

## Changes of Proline Content and Activity of Antioxidative Enzymes in Two Canola Genotype under Drought Stress

Heshmat Omid  
College of Agricultural Sciences, Shahed University,  
P.O. Box 18151/159, Tehran, Iran

---

**Abstract:** The aim of present study was to get further information on the ability of drought stress applied to retard senescence and to frustrate inhibitory effects of drought-stress via comparing two canola genotypes differing in drought tolerance. The objective of this work was to evaluate the effects of the drought stress (control, -0.5, -1 and -1.5 Mpa) on two canola genotypes (Okapi and RGS). The experiment was factorial with four replications at experimental greenhouse at Center of Agriculture Research-Shahed, Iran, during 2006-2007. Morphological parameters of root and shoot dry weight, proline content osmolyte and enzyme activities of Ascorbate peroxidase (APx), Guaiacol peroxidase (GAX) and Catalase (Cat) in shoot and root of tissues canola genotypes were highly significant differences for main and interaction effects of two factors (Drought (D) and Genotype (G)) studied. Drought decreased the roots and shoots dry weight of canola plants and this effect was particularly significant at high level of stress. So, results indicated that proline content, ascorbate peroxidase (APx) and Guaiacol peroxidase (GAX) and Catalase (Cat) activity in root and shoot increased with enhancement of drought stress in the two genotypes. Of course, the proline content and antioxidant activities in Okapi genotype were more than RGS genotype. These results showed that Okapi genotype tolerated drought stress than RGS with increasing of proline content and antioxidant activities including GAX and APx. Shoot always maintained higher level of free Catalase (CAT) contents than the root. Therefore, the evaluation of both antioxidant enzymes and proline content can be suggested for Mechanisms of drought stress tolerance.

**Key words:** Free radical-scavenging, *Brassica napus* L., hydrogen peroxide, proline and osmotic adjustment

---

### INTRODUCTION

Plants are often exposed to environmental stresses through a life cycle. Several environmental factors adversely affect plant growth and development and final yield performance of a oilseed crop. Antioxidants enzyme and organic osmolytes such as proline are known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses.

Among these stresses, in particular drought is the most serious problem that limits plant growth and crop productivity in agriculture. Drought stress is one of the major factors in limiting crop production, particularly in arid and semi-arid locations (Ashraf and Foolad, 2007). In such regions, germination and seedling establishment constitute are the most critical periods in the life-cycle of plants (Omid *et al.*, 2008). Consequently, adaptations during these stages can determine their natural distribution.

Canola is one of the most important oil seeds growing in many parts of Iran. It is very important to grow canola with high oil levels for agronomical benefits (Omidi *et al.*, 2008). A large part of vegetable oil for human consumption in Iran is imported to the country and hence cultivation and appropriate management of oil seeds to increase yield is very important (Omidi *et al.*, 2010). In recent years, due to the adaptation of canola to different climatic conditions, its production has been the center of attention and its cultivation area has increased from 19 000 ha in 2000 to 300 000 ha in 2008 (Omidi *et al.*, 2010).

Drought stress lowers plant production worldwide. Drought stress results in a wide variety of physiological and biochemical changes in plants. Among them, the accumulation of low molecular weight solutes, such as proline commonly referred to as compatible solutes. Proline accumulation in plant cells exposed to water stress is a widespread phenomenon and is often considered to be involved in stress resistance mechanisms, although its precise role continues to be controversial (2-5). Water scarcity has been considered a limiting factor to crop production in arid and semiarid regions of the world (Hsu *et al.*, 2003; Mudgal *et al.*, 2010). Under environmental stress conditions, many plants accumulate several kinds of compatible solutes such as proline, glycinebetaine (from now on betaine), sugars and polyols (Ashraf and Foolad, 2007).

Oilseed crop studies published to date have concentrated on the various species in response to different biotic and abiotic factors and many functional proteins have been identified, e.g., many kinds of proteins up-regulated in response to stress in *Suaeda aegyptiaca* leaves (Askari *et al.*, 2006), rice leaves (Kim *et al.*, 2005a, b) and rice roots (Yan *et al.*, 2005) and proteins involved in responses to osmotic stress in *Arabidopsis* (Ndimba *et al.*, 2005). The responses of plants to stress conditions have evolved a variety of physiological and biochemical processes, e.g., solute accumulation and the development of enzymatic antioxidant systems (Ashraf and Foolad, 2007). The amino acid proline (Pro) is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Khedr *et al.*, 2003). Plants employ antioxidant defense mechanisms against oxidative damage of reaction oxygen species. Proline and betaine enhance antioxidant defense systems in plant responses to various oxidative stresses (Khedr *et al.*, 2003; Demiral and Turkan, 2004; Okuma *et al.*, 2004; Park *et al.*, 2006; Molinari *et al.*, 2007).

Some of the proteins made in response to such stresses are enzymes that synthesize compounds believed to serve as osmoprotectants, including proline, polyamines, glycine, betaine and soluble sugars. Soluble sugars accumulate in the leaves of many plant species when they experience water stress (Udomchalothorn *et al.*, 2009). Earlier reports have shown that both proline and betaine improve salt tolerance in tobacco BY-2 cells by increasing the activity of enzymes involved in the antioxidant defense and methylglyoxal detoxification systems (Hoque *et al.*, 2007a, b). Recent research has focused the protective roles of proline and betaine in cell death against various oxidative stresses (Chen and Dickman, 2005). The expressions of antioxidant enzymes are up-regulated upon some abiotic stress, including, e.g., responses in shoot and root tissues, cells and organelles in the model plant *Arabidopsis* (Hsu *et al.*, 2003; Dixon *et al.*, 2005; Schurmann, 2003), responses to drought stress in maritime pine (Costa *et al.*, 1998), sugar beet (Hajheidari *et al.*, 2005), rice (Ali and Komatsu, 2006), *Elymus elongatum* (Gazanchian *et al.*, 2007) and wheat (Hajheidari *et al.*, 2007), responses to salt stress in rice (Ashraf and Foolad, 2007) and *S. aegyptiaca* (Kim *et al.*, 2005a, b) and responses in poplar to heat stress (Ferreira *et al.*, 2006) and ozone exposure (Ozden *et al.*, 2009). The association between drought stress damage and senescence, on one hand and osmotic adjustment mechanisms and biochemical processes on the other, are little considered. Several recent studies focus on evaluation of water deficit on oilseed

establishment adaptation. However, we hypothesized that solute accumulation and allocation under drought conditions depend on aging process of plant tissue.

In many researches, drought tolerance in plants have been studied in relation to regulatory mechanisms of osmotic (Ashraf and Foolad, 2007). drought stress, like other abiotic stresses, can also lead to oxidative stress through the increase in reactive oxygen species (ROS), such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals (OH), which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Apel and Hirt, 2004; Mudgal *et al.*, 2010; Nojavan and Khorshidi, 2006).

To be able to endure oxidative damage under conditions which favors increased oxidative stress such as drought, plants must possess efficient antioxidant system. Plant cells have evolved a complex antioxidant system, which is composed of lowmolecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) (Apel and Hirt, 2004; Bhardwaj *et al.*, 2007).

Canola (*Brassica napus* L.) species is considered to be a relatively moderately drought-sensitive genus within which there is certain variability towards drought tolerance. Because the canola species used in this study were cultivated under various conditions throughout the world, comparisons between varieties are questionable; nonetheless, identification of varietal differences in response to drought between some canola species was the aim of the present study.

The recent study was conducted in order to assess two canola varieties for their drought tolerance and to give more information on the significance of osmotic adjustment and osmolytes accumulation under drought stress. In this case, the following questions were therefore addressed: (1) how much these processes are influenced by Root-Shoot tissue senescence and (2) what is the degree of tolerance of the two tested varieties? For this purpose we investigated the changes in Enzyme assays status and solutes accumulation especially proline, in canola roots and shoots and to relate them to defense strategies in terms of osmotic adjustment of each variety against drought stress.

## MATERIALS AND METHODS

### Experimental Design

The experiments were carried out at the Basic Science Experimental Station (BSES), 25 km, South of Tehran, Iran in 2006-2007 and. The experimental design was Factorial of drought stress and canola genotypes with four replications. The four drought stress, were including: No stress-(control), minimum stress (-0.5 Mpa), moderate (-1 Mpa) and High stress (-1.5 Mpa). The canola autumn genotypes were consist of RGS and Okapi.

### Plant Material and Growth Conditions

The one hundred seeds of both genotypes were surface sterilized with 5% sodium hypochlorite for 5 min and then thoroughly rinsed with distilled water before further experimentation. Seeds of canola genotypes were grown in the containers with Hoagland nutrient solution. Surface-sterilized seeds were germinated in the dark on sand, moistened with distilled water. Ten day old seedlings with the uniform size were transferred to hydroponic culture in plastic container with 2 L of nutrient solution. Plants were grown under greenhouse conditions with a 14 h photoperiod of natural daylight, maximum and minimum temperatures were 26 and 18°C, respectively and relative humidity was 60-80% on average and the average of photosynthetically active radiation was 500 mol/m<sup>2</sup>/sec. Canola seedlings were grown under controlled conditions as previously described for

3 weeks. Four stress treatments were imposed by adding D0 = 0 (control), D1 = 20.33, D2 = 29.61 and D3 = 36.80 g/100<sup>o</sup> PEG 6000 to the nutrient solution after 10 day old plants. The Osmotic Potential (OP) of the treatments was calculated using (Michel and Kaufmann, 1973). Twenty days after drought treatment, the plants were harvested. The Shoot and root samples collected from both varieties were frozen in liquid nitrogen and stored at -20°C in order to disrupt cell membranes. Shoot tissues were thawed and centrifuged at 1200×g for 25 min at 4°C to extract the cell sap. The analysis of biochemical solutes were performed in roots and Shoot stages during the experiment. Moreover, the proline concentrations were determined from the primary shoot and root apex.

### **Proline Content**

Proline content was estimated using ninhydrin reaction method (Bates *et al.*, 1973), except that phosphoric acid was omitted to avoid interference with concentrated sugars (Magne and Larher, 1992). Shoot discs were placed into tubes (five discs per tube) and 2 mL of distilled water were added. The tubes were kept for 30 min in a boiling water bath to extract hot water soluble compounds and then cooled to room temperature. One hundred microliter of the water extract was mixed with 400 mL of distilled water and 1 mL of ninhydrin reagent (0.5 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of distilled water). The mixture was boiled for 20 min in a water bath and then cooled on ice-water. The chromophore formed was extracted with 3 mL of toluene by vigorous shaking and tubes were placed in the dark for a minimum of 2 h. Absorbance of the resulting organic layer was measured at 520 nm. Calibrations were made with 1 mM L-proline as a standard.

### **Enzyme Assays**

Fresh shoot samples (0.4 g) were ground in liquid nitrogen using a mortar and pestle and the ground samples were homogenized in an ice bath in 4 mL single extraction solution containing 50 mM Tris-HCl (pH 7.0), 1 mM EDTA, 20% glycerin, 1 mM Ascorbic acid (AsA), 1 mM dithiothreitol (DTT), 1 mM glutathione (GSH) and 5 mM MgCl<sub>2</sub> and extracted at 48°C. The homogenate was centrifuged at 8,000 g at 48°C for 15 min. This method was modified as described by Knorzer *et al.* (1996). The supernatant was stored in volumes of 0.4 mL at 70°C for the analysis of soluble protein and the following determination of antioxidant enzymes. All experiments were performed at 25°C and completed within a day.

#### **Catalase (EC 1.11.1.6, CAT)**

A modification of the procedure of Aebi (1984) was used to analyze the CAT activity. The CAT activity was determined by directly measuring the decomposition of H<sub>2</sub>O<sub>2</sub> at 240 nm. The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.0) and 0.1 mM EDTA. The reaction mixture and 750 mM H<sub>2</sub>O<sub>2</sub> were warmed up at 25°C. Then, 50 µL enzyme solution was mixed with 2.9 mL reaction mixture and 50 µL 750 mM H<sub>2</sub>O<sub>2</sub> was added to start the reaction. The absorbance at 240 nm was read every 30 sec. The CAT activity was followed by a decrease of absorbance between 0.5 and 3 min. The CAT activity was calculated and expressed as nmol H<sub>2</sub>O<sub>2</sub> mg<sup>-1</sup> protein min<sup>-1</sup>.

#### **Guaiacol Peroxidase (EC 1.11.1.7, GPx)**

The Guaiacol peroxidase (GPx) activity was measured using a modification of the method of Chance and Maehly (1955). The assay mixture contained 50 mM Tris-HCl (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol and 5 mM H<sub>2</sub>O<sub>2</sub>. Then, 50 µL enzyme solution was added to start the reaction in a total volume of 3.0 mL. Changes in the absorbance of the brown guaiacol at 470 nm between 0.5 and 3.5 min were recorded for calculating the GPx activity, which was expressed as micromol guaiacol mg<sup>-1</sup> protein min<sup>-1</sup>.

### Ascorbate Peroxidase (EC 1.11.1.11, APx)

The APx activity was measured using a modification of the procedure of Nakano and Asada (1981). The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM H<sub>2</sub>O<sub>2</sub>. Then, 2.93 mL of the reaction mixture was homogenized with 50 µL enzyme solution and the reaction was started by 20 µL 30 mM AsA (a total volume of 3 mL). The H<sub>2</sub>O<sub>2</sub>-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm within 1 min. The APx activity was calculated and expressed as µmol AsA mg<sup>-1</sup> protein min<sup>-1</sup>.

### Statistical Analysis

The results of the morphological and biochemical parameters were statistically analyzed by one-way ANOVA and Least Significant Difference (LSD) test to determine significant differences among group means (SAS Institute, 1988). A p-value of 0.05 was considered statistically significant. The statistical package SAS, version 9.1 (SAS/STAT Software for PC. SAS Institute Inc., Cary, NC, USA) was used for all the applied statistical analyses (Steel and Torrie, 1980).

## RESULTS

Effects of the four Drought stress (D) treatments on the growth of canola genotypes is shown in Table 1. The application of stress significantly affected plant growth components such as root and shoot dry weight (DW), proline content and antioxidant enzymes activity of canola genotypes (Table 1-2, Fig. 1-4). Drought decreased the roots and shoots dry weight

Table 1: Results of variance analysis (ANOVA) of Drought stress (D), genotype (G) and their interaction for shoot and root dry weight, proline concentration and antioxidant activity

Dependent variables	Independent variable		
	Drought stress (D)	Genotype (G)	D×G
Root dry weight	1.421**	4.169**	0.840**
Shoot dry weight	1.975**	0.031ns	0.023ns
Root proline content	459.962**	1489.761**	126.741**
Shoot proline content	235.458**	587.548**	97.254**
Root GPx	14.289**	399.674**	1.299**
Shoot GPx	0.997**	78.125**	0.103**
Root APx	0.009**	0.004**	0.006**
Shoot APx	0.034**	0.004**	0.001**
Root CAT	0.009**	0.024**	0.026**
Shoot CAT	0.004**	0.034**	0.021**

Numbers represent F-values at 5% level. \* and \*\*significant at p<0.05 and p<0.01; ns: Non-significant. GPx: The guaiacol peroxidase; APx: Ascorbate peroxidase, CAT: Catalase

Table 2: The interaction effects of drought stress and varieties on canola antioxidant enzymes activity

Genotype	Drought stress (Mpa)	Gpx (Mmolmin H <sub>2</sub> O <sub>2</sub> /g/FW)		APx (µmolmin H <sub>2</sub> O <sub>2</sub> /g/FW)		CAT. (µmolmin H <sub>2</sub> O <sub>2</sub> /g/FW)	
		Root	Shoot	Root	Shoot	Root	Shoot
Okapi	Control	13.04c	4.11d	0.150a	0.067d	2.67bc	0.73bc
	-0.5	13.89a	4.24c	0.07c	0.075d	3.28ab	1.16ab
	-1	13.29b	4.76b	0.037d	0.140c	3.39ab	1.815a
	-1.5	10.02d	5.11a	0.027d	0.187a	3.81a	1.65a
RGS	Control	5.95f	1.21g	0.115b	0.035e	2.12c	0.56c
	-0.5	4.05g	1.25g	0.065c	0.028e	2.79bc	1.16abc
	-1	5.95f	1.55f	0.075c	0.145c	3.13bc	1.62a
	-1.5	6.01e	1.72e	0.117b	0.170b	2.65bc	1.08abc

Means within each column followed by the same letter are not statistically different at 0.05

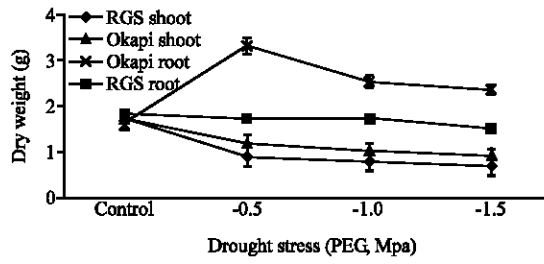


Fig. 1: Change of Dry Weight (DW) of root and shoot canola genotypes under drought stress

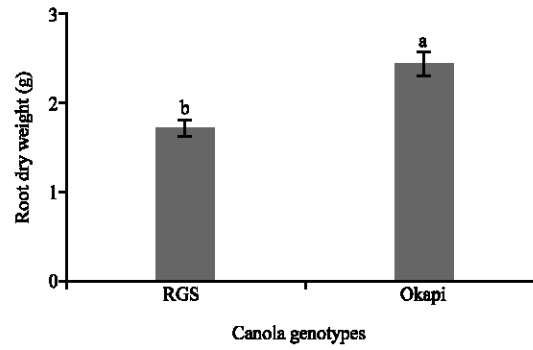


Fig. 2: Root dry weight of canola genotypes

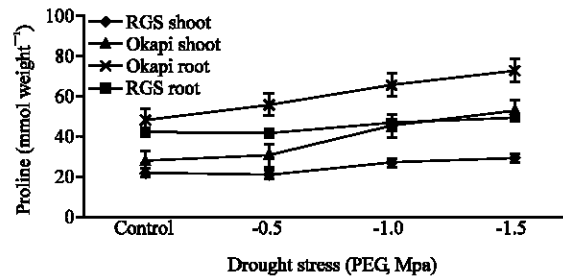


Fig. 3: Effect of drought stress on changes in root and shoot proline content of canola genotypes

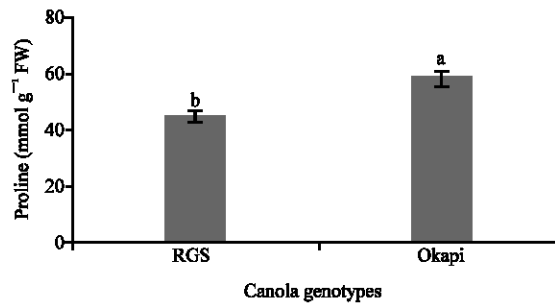


Fig. 4: Proline content under canola genotypes

of canola plants and this effect was particularly significant at high level of stress. The growth of shoot canola seedling was decreased by drought levels of -0.5 Mpa and above; this decrease was severe at -1.5 Mpa, whereas a level of -1 Mpa was lethal to the plants (Fig. 1). At all levels of drought, the shoot dry weight decrease more and this showed the sensitivity of shoot than root to drought stress.

The root dry matter in the Okapi genotype was higher than in the RGS genotype (Fig. 2). This study showed that canola genotypes differ in their capacity to utilize water content under drought stress. At higher water availability, Okapi also had the greatest root dry weight but there were no differences in shoot growth of the two canola genotypes. When water availability was low, the canola RGS genotype achieved the lowest shoot growth and Okapi the highest root growth, respectively. Okapi was characterized by lower water content concentrations in the roots than the RGS canola genotypes which suggest that this genotype can achieve high growth rates at low plant drought stress content (Fig. 1-2). The interaction of genotypes and drought stress on dependent variable of Dry Weight (DW) showed the highest significant reduction of RGS shoot among the genotypes tested in drought treatment compared to the unstressed plants.

### **Proline Content**

There were highly significant differences for root and shoot proline content for two factors (drought and genotype) studied. In this study, proline content in root was substantially higher than in shoot genotypes. Proline content increased in root than shoot significantly when exposed to drought stress level (Fig. 3). The content of proline was higher in the Okapi genotype than in the RGS genotype (Fig. 4). With a drought concentration of -0.5 Mpa treatment in the nutrient solution, the proline concentration in root and shoot in canola genotypes increased to twofold values compared to the control treatment (Fig. 3) and drought stress had a significant effect on it (Table 1). Among the genotypes, Okapi had the maximum concentration of proline in root and shoot than RGS in all drought treatment (Table 1, Fig. 3-4).

### **Antioxidant Enzymes Activity**

Water deficit increased CAT activity about 1.26 multiple more than the control plants and Apx activity increased in shoot of stressed canola plants about at least 1.98 multiple higher than the control plants, as shown in Table 2. Moderate stress (-1 Mpa) notably increased the specific GAX peroxidase and CAT activities in the canola plants. In contrast, these antioxidant enzymes were increased under high stress conditions, particularly changed in the plants treated with high level of stress (Table 2). By increasing drought levels from 0 to -1.5 Mpa, the activity of two antioxidant enzymes (APX and CAT) in shoot and root increased but GPX in two genotypes decreased. The enzymatic activity (APx and GAx) of shoot and root plants treated in combination with drought stress was increased by all drought levels, as shown in Table 2. The result showed that canola genotypes in case of antioxidant enzyme activities responses, in which Okapi genotype had more Guaiacol peroxidase activity than RGS genotype. The enzymatic activity (APx and CAT) of canola genotypes were different and Okapi genotype (as tolerant) in shoot and roots organs had the most enzymatic activity (Table 2).

### **Correlations Coefficients**

Correlation coefficients among canola seedling traits and antioxidant enzymes activity shown in Table 3. In this study, results showed a negative relationship between root dry weight and the shoot dry weight ( $r^2 = -0.44^{**}$ ), GPx in shoot ( $r^2 = -0.576^{***}$ ) and GPx in

Table 3: Correlation coefficients among canola seedling traits and antioxidant enzymes activity

Attribute	Root dry weight	Shoot dry weight	Proline	Gpx shoot	Gpx root	Apx. root	Apx. shoot	Cat. shoot
Shoot dry weight	-0.44**							
Proline	-0.329	-0.247						
GPx Shoot	-0.576***	0.108	0.741***					
GPx Root	-0.672***	-0.091	0.758***	0.965***				
APx. Root	-0.196	0.541***	-0.600***	-0.383*	-0.245			
APx. Shoot	-0.088	-0.238	0.654***	0.367*	0.369*	-0.369*		
Cat. Shoot	0.090	-0.238*	0.328*	0.326	0.232	-0.607***	0.393*	
Cat. Root	-0.010	-0.226	0.426**	0.409***	0.490	-0.530***	0.468***	0.531***

\*, \*\*and \*\*\*significant at the 5, 1 and 0.01 % levels of probability, respectively

canola root ( $r^2 = -0.672^{***}$ ). Additionally, we found a strong negative correlation between proline accumulation and Apx root ( $r^2 = -0.600^{***}$ ) under stress. The Proline content was significantly correlated with Gpx. shoot content ( $r^2 = 0.741^{***}$ ), Gpx root activity ( $r^2 = 0.758^{***}$ ) and APx. shoot ( $r^2 = 0.654^{***}$ ) of the plants.

## DISCUSSION

Drought is a major factor in reducing the growth and productivity of plants and involves different responses: dehydration of the cells as a result of the low water potential and nutritional imbalance caused by reducing of nutrition absorb elements by unavailable moisture in the uptake and translocation of essential nutrients (Ehsanpour and Fatahian, 2003; Zhu, 2002). The physiological and biochemical responses of *B. Napus* genotypes were investigated under drought stress. The shoot and root dry weight of each genotype were significantly affected by stress (Fig. 1-2). Shoot and root and dry weight of stressed plants was reduced compared to unstressed plants. There are several reports to show contradictory results on the effect of drought and salt stress on biomass accumulation (Hsu *et al.*, 2003). Some studies have reported that biomass accumulation increased (Demiral and Turkan, 2004) while others have found that it decreased (Ali and Komatsu, 2006) or remained unchanged (Zhu, 2002) during stress condition.

The increase in drought stress, increased proline concentration in both root and shoot tissues of canola genotypes (Fig. 3-4). The accumulation of free proline is a common response to a wide range of biotic and abiotic stresses (Bohnert *et al.*, 1995). A positive correlation between the proline content and salt-stress tolerance has been previously observed in a wide range of plant species (Bohnert *et al.*, 1995). Many studies have suggested that proline is involved in intracellular osmotic adjustments between the cytoplasm and vacuole. It has also been proposed that proline can stabilize the cellular structure and scavenge free radicals or act as a storage compound for carbon and nitrogen to allow recovery of lipids from stress (Delauney and Verma, 1993). The synthesis, accumulation and degradation of proline in plants are highly regulated in response to environmental changes. When the synthesis of proline is induced by osmotic stress, the P5CS gene has a role as a rate-limiting enzyme in the salt-stress pathway in plants. A correlation between levels of transcription of the P5CS gene and proline content has been shown in a number of plants. This result suggests that up to a certain level of drought stress (-1 Mpa) treatment, canola plants can produce higher amount of proline in protecting cells against drought stress. However, under control and almost low stress condition (up to -0.5 Mpa of drought) proline content was not increased (Fig. 4), which might be the possible reason of not a success of intracellular osmotic adjustments, thus resulting unreduced in plant growth and decrease of growth plantlet. This has been previously observed by findings of Bohnert *et al.* (1995).



The mechanisms of Pro action are not fully understood, but it has been suggested that in addition to its role as an osmolyte for osmotic adjustment, Pro contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). The variation in drought tolerance between the Okapi and the genotypes' of RGS could be due to differences in the level of soluble components e.i. proline content and thus drought tolerance may be partly attributed to an increased level of solutes, which protect and support cellular structures under stress (Nojavan and Khorshidi, 2006).

Water deficit could cause oxidative damage. Therefore, plant cells need different mechanisms, which enable the detoxification of excess ROS and keep the balance of the formation and removal of ROS. The increased activities of CAT, APx and GPx, detected in this study are presumed to limit cellular damage and enhance the plant's antioxidative capacity to defend stress. Some proteins, a part of the plant antioxidant system, are rapidly activated and up regulated in response to oxidative stress generated by drought stress in canola seedling, including, ascorbate, glutathione (Bhardwaj *et al.*, 2007). The most important reducing substrate for H<sub>2</sub>O<sub>2</sub> detoxification in plant cells is ascorbate. Ascorbate peroxidase reduces H<sub>2</sub>O<sub>2</sub> to water, with the concomitant generation of monodehydroascorbate (Aebi, 1984). Thioredoxin peroxidase is a part of the thioredoxin antioxidant system, acting after the oxidative stress is generated in the electron transport chain through salt exposure and inducing redox changes in different compartments (Delauney and Verma, 1993). It has been indicated that drought stress affects the physiology and biochemistry of plant cells under in vivo and in vitro conditions. In this context, increased proline and activities of CAT, APx, GPx has been reported in plants grown under stress (Hoque *et al.*, 2007a). Drought stimulated the accumulation of the ROS including H<sub>2</sub>O<sub>2</sub> in plants cells. The metabolism of H<sub>2</sub>O<sub>2</sub> is dependent on various functionally interrelated antioxidant enzymes such as catalases and peroxidases. These enzymes are involved in elimination of H<sub>2</sub>O<sub>2</sub> from stressed cells (Kim *et al.*, 2005a, b; Nojavan and Khorshidi, 2006).

Present results demonstrated that catalase (CAT) and ascorbate peroxidase (APX) activities were significantly increased (Table 2) and guaiacal peroxidase (GPX) decreased in shoot and root of canola genotypes under drought stress. These results about guaiacal peroxidase activity were supported by the findings of Hoque *et al.* (2008). The reductions of GPX activity suggest that these enzymes were unable to completely neutralize H<sub>2</sub>O<sub>2</sub> resulted from the oxidative stress. Thus, results suggest that CAT and APX activities coordinated with SOD activity play a central protective role in the O<sub>2</sub>-and H<sub>2</sub>O<sub>2</sub> scavenging process which has been reported similar by findings (Hoque *et al.* 2007b). When the activity values of the H<sub>2</sub>O<sub>2</sub> scavenging enzymes were compared (Table 2) it was observed that CAT and APX had a much higher scavenging activity than GPX in shoot and root of stressed canola plants.

It has been demonstrated that drought can limit growth, fresh and dry weight of canola genotypes and lead to an increase in ROS generation, which are responsible for a secondary oxidative stress that can damage cellular structure and metabolism. The efficiency of the latter process is dependent upon the plant antioxidant defense mechanisms. The obtained results showed that the differences in the antioxidative enzyme activities of shoot and root may, at least in part, explain the greater tolerance of Okapi to drought stress when compared to other genotype. RGS however had the highest decreasing in fresh and dry weight but it showed the highest CAT activity at high drought level in root, the highest activity of GPx I in drought treatments and the highest proline content in shoot when subjected to drought stress. It can be deduced that antioxidant activity especially CAT and APX activities play a protective role to drought tolerance in canola genotypes. Among the genotypes, Okapi genotype used these two mechanisms to drought tolerance.

## CONCLUSION

As conclusion, the different organs of canola plant treated (roots or leaves) are considered in drought, the accumulation and remobilization of the major solutes contributing to the osmotic adjustment were shown to be affected by shoot and root tissue senescence. The later growth stages, of both organs seem to be more protected but sometimes more reactive toward the application of drought stress. Results of this research suggest also that osmolytes accumulation such as proline and antioxidant enzymes activity as GPx, CAT and APX could be a part of the drought resistance mechanisms developed by canola tolerant genotype and could be exploited in breeding programs for improved drought stress tolerance.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. Tahmasebi and Mr. Kazemi for their assistance in the laboratory and field, respectively. In addition Dr. Karami and Dr. Habibi are thanked for their helpful correspondence.

## REFERENCES

- Aebi, H., 1984. Catalase *in vitro*. *Methods Enzymol.*, 105: 121-126.
- Ali, G.M. and S. Komatsu, 2006. Proteomic analysis of rice leaf sheath during drought stress. *J. Proteome Res.*, 5: 396-403.
- Apel, K. and H. Hirt, 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Ann. Rev. Plant Biol.*, 55: 373-399.
- Ashraf, M. and M.R. Foolad, 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59: 206-216.
- Askari, H., J. Edqvist, M. Hajheidari, M. Kafi and G.H. Salekdeh, 2006. Effects of salinity levels on proteome of *Suaeda aegyptiaca* leaves. *Proteomics*, 6: 2542-2554.
- Bates, L.S., R.P. Walderren and I.D. Teare, 1973. Rapid determination of free proline for water-studies. *Plant Soil*, 39: 205-207.
- Bhardwaj, R., N. Arora, P. Sharma and H.K. Arora, 2007. Effects of 28-homobrassinolide on seedling growth, lipid peroxidation and antioxidative enzyme activities under nickel stress in seedlings of *Zea mays* L. *Asian J. Plant Sci.*, 6: 765-772.
- Bohnert, H.J., D.E. Nelson and R.G. Jensen, 1995. Adaptations to environmental stresses. *Plant Cell*, 7: 1099-1111.
- Chance, B. and A.C. Maehly, 1955. Assay of catalase and peroxidases. *Methods Enzymol.*, 2: 764-765.
- Chen, C. and M.B. Dickman, 2005. Proline suppresses sap otopsisin the fungal pathogen *Colletotrichum trifolii*. *Proc. Natl. Acad. Sci. USA.*, 102: 3459-3464.
- Costa, P., N. Bahrman, J.M. Frigerio, A. Kremer and C. Plomion, 1998. Water-deficit-responsive proteins in maritime pine. *Plant Mol. Biol.*, 38: 587-596.
- Delauney, A.J. and D.P.S. Verma, 1993. Proline biosynthesis and osmoregulation in plants. *Plant J.*, 4: 215-223.
- Demiral, T. and I. Turkan, 2004. Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment. *J. Plant Physiol.*, 161: 1089-1100.
- Dixon, D.P., M. Skipsey, N.M. Grundy and R. Edwards, 2005. Stress-induced protein sglutathionylation in *Arabidopsis*. *Plant Physiol.*, 138: 2233-2244.

- Ehsanpour, A.A. and N. Fatahian, 2003. Effect of salt and proline on *Medicago sativa* callus. *Plant Cell Tissue Organ. Cult.*, 73: 53-56.
- Ferreira, S., K. Hjemo, M. Larsen, G. Wingsle and P. Larsen *et al.*, 2006. Proteome profiling of *populus euphratica* Oliv. upon heat stress. *Ann. Bot.*, 98: 361-377.
- Gazanchian, A., M. Hajheidari, N.K. Sima and G.H. Salekdeh, 2007. Proteomeresponse of *elymus elongatum* to severe water stress and recovery. *J. Exp. Bot.*, 58: 291-300.
- Hajheidari, M., A. Eivazi, B.B. Buchanan, J.H. Wong, I. Majidi and G.H. Salekdeh, 2007. Proteomics uncovers a role for redox in drought tolerance in wheat. *J. Proteome Res.*, 6: 1451-1460.
- Hajheidari, M., M. Abdollahian-Noghabi, H. Askari, M. Heidari and S.Y. Sadeghian *et al.*, 2005. Proteome analysis of sugar beet leaves under drought stress. *Proteomics*, 5: 950-960.
- Hoque, M.A., E. Okuma, M.N.A. Banu, Y. Nakamura, Y. Shimoishi and Y. Murata, 2007a. Exogenous proline mitigates the detrimental effects of salts more than exogenous betaine by increasing antioxidant enzyme activities. *J. Plant Physiol.*, 164: 553-561.
- Hoque, M.A., M.N.A. Banu, E. Okuma, K. Amako, Y. Nakamura and Y. Shimoishi, 2007b. Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. *J. Plant Physiol.*, 164: 1457-1468.
- Hoque, M.A., M.N.A. Banu, Y. Nakamura, Y. Shimoishi and Y. Murata, 2008. Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *J. Plant Physiol.*, 165: 813-824.
- Hsu, S.Y., Y.T. Hsu and C.H. Kao, 2003. The effect of polyethylene glycol on proline accumulation in rice leaves. *Biol. Plant*, 46: 73-78.
- Khedr, A.A., M.A. Abbas, A.A. Abdel Wahid, W.P. Quick and G.M. Abogadallah, 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L. to salt-stress. *J. Exp. Bot.*, 54: 2553-2562.
- Kim, D.W., R. Rakwal, G.K. Agrawal, Y.H. Jung and J. Shibato *et al.*, 2005a. A hydroponic rice seedling culture model system for investigating proteome of salt stress in rice leaf. *Electrophoresis*, 26: 4521-4539.
- Kim, S., J. Lim, M. Park, Y. Kim, T. Park, Y. Seo and S. Yun, 2005b. Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. *J. Biochem. Mol. Biol.*, 38: 218-224.
- Knorzer, O.C., J. Durner and P. Boger, 1996. Alterations in the antioxidative system of suspension-cultured soybean cells (*Glycine max*) induced by oxidative stress. *Physiol. Plant.*, 97: 388-396.
- Magne, C. and F. Larher, 1992. High sugar content of extracts interferes with colorimetric determination of amino-acids and free proline. *Anal. Biochem.*, 200: 115-118.
- Michel, B.E. and M.R. Kaufmann, 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiol.*, 51: 914-916.
- Molinari, H.B.C., C.J. Marur, E. Daros, M.K.F. Campos and J.F.R.P. Carvalho *et al.*, 2007. Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum* sp.): Osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiol. Plant.*, 130: 218-229.
- Mudgal, V., N. Madaan and A. Mudgal, 2010. Biochemical mechanisms of salt tolerance in plants: A review. *Int. J. Bot.*, 6: 136-143.
- Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant Cell Physiol.*, 22: 867-880.

- Ndimba, B.K., S. Chivasa, W.J. Simon and A.R. Slabas, 2005. Identification of arabidopsis salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. *Proteomics*, 5: 4185-4196.
- Nojavan, A.M. and M. Khorshidi, 2006. An investigation of vanillin imposed oxidative stress in corn (*Zea mays* L.) and the activities of antioxidative enzymes. *Pak. J. Biol. Sci.*, 9: 34-38.
- Okuma, E., Y. Murakami, Y. Shimoishi, M. Tada and Y. Murata, 2004. Effects of exogenous sap placcation of proline and betaine on the growth of tobacco cultured cells under salineconditions. *Soil Sci. Plant Nutr.*, 50: 1301-1305.
- Omid, H., Z. Tahmasebi, H. Torabi and M. Miransari, 2008. Soil enzymatic activities and available P and Zn as affected by tillage practices, canola (*Brassica napus* L.) cultivars and planting date. *Eur. J. Soil Biol.*, 44: 443-450.
- Omid, H., Z. Tahmasebi, H.A.N. Badi, H. Torabi and M. Miransari, 2010. Fatty acid composition of canola (*Brassica napus* L.), as affected by agronomical, genotypic and environmental parameters. *Comptes Rendus Biologies*, 333: 248-254.
- Ozden, M., U. Demirel and A. Kahraman, 2009. Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H<sub>2</sub>O<sub>2</sub>. *Sci. Hort.*, 119: 163-168.
- Park, E.J., Z. Jeknic and T.H.H. Chen, 2006. Exogenous Application of glycinebetaine increases chilling tolerance in tomato plants. *Plant Cell Physiol.*, 47: 706-714.
- SAS Institute Inc., 1988. SAS/STAT User's Guide. 4th Edn., Version 6, Statistical Analysis Institute Inc., Cary, North Carolina.
- Schurmann, P., 2003. Redox signaling in the chloroplast: The ferredoxin/thioredoxin system. *Antioxid. Redox Signal*, 5: 69-78.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill, New York, USA., ISBN-13: 978-0070609259.
- Udomchalothorn, T., S. Maneeprasobuk, E. Bangyeekhun, P. Boon-Long and S. Chadchawan, 2009. The role of the bifunctional enzyme, fructose-6-phosphate-2-kinase/fructose-2, 6-bisphosphatase, in carbon partitioning during salt stress and salt tolerance in Rice (*Oryza sativa* L.). *Plant Sci.*, 176: 334-341.
- Yan, S.P., Z.C. Tang, W. Su and W. Sun, 2005. Proteomic analysis of salt stress-responsive proteins in rice root. *Proteomics*, 5: 235-244.
- Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Ann. J. Plant Biol.*, 53: 247-273.