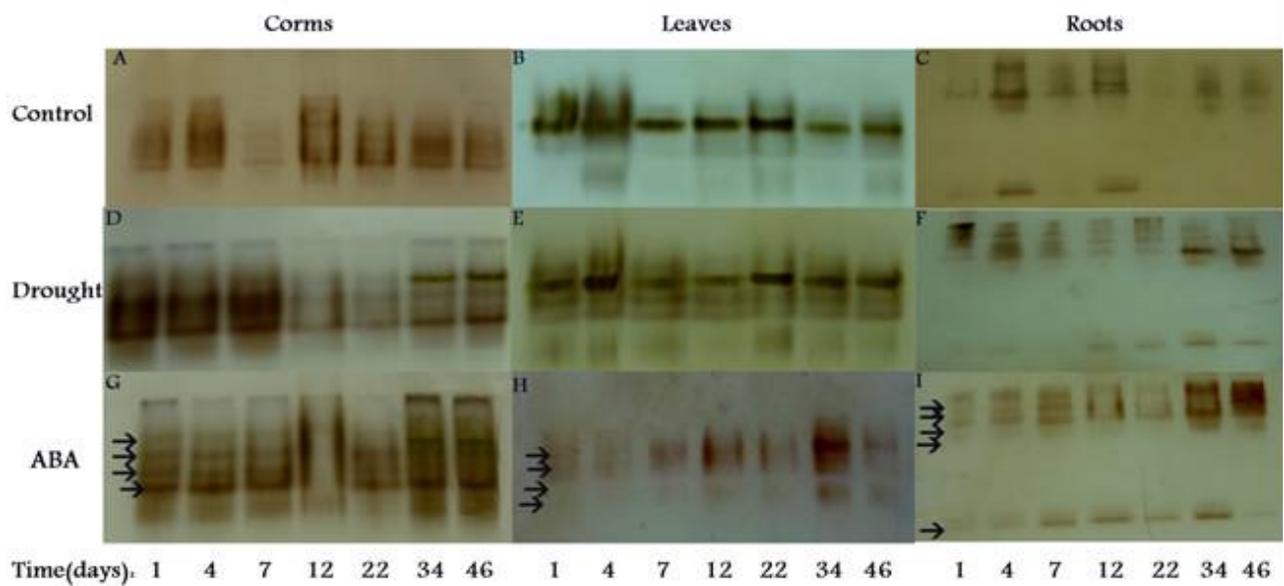


**Figure 6.** Activity staining for POX in corms, leaves and roots of *C. sativus* L. under drought and ABA.

corm under drought was more prominent than that of ABA.


## Conclusions

Growth and RWC decreased while antioxidative enzymes increased with progressive drying. From the aforesaid discussion, it is evident that foliar spray of ABA and drought increased activities of antioxidant enzymes such as SOD and POX. The ABA treatment enhanced drought tolerance as indicated by higher RWC and activity of antioxidant enzymes in all organs when com-pared with plants without ABA.

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