

## Effect of Super Absorbent Application on Antioxidant Enzyme Activities in Canola (*Brassica napus* L.) Cultivars under Water Stress Conditions

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**Abstract: Problem statement:** Drought stress significantly limits Canola (*Brassica napus* L.) growth and crop productivity. Hence, efficient management of soil moisture and study metabolic changes which occur in response to drought is important for agricultural production of this Crop. **Approach:** For a better understanding of drought tolerance mechanisms and improving soil water content management strategies, an experiment was laid out in a randomized complete block design with factorial split arrangement with three replications. **Results:** Irrigation strategy and super absorbent application were allotted to main plots. Irrigation strategy had two levels: 80% of evaporation as control (I<sub>1</sub>), drought stress started from flowering stage (I<sub>2</sub>) Application of super absorbent had two levels: Non-application of super absorbent as control (S<sub>1</sub>), application of super absorbent with 7% concentration. Cultivars (Rgs003 (V<sub>1</sub>), Sarigol (V<sub>2</sub>), Option500 (V<sub>3</sub>), Hyola401 (V<sub>4</sub>), Hyola330 (V<sub>5</sub>), Hyola420 (V<sub>6</sub>) were allotted to sub plots. Plants under water deficit stress and application of super absorbent showed a significant increase and decrease, respectively, in SOD, CAT and GPX activities in leaves compared with control plants. In this context, plants with higher levels of antioxidants showed higher resistance to these stress conditions and higher yield and dry matter allocation to grain filling process i.e. harvest index. **Conclusion/Recommendations:** Our results suggested that drought stress leads to production of oxygen radicals, which results in increased lipid peroxidation and oxidative stress in the plant. In conclusion of present study, Application of super absorbent polymer could reserve different amounts of water in itself and so increases the soil ability of water storing and preserving and at last in water deficiency, produce plant water need and approve its growth under postanthesis water deficiency.

**Key words:** Canola, super absorbent, drought stress, agronomic characters, antioxidant enzymes

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### INTRODUCTION

Drought stress significantly limits plant growth and crop productivity. However in certain tolerant/adaptable crop plants morphological and metabolic changes occur in response to drought, which contribute towards adaptation to such unavoidable environmental constraints<sup>[8,53]</sup>. Efficient management of soil moisture is important for agricultural production in the light of scarce water resources. Soil conditioners, both natural

and synthetic, contribute significantly to provide a reservoir of soil water to plants on demand in the upper layers of the soil where the root systems normally develop. These polymeric organic materials and hydro gels apart from improving the soil physical properties, also serve as buffers against temporary drought stress and reduce the risk of plant failure during establishment<sup>[17,31]</sup>. This is achieved by means of reduction of evaporation through restricted movement of water from the sub-surface to the surface layer<sup>[41]</sup>.

Brassica oilseed species now hold the third position among the oilseed crops and are an important source of vegetable oil<sup>[5]</sup>. Drought stress invariably leads to oxidative stress in the plant cell due to higher leakage of electrons towards O<sub>2</sub> during photosynthetic and respiratory processes leading to enhancement in Reactive Oxygen Species (ROS) generation<sup>[3]</sup>. The ROS such as O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub> and OH radicals, can directly attack membrane lipids, inactive metabolic enzymes and damage the nucleic acids leading to cell death<sup>[37]</sup>. The reaction of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development<sup>[14]</sup>. Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, glutathione reductase<sup>[32]</sup>). The degree to which the activities of antioxidant enzymes and the amount of antioxidants are elevated under drought stress is extremely variable among several plant species<sup>[53]</sup> and even between the two cultivars of the same species<sup>[7]</sup>. However, under conditions of environmental stress, production of ROS can increase and endogenous protective activity may then become inadequate. Various associations between water stress and endogenous levels of water-soluble antioxidants have been described<sup>[53]</sup>. Environmental stresses including drought and temperature affect nearly every aspect of the physiology and biochemistry of plants and significantly diminish yield. Many arid and semi-arid regions in the world contain soils and water resources that are too saline for most of the common economic crops, which affect plants through osmotic effects, ion specific effects and oxidative stress<sup>[39,43]</sup>. Much of the injury to plants exposed to stress is connected with oxidative damage at the cellular level<sup>[22]</sup>. If there is a serious imbalance in any cell compartment between the production of Reactive Oxygen Species (ROS) and antioxidant defense, oxidative stress and damage occurs<sup>[37]</sup>. Even under normal growth conditions, low amounts of ROS such as superoxide radical (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH.) and singlet oxygen (1 O<sub>2</sub>) are metabolic byproducts of plant cells<sup>[15]</sup>. Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic<sup>[44,18]</sup>. When ROS increases, chain reactions start, in which Superoxide Dismutase (SOD) catalyzes the dismutation of O<sub>2</sub><sup>-</sup> radicals to molecular O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub><sup>[36]</sup>. The H<sub>2</sub>O<sub>2</sub> is then detoxified in the ascorbate-glutathione cycle<sup>[4,37]</sup> (which involves the oxidation and re-reduction of ascorbate and glutathione through the Ascorbate Peroxidase (APX) and Glutathione Reductase (GR)

action<sup>[21,38]</sup>. Drought stress induces cellular accumulation of ROS which can damage membrane lipids, proteins and nucleic acids<sup>[1,7,27-29,35]</sup>. A correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in some plant species<sup>[19,25,28]</sup>. Several studies have pointed out that drought-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to drought treatment, whereas drought-sensitive species failed to do so<sup>[18,49]</sup>. To be able to endure oxidative damage under (unfavorable conditions such as high/low temperatures, water deficit and salinity, plants must possess efficient antioxidant system<sup>[46]</sup>. In addition, plants are subjected to the interaction of two or more environmental stress factors under natural conditions and many studies have been carried out to study the effects of these stress factors on plant metabolism separately. Therefore, the aim of the study was to investigate the effect of long-term drought stress and temperature interaction on antioxidant enzyme activities (APX, GR and POD) in the canola plants. Therefore, the primary objective of the present investigation was to examine the effect of drought stress on the activities of different antioxidant enzymes and biochemical exchanges in leaves of canola. The research was aimed also whether a super absorbent polymer supply to plant might be a strategy for increasing the drought tolerance.

## MATERIALS AND METHODS

The experiment was carried out in Seed and Plant Improvement Institute, Kraraj, Iran during 2007-2008. The site is located at 35°59'N latitude, 50°75'E longitudes and an altitude of 151 m above the sea level. This region has a semi-arid climate (354 mm rainfall yearly). The soil of experimental site was clay loam with a clay type of montmorillonite, low in nitrogen (0.06-0.07%), low in organic matter (0.56-0.60) and alkaline in reaction with a PH of 7.9 and Ec = 0.66 dS m<sup>-1</sup>. The soil texture is sand loam with 10% neutralizing substances. Experiment was laid out in a randomized complete block design with factorial split arrangement with tree replications. Irrigation strategy and super absorbent application were allotted to main plots. Irrigation strategy had two levels: 80% of evaporation as control (I<sub>1</sub>), drought stress started from flowering stage (I<sub>2</sub>) Application of super absorbent had two levels: Non-application of super absorbent as control (S<sub>1</sub>), application of super absorbent with 7% concentration. cultivars (Rgs003 (V<sub>1</sub>), Sarigol (V<sub>2</sub>), Option500 (V<sub>3</sub>), Hyola401 (V<sub>4</sub>), Hyola330 (V<sub>5</sub>), Hyola420 (V<sub>6</sub>) were allotted to sub plots. Then 7% concentration of super

absorbent for each plot was noticed. After calculation, super absorbents were poured in necessary amount on each pail separately and sufficient water was applied. Then 30 min was left till super absorbents absorb water completely and then were poured on the whole plot monotonously and accurately. After settling each plot was covered with soil. Irrigation of control group was done with seven days apart. Measured parameters where Grain yield, harvest index, the amount of biochemical characters (super oxide dismutase, catalase and glutathione per oxidase).

**Sampling:** After drought stress treatment, three leaves of each plant were removed. The samples were washed and then frozen in liquid N<sub>2</sub> and then stored at -80°C pending biochemical analysis.

**Preparation of extracts:** Leaf sample was homogenized in a mortar and pestle with 3 mL ice-cold extraction buffer (25 mM sodium phosphate, pH 7.8). The homogenate was centrifuged at 18000 g for 30 min at 48°C and then supernatant was filtered through paper. The supernatant fraction was used as a crude extract for the assay of enzyme activity. All operations were carried out at 48°C.

**Assay of antioxidant enzymes:** Catalase activity was estimated by the method of Cakmak and Horst<sup>[13]</sup>. The reaction mixture contained 100 crude enzyme extract, 500 µL 10 mM H<sub>2</sub>O<sub>2</sub> and 1400 µL 25 mM sodium phosphate buffer. The decrease in the absorbance at 240 nm was recorded for 1 min by spectrophotometer, model Cintra 6 GBC (GBC Scientific Equipment, Dandenong, Victoria, Australia). CAT activity of the extract was expressed as CAT units per milligram of PROT. Superoxide dismutase activity was determined with the reaction mixture contained 100 µL 1 µM riboflavin, 100 µL 12 mM L-methionine, 100 µL 0.1 mM EDTA (pH 7.8), 100 µL 50 mM Na<sub>2</sub> CO<sub>3</sub> (pH 10.2) and 100 µL 75 µM Nitroblue Tetrazolium (NBT) in 2300 µL 25 mM sodium phosphate buffer (pH 6.8), 200 µL crude enzyme extract in a final volume of 3 mL. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit by 50% the photo reduction of NBT to blue formazan. The SOD activity of the extract was expressed as SOD units per milligram of PROT. Peroxidase activity was

determined by the oxidation of guaiacol in the presence of H<sub>2</sub>O<sub>2</sub>. The increase in absorbance was recorded at 470 nm<sup>[24]</sup>. The reaction mixture contained 100 µL crude enzyme, 500 µL H<sub>2</sub>O<sub>2</sub> 5 mM, 500 µL guaiacol 28 mM and 1900 µL potassium phosphate buffer 60 mM (pH 6.1). POX activity of the extract was expressed as POX units per mg.

**Statistical analysis:** All data were analyzed using SAS software Each treatment was analyzed in three replication. When ANOVA showed significant treatment effects. Duncan's multiple range test was applied to compare the means at  $p < 0.05$ <sup>[54]</sup>.

## RESULTS

The results showed that water deficit stress affected all parameters measured; the effect of three-way interaction between irrigation × super absorbent × varieties was significant for all variables except SOD and GPX activities in both years (Table 1).

Results showed a significant difference between irrigation treatments, concentration of super absorbent and varieties studied of yielding and measured biochemical characteristic. As it was shown, water deficit stress decreases yield and its components, increases biochemical characters and in other hand, using 7% super absorbent in field situation increases agricultural characters, also field results show that in water deficit stress and non application of super absorbent, the best record in this exam belongs to Hyola330 because antioxidant enzymes increased in this condition in Hyola330 variety and the most sensitive record was Sarigol variety because antioxidant enzymes decrease in this condition. Plants under water deficit stress showed decrease in yield and harvest index parameters.

Also results three-way interaction between irrigation × super absorbent × variety show that in water deficit stress and application of super absorbent could cause of improvement in yield and harvest index (Table 2). However, results showed that the highest yield and harvest index were obtained in Option500 variety. On the other hand study of material effects of interaction irrigation with polymer showed that in both conditions (normal and stress) this polymer has increased yield rate in every variety but yield and harvest index was increased in 2008 year. Natural rainfall varied between the 2007 and 2008 seasons (Total rainfall during the growing season was 149.1 mm in 2007 and 186.6 mm in 2008). In addition to greater rainfall in 2007, there was also a more uniform rain distribution compared to 2008.

Table 1: Analysis of variance for experimental triets

Treatment	df	MS				
		Yield	Harvest index	Superoxide dismutase enzyme	Catalase enzyme	Peroxidase glutathione enzyme
Year	1	26117465.78***	142.291000***	470367.361***	1254.222ns	16.660ns
Error	4	530869.0300	0.000100	9200.174	542.138	26.759
Irrigation	1	63670159.41***	146.872000***	7592780.250***	22199.013***	1993.324***
Super absorbent	1	62248812.54***	137.250000***	202200.111***	922.843***	324.720***
Irrigation* super absorbent	1	12858260.46***	1223.373000***	215605.444***	1175.346***	113.777***
Year* irrigation	1	2102910.85***	0.187000***	4.694ns	3.737ns	3.770***
Year* super absorbent	1	2069593.94***	0.175000***	2240.444ns	2.325ns	0.691ns
Year* super absorbent * irrigation	1	419979.60***	1.559000***	18.778ns	10.112ns	0.140ns
Error	12	28979.07***	0.000100	6081.961	3.337	0.354
Variety	5	6499892.94***	2850.732310***	654781.611***	751.127***	91.533***
Irrigation* variety	5	1388768.81***	77.728760***	527706.100***	423.493***	52.024***
Super absorbent* variety	5	1381621.11***	73.261030***	9602.128ns	434.606***	2.813***
Year* variety	5	225859.71***	3.637130***	7494.944ns	8.302***	0.025ns
Irrigation* super absorbent* variety	5	4582683.29***	135.090230***	13554.728ns	144.352***	0.760***
Year* variety* irrigation	5	48946.61***	0.098980***	8953.144ns	6.112***	0.228***
Year* variety* super absorbent	5	50239.94***	0.093470***	8499.794ns	7.755***	0.362***
Year*variety*superabsorbent* irrigation	5	159016.29***	0.172360***	8222.661ns	6.330***	0.040ns
Error	80	3188.80	0.000100	7042.930	1.503	0.042
Total	143					
CV		2.4200	0.038000	5.480	1.190	1.040

ns, \*and \*\*\*: Non significant and significant at the 5 and 1% levels of probability, respectively

Table 2: Effects of Irrigation Regimes (IR) and super absorbent concentration (SU) and variety (VA) on Yield and harvest index and antioxidant enzymes in canola in 2007 and 2008

IR	SU	VA	Yield	Yield	Harvest index	Harvest index	CAT	CAT
			(Kg ha <sup>-1</sup> ) 2007	(Kg ha <sup>-1</sup> ) 2008	(%) 2007	(%) 2008	(u mg <sup>-1</sup> protein) 2008	(u mg <sup>-1</sup> protein) 2008
I <sub>1</sub>	S <sub>1</sub>	V <sub>1</sub>	1419.83d	2058.73d	11.328f	12.167f	94.966700d	88.79670c
		V <sub>2</sub>	1312.13e	1902.60e	12.885e	13.839e	96.353300d	89.81000c
		V <sub>3</sub>	2467.50a	3577.90a	26.250d	28.195d	103.356700b	96.84670b
		V <sub>4</sub>	1341.47e	1945.13e	29.025c	31.175c	101.303300c	95.74670b
		V <sub>5</sub>	1889.70b	2728.07b	35.739a	38.386a	108.653300a	101.76000a
		V <sub>6</sub>	1538.30c	2230.50c	29.123b	31.279b	88.530000e	82.01670d
	S <sub>2</sub>	V <sub>1</sub>	2320.60d	3364.87d	17.560e	18.860e	93.280000b	96.63330a
		V <sub>2</sub>	2065.93e	2931.87e	13.700f	14.720f	79.363300e	72.71330f
		V <sub>3</sub>	2878.67c	4174.07c	26.250d	28.190d	96.433300a	88.49000b
		V <sub>4</sub>	3632.70b	5267.43b	47.560a	51.090a	86.270000d	78.84330d
		V <sub>5</sub>	4817.40a	6985.23a	45.550b	48.930b	78.773300e	74.65330e
		V <sub>6</sub>	3642.60b	5281.77b	38.750c	41.620c	89.676700c	83.68670c
I <sub>2</sub>	S <sub>1</sub>	V <sub>1</sub>	1126.07c	1632.77c	22.116e	23.754e	120.567000c	114.65000c
		V <sub>2</sub>	644.370f	934.330f	14.956f	16.063f	101.403000e	95.45000f
		V <sub>3</sub>	832.200e	1206.70e	24.996d	26.848d	109.567000d	102.92330e
		V <sub>4</sub>	1219.10b	1767.70b	33.686b	36.182b	127.507000b	121.53670b
		V <sub>5</sub>	1527.60a	2215.00a	41.140a	44.188a	134.387000a	127.78330a
		V <sub>6</sub>	1028.17d	1490.83d	29.496c	31.680c	113.197000d	108.5900d
	S <sub>2</sub>	V <sub>1</sub>	1417.07d	2054.77d	11.320f	12.160f	122.476700b	116.83330a
		V <sub>2</sub>	1298.33e	1882.57e	12.790e	13.730e	108.660000e	102.88670e
		V <sub>3</sub>	2454.23a	3558.63a	26.160d	28.090d	115.540000d	108.78000d
		V <sub>4</sub>	1321.47e	1916.13e	28.750c	30.880c	120.403300bc	114.02330b
		V <sub>5</sub>	1880.40b	2726.60b	36.150a	38.830a	126.430000a	118.82000a
		V <sub>6</sub>	1519.40c	2203.13c	28.800b	30.930b	118.676700c	111.84000c

For a given means within each column of each section followed by the same letter are not significantly different (p<0.05)

Plants under water deficit stress showed a significant increase in SOD, CAT and GPX activities in leaves compared with control plants (Table 2). In water deficit stress conditions Hyola330 with product of

Antioxidant enzymes could increase yield and harvest index. Application of super absorbent to stressed plants decreased the Antioxidant enzymes activity in the leaves, that this conditions Option500 increased product

of Antioxidant enzymes. In this context, plants with higher levels of antioxidants, either constitutive or induced, have been reported to possess SOD resistance to these stress conditions and higher yield and dry matter allocation to SODain filling process i.e., harvest index (Table 1 and 2)<sup>[19,50]</sup>. H<sub>2</sub>O<sub>2</sub> can be removed using the ascorbate-glutathione cycle [ascorbic acid (ASA)-GSH cycle] which APX and SOD are the key enzymes in this cycle<sup>[38]</sup>. In the present study, water stress and lack of super absorbent led to a significant increase in the APX compared to the respective controls, although there were some variations among canola cultivars and super absorbent (Table 1 and 2). The diverse responses of the APX and SOD enzyme activities in the plants subjected to saline conditions suggest that oxidative stress is an important component of drought stress<sup>[51]</sup>. These results are in agreement with those of Stepien and Klobus<sup>[51]</sup>, who have propounded that the APX and SOD and CAT action suggests that the more active ascorbate-glutathione cycle may be related to the development of relatively higher drought tolerance in maize. The constitutive and the drought-induced APX and SOD activities were remarkably higher in the cultivars SOD at 35 compared to 25°C. These results may point out that the lack of superabsorbent provokes antioxidant enzyme responses (Table 2 and 3). Our results are consistent with other parameters of these six canola cultivars i.e., stress

caused a decline in the K<sup>+</sup>/Na<sup>+</sup> ratio, plant height, fresh and dry biomass of the shoot and an increase in the relative leakage ratio and the contents of proline and Na<sup>+</sup><sup>[16]</sup>. POD activity decreased considerably upon drought treatments under both superabsorbents levels in all cultivars (Table 2). Drought and superabsorbent treatment increased the activity in this canola cultivar by 1.5 fold. Conversely, Ben Amor *et al.*<sup>[7]</sup> found that peroxidase activity in the *Cakile maritime* increased SODadually with time and with increasing Drought concentrations up to 400 mmol L<sup>-1</sup>, whereas POD unexpectedly started to decrease in plants treated with 400 mmol L<sup>-1</sup> Drought.. Our results suggest that drought stress directly or indirectly leads to production of oxygen radicals, which results in increased lipid peroxidation and oxidative stress in the plant. Drought stress may also lead to stomata closure, which reduces CO<sub>2</sub> availability in the leaves and inhibits carbon fixation. This exposes the chloroplast to excessive excitation energy, which in turn could increase the generation of free radicals and induce oxidative stress<sup>[26]</sup>. The canola plant which is considered moderately drought tolerant<sup>[43]</sup> might have inadequate ROS scavenging system, in addition to other tolerance mechanisms, to cope with stress. The increase in SOD activity was reported in tolerance basmati rice variety<sup>[52]</sup>. In our study, super absorbent polymer decreased the activity of these enzymes maybe by elimination of free radicals.

Table 3: Effects of Irrigation Regimes (IR) and super absorbent concentration (SU) and variety (VA) on yield and harvest index and antioxidant enzymes in canola in 2007 and 2008

IR	SU	VA	SOD (u mg <sup>-1</sup> protein) 2007	SOD (u mg <sup>-1</sup> protein) 2008	GPX (u mg <sup>-1</sup> protein) 2007	GPX (u mg <sup>-1</sup> protein) 2008
I <sub>1</sub>	S <sub>1</sub>	V <sub>1</sub>	1363.00bc	1260.000c	16.31330c	15.93670c
		V <sub>2</sub>	1340.33cd	1235.000d	16.44670c	15.52330c
		V <sub>3</sub>	1313.67de	1203.333e	15.93670c	15.49670c
		V <sub>4</sub>	1382.67ab	1271.000b	17.40670b	16.87330b
		V <sub>5</sub>	1413.670a	1307.667a	18.22330a	17.82670a
		V <sub>6</sub>	1296.330e	1192.000f	17.24330b	16.56670b
	S <sub>2</sub>	V <sub>1</sub>	1313.000e	1194.000d	15.08330d	14.92667c
		V <sub>2</sub>	1395.000a	1271.000a	15.77330b	15.32000b
		V <sub>3</sub>	1387.000b	1268.000a	15.44000c	14.88000c
		V <sub>4</sub>	1393.000a	1264.000a	16.18000a	15.86333a
		V <sub>5</sub>	1348.000c	1231.333b	15.58000c	15.68667a
		V <sub>6</sub>	1331.333d	1212.333c	14.95330d	15.40000b
I <sub>2</sub>	S <sub>1</sub>	V <sub>1</sub>	1846.000b	1605.000d	26.35000c	25.76330c
		V <sub>2</sub>	1610.300b	1474.000f	22.31330f	21.48670f
		V <sub>3</sub>	1702.000b	1561.333e	23.18000e	22.46330e
		V <sub>4</sub>	1966.300b	2152.667b	29.18330b	28.14330b
		V <sub>5</sub>	2408.000a	2263.000a	32.54670a	30.93670a
		V <sub>6</sub>	1794.700b	1635.000c	24.87670d	23.17670d
	S <sub>2</sub>	V <sub>1</sub>	1657.333c	1532.000c	21.19670c	20.06667c
		V <sub>2</sub>	1429.000f	1297.333f	18.18000f	17.42667f
		V <sub>3</sub>	1508.667e	1394.000e	19.57000e	18.51000e
		V <sub>4</sub>	2096.333b	1971.333b	23.65670b	22.60667b
		V <sub>5</sub>	2211.000a	2085.000a	26.62670a	25.76667a
		V <sub>6</sub>	1562.667d	1445.667d	20.07670d	19.36333d

For a given means within each column of each section followed by the same letter are not significantly different (p<0.05)

Application of super absorbent polymer could reserve different amounts of water in itself and so increases the soil ability of water storing and preserving and at last in water deficiency, produce plant water need and approve its growth. Thus in drought stress application of super absorbent caused yield and harvest index. Results are in comparison with Padman studies<sup>[45]</sup>, based on increasing the seed yield in improved treatment with this substance. Because, for inducing high yield, adequate water is necessary, these substances result in better and more effective use of water and nutrition with increasing the available water for plant and at least increase the yield. In notification to this harvest index, that is actually the proportion of seed yield to biologic yield, with better access of plant to humidity and nutrition by super absorbent, rate of both qualities increases and at last the harvest index rate increases. The result of decrease in harvest index during stress is compatible with Turk *et al.*<sup>[51]</sup> result. They concluded that due to stress and water deficiency certainly the transmission of photosynthetic substances to shoot organs decrease and in the end yield components reduce. Indeed with reduction of these components the rate of harvest index decreases.

## DISCUSSION

Canola yield is to a very large degree a result of the interaction of nitrogen and carbon acquisition throughout the life cycle and a partitioning of these resources to seed production. Thus, effects of irrigation regimes and reservoir of soil water and micronutrients to plants on successful acquisition of these resources in different genotypes may be useful as tools for improved yield and water use efficiency. The stress treatments decrease the number of days required for canola plants to reach 50% flowering or maturity by an average of 4-7 days compared with the unstressed control. It has been reported in faba bean (*Vicia faba* L.)<sup>[40]</sup>. Acceleration of flowering and/or maturity probably contributed to reduce the impact of drought stress in canola varieties. The decrease in yield and yield components in different safflower genotypes due to water deficiency has also been reported by other researchers<sup>[33,34,54]</sup>. Anyia and Herzog<sup>[2]</sup> indicated that water deficit caused between 11 and more than 40% reduction of biomass across the genotypes of cowpea (*Vigna unguiculata* (L.) Walp.) due to decline in leaf gas exchange and leaf area. The increase in resistance to drought stress is associated with the antioxidant activity. According to these results it can be suggested that usage of super absorbent polymer can reduce the

harmful effects of ROS and improves plant resistance. Plants resort to a range of distinct acclimation strategies in response to abiotic environmental stresses such as drought, dehydration, cold, heat and excessive osmotic pressure<sup>[42]</sup>. Drought stress is an intricate phenomenon which includes osmotic stress, specific ion effect, nutrient deficiency thereby affecting various physiological and biochemical mechanisms associated with plant resistance development<sup>[46]</sup>. It has been suggested that salinity causes oxidative stress by inhibition of the CO<sub>2</sub> assimilation, exposing chloroplasts to excessive excitation energy, which in turn increases the generation of ROS from triplet chlorophyll<sup>[25]</sup>. Several researchers have suggested that drought tolerance is often correlated with a more efficient antioxidative system<sup>[6,11,19,25]</sup>. Some canola cultivars increased their enzyme activities as a consequence of stress, however, these responses might not be enough to overcome the detrimental effects of long-term stress or to allow survival of the plants as it was observed that all canola cultivars lost their vitality under the highest stressful conditions at the end of experiment. Foyer *et al.*<sup>[23]</sup> proposed that the absence of a rapid increase in the level of transcripts of the antioxidant enzymes could be related to the role of ROS in signal transduction. This difference between transcript levels and enzyme activities during Drought treatment may result from a higher turnover of these enzymes and/or an increase of their inactivation by H<sub>2</sub>O<sub>2</sub><sup>[47]</sup>. Abiotic stress, such as drought stress cause molecular damage to plant cells either directly or indirectly through the formation of ROS. In the present study, the plants exposed to drought showed a significant increase in SOD, CAT and GPX activity in the leaves. The enzymes assayed are scavengers of free radical species. SOD converts one form of ROS (O<sub>2</sub><sup>-</sup>) to another equally toxic one (H<sub>2</sub>O<sub>2</sub>). Hydrogen peroxide is converted to oxygen and water by CAT and POX, which use ascorbate as the hydrogen donor<sup>[30]</sup>. In conclusion, the results of the present study clearly showed that there was differential accumulation of H<sub>2</sub>O<sub>2</sub> as well as genotypic variations in H<sub>2</sub>O<sub>2</sub>-scavenging enzymes in canola cultivars SODown under different drought stress and high superabsorbent conditions.

## CONCLUSION

In conclusion this study has shown that application of super absorbent polymer can increase the survival capacity of canola plants under conditions of drought stress.

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