

# CONTRASTIVE RESPONSE OF *BRASSICA NAPUS* L. TO EXOGENOUS SALICYLIC ACID, SELENIUM AND SILICON SUPPLEMENTATION UNDER WATER STRESS

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**Abstract:** The present research was designed to determine the effects of exogenous salicylic acid (SA), selenium (Se) and silicon (Si) on the resistance of canola (*Brassica napus* L. cv Okapi) seedlings to salt stress. Foliar application of SA (0.1 mM) in canola plants under drought stress for 25 days exhibited a significantly positive effect on shoot dry mass and raised the levels of total chlorophyll as well as boosting the activity of superoxide dismutase (SOD) and catalase (CAT). In addition, soil application of silicon (0.35 g Na<sub>2</sub>SiO<sub>3</sub>/kg soil) had ameliorative effects on canola root growth under drought. It is concluded that SA and Si enhanced the salt tolerance of canola by protecting the cell membrane against lipid peroxidation. However, the foliar application of Se (10 mg/l) had no ameliorative effects on canola growth and antioxidant capacity under drought stress, as could be judged by accumulation of malondialdehyde (MDA).

**Key words:** Antioxidant defense system; canola; exogenous salicylic acid; drought stress; sodium selenate.

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## INTRODUCTION

Stress factors such as drought trigger common reactions in plants and lead to cellular damages mediated by reactive oxygen species (ROS). During drought stress, an abscisic acid (ABA) signal causes stomatal closure and the light-exposed, reduced photosynthesis apparatus may experience oxidative stress. Drought induces the formation ROS, through enhanced leakage of electrons to molecular oxygen (Arora et al., 2002). Extreme ROS production can cause oxidative stress, which damages plants by oxidizing photosynthetic pigments and membrane lipids (Yordanov et al., 2000).

To avoid oxidative damage, plants have a complex antioxidant system that can be enzymatically comprised by enzymes such as superoxide dismutase (SOD) and catalase (CAT). Another strategy that partially moderates the adverse effects of drought stress on plants is the nutrient supply (Kong et al., 2005). Beneficial elements for plants such as aluminum (Al), cobalt (Co), sodium (Na), selenium (Se), and silicon (Si) all have documented positive effects on plant growth and stress resistance (Epstein, 2009). Currently, numerous studies have been made with trace elements to improve the response of plants subjected to drought, as in the

case of Se (Feng et al., 2013) and silicon (Habibi and Hajiboland, 2013).

It has been reported that silicon plays a more important role in plant's water status and ion balance in plant systems to reduce the tensions of drought (Sonobe et al., 2011). In previous work, we concluded that supplementation of water-deficient pistachio plants with Si alleviates the adverse effects of drought due to its enhancement of photochemical efficiency and photosynthetic gas exchange, as well as an activation of the antioxidant defense capacity in this species (Habibi and Hajiboland, 2013).

Recent research has demonstrated that Se is not only capable of promoting growth and development of plants but can also increase the resistance and antioxidant capacity of plants subjected to various stresses. Our previous study suggests that selenium application can improve the antioxidant defense system under drought-stress conditions, and it may be recommended for arid and semiarid regions (Habibi, 2013).

**Table 1.** Some chemical properties of soil used in the experiment. The soil samples were taken before application of sodium silicate. The data given are means  $\pm$  SD of four replicates.

pH	7.80 $\pm$ 0.07
Organic matter (% w/w)	2.12 $\pm$ 0.05
EC (dS/m)	1.08 $\pm$ 0.01
FC (%)	21.0 $\pm$ 0.50
Total N (% w/w)	0.13 $\pm$ 0.02
Available K (g/kg)	0.12 $\pm$ 2.37
CaCl <sub>2</sub> -extractable Si (g/kg)	0.03 $\pm$ 0.00

The role of salicylic acid in plant tolerance to abiotic stresses such as ozone, heat, heavy metal and osmotic stress has been reported (Wang et al., 2010; Kadioglu et al., 2011). A survey of literature indicates that salicylic acid plays a key

role in providing tolerance to the plants exposed to water stress (Hayat et al., 2008; Kadioglu et al., 2011). Salicylic acid was found to enhance the activities of antioxidant enzymes such as peroxidase (POD), SOD and CAT, when sprayed exogenously to the drought-stressed plants of tomato (Hayat et al., 2008). Our previous work suggested that the improvement of SA on drought tolerance of barley plants was associated with the increase of antioxidant defense abilities and maintenance of photosynthesis under drought (Habibi, 2012).

Canola (*Brassica napus* L.) is an important crop for rotation with wheat. The increase in plant resistance to drought is an important way to overcome drought problems. Therefore, the aim of the present study was to determine whether the application of exogenous salicylic acid, selenium and silicon can improve the response of severe water stress in canola (*Brassica napus* L. cv Okapi). In addition of monitoring the shoot and root growth, we examined the effect of these exogenous protectants on the antioxidant defense system during drought stress in canola plants, which may help elucidate the physiological mechanism of exogenous protectants in improvement of the drought tolerance of plants

## MATERIALS AND METHODS

### Plant growth and treatments

Seeds of canola (*Brassica napus* L. cv Okapi) were sown in the top parts of cylindrical plastic pots; four seeds were planted in each pot. Pots were 14 cm in diameter and 105 cm in depth, filled with 15 kg sandy loam soil. Some chemical properties of soil used for the present experiment are shown in Table 1. Before filling the pots, the soils of Si treatments were fertilized

with 0.35 g sodium metasilicate ( $\text{Na}_2\text{SiO}_3$ )/kg soil (3.44 mmol/dm soil, 2.73 mmol/kg soil). Equimolar concentrations of NaCl were applied for balancing Na amounts in the soils of control pots without Si. After emergence, the seedlings were thinned to one plant per pot and plants were watered every 5 days to maintain at 90% of FC before starting treatments. Plants were grown under field conditions located near the city of Miandoab, NW Iran (46°6' E and 36°46' N) with day/night temperature of 20-35/17-20°C, relative humidity of 35-45% and daily photon flux density of about 1 200-1 700  $\mu\text{mol}/\text{m}^2 \text{ s}$  throughout the experimental period. Thirty-two days after sowing, water holding was applied in the water-stressed group of plants, and SA (0.1 mM) and Se (10 mg/l) was applied on the foliage with a hand sprayer. The volume of the spray was 25 ml

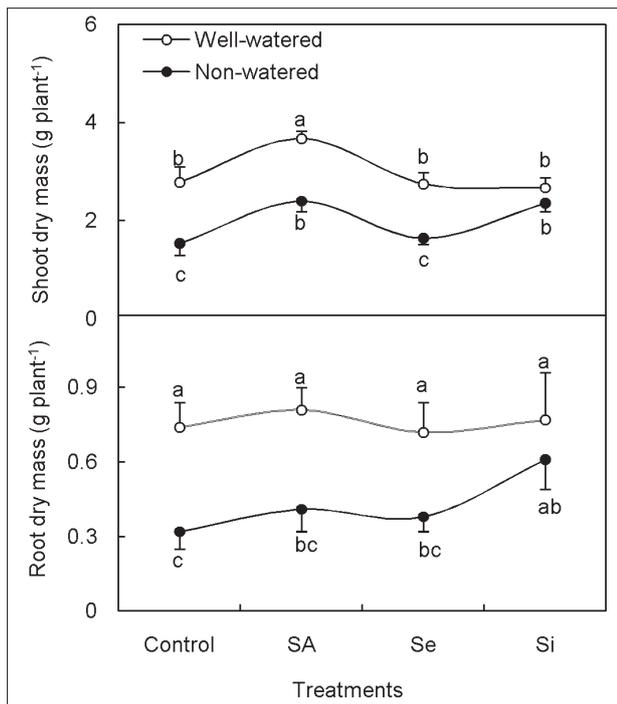
per pot. At 57 days after sowing (25 d after SA and Se application and drought treatment), measurements were done and the recent fully expanded leaves were collected and frozen in liquid  $\text{N}_2$  immediately until analysis.

**Analysis of growth parameters**

Leaves were washed with distilled water, blotted dry on filter paper and after determination of fresh mass (FM) were dried for 48 h at 70°C for determination of dry mass (DM).

**Assay of antioxidative enzymes activity and related metabolites**

The activity of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) was determined according to methods described elsewhere (Habibi and Hajiboland, 2013). For the determination of superoxide dismutase (SOD, EC 1.15.1.1) activity, the enzyme was extracted in 25 mM HEPES pH 7.8 with 0.1 mM EDTA and the supernatant was added to the reaction mixture containing 0.1 mM EDTA, 50 mM  $\text{Na}_2\text{CO}_3$  pH 10.2, 13 mM methionine, 63  $\mu\text{M}$  nitroblue tetrazolium chloride (NBT) and 13  $\mu\text{M}$  riboflavin. One unit of SOD was defined as the amount of enzyme that produced a 50% inhibition of NBT reduction under assay conditions. For the determination of catalase (CAT, EC 1.11.1.6) activity, samples were homogenized with 50 mM phosphate buffer pH 7.0 and assayed spectrophotometrically by following the degradation of  $\text{H}_2\text{O}_2$  at 240 nm. The reaction medium contained 50 mM phosphate buffer pH 7 and 10 mM  $\text{H}_2\text{O}_2$ . The glutathione peroxidase (GSH-Px, EC 1.11.1.9) activity was measured by a modification of the method of Flohé and Günzler (1984) using  $\text{H}_2\text{O}_2$  as substrate. The enzyme was extracted in 50 mM phosphate buffer pH 7.0 and the supernatant



**Fig. 1.** Effects of SA, Se and Si application on dry mass of canola under drought conditions. Bars indicated with the same letter are not significantly different ( $P < 0.05$ ). Values are the mean  $\pm$  SD ( $n=4$ ).

was added to the reaction mixture contained 0.2 ml of the supernatant, 0.4 ml GSH (0.1 mM) and 0.2 mL  $\text{KNaHPO}_4$  (0.067 M). The above reagents without supernatant extract were used for the non-enzyme reaction. After preheating the mixture in a water bath at 25°C for 5 min, 0.2 ml  $\text{H}_2\text{O}_2$  (1.3 mM) was added to initiate the reaction. The reaction was stopped by adding 1 ml 1% trichloroacetic acid and the mixture was put into an ice bath for 30 min. Then the mixture was centrifuged for 10 min at 1 100 g, 0.48 ml the supernatant was placed into a cuvette and 2.2 ml of 0.32 M  $\text{Na}_2\text{HPO}_4$  and 0.32 ml of 1.0 mM DNTB were added for color development. The absorbance at wavelength 412 nm was measured after 5 min. The enzyme activity was calculated as a decrease in GSH within the reaction time when compared with that in the non-enzyme reaction. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric

acid. The hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) contents in the leaves were assayed according to the method of Velikova et al. (2000). Leaves were homogenized in ice bath with 0.1% (w/v) TCA. The extract was centrifuged at  $12\,000 \times g$  for 15 min, after which 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI was added to 0.5 ml of the supernatant; the reaction was improved for 1 h in the dark and measured spectrophotometrically at 390 nm. The content of  $\text{H}_2\text{O}_2$  was given on a standard curve.

### Determination of total chlorophyll and total free amino acids

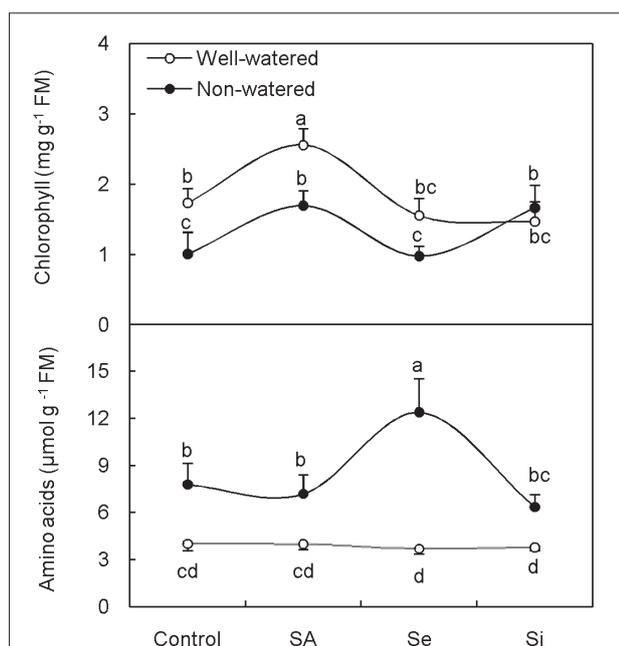
Leaf concentration of total Chl was determined after extraction of pigments in cold acetone and allowing the samples to stand for 24 h in the dark at 4°C (Lichtenthaler and Wellburn, 1985). The content of total free  $\alpha$ -amino acids was assayed using ninhydrin colorimetric method. Glycine was used for production of standard curve (Hwang and Ederer, 1975). Soluble protein was estimated spectrophotometrically by the Bradford method (1976).

Experiments were undertaken in complete randomized block design. All experiments were conducted using four independent replications. Statistical analyses were carried out using sigma stat (3.5) with Tukey's test ( $P < 0.05$ ).

## RESULTS

### Effects of exogenous protectants on the growth parameters, total chlorophyll and amino acids contents of canola under drought

The data in Fig. 1 show that drought stress remarkably reduced the shoot dry matter. Exogenous SA applied alone significantly increased



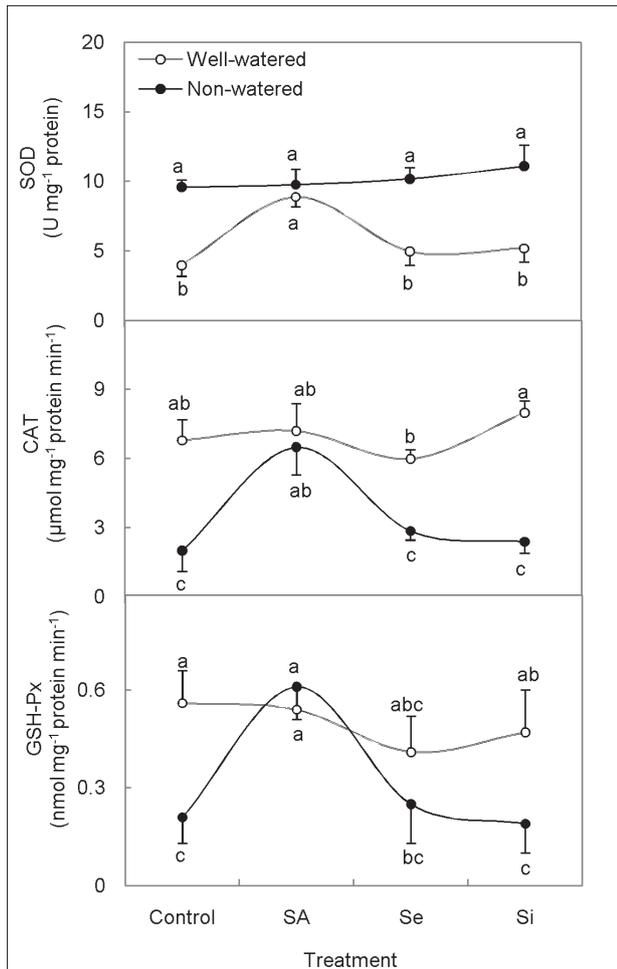
**Fig. 2.** Effects of SA, Se and Si application on the concentration of chlorophyll and amino acids. Bars indicated with the same letter are not significantly different ( $P < 0.05$ ). Values are the mean  $\pm$  SD ( $n=4$ ).

the shoot dry matter under well-watered conditions. Salicylic acid-supplied plants showed higher shoot dry matter compared to those without application of SA under drought conditions. Drought stress also remarkably decreased the root dry matter. However, the decrease extent in the silicon treatment was less than that in the non-silicon treatment. The content of total chlorophyll was significantly decreased by drought stress, and the decrease extent in the silicon and salicylic acid treatments was obviously less than that in the non-silicon and non-SA treatment (Fig. 2).

The content of total chlorophyll was not influenced significantly by Se application under well-watered and non-watered conditions. An increase in the amount of total amino acids was observed under water-stress conditions compared with the control. When SA, Se and Si were applied singly, the amount of total amino acids was not influenced under well-watered conditions. In contrast, Se application significantly increased the root amino acid content after 25 days water stress.

**Effects of exogenous protectants on some antioxidative enzymes activities, MDA and H<sub>2</sub>O<sub>2</sub> contents of canola plants under drought**

Under well-watered conditions, activity of SOD was not influenced significantly by Se and Si applications (Fig. 3). However, a significant rise in the activity of SOD was observed under well-watered conditions. In contrast, the activity of SOD was also increased by SA, Se and Si application in water-deficit plants. After 25 days water stress, the activity of CAT and GSH-Px in canola leaves was even lower than that in well-watered plants. The activity of CAT and GSH-Px in water-stressed plants did not differ from that in Si-supplemented water-deficit plants. A significant rise in the activity of GSH-Px was observed in the Se-supplemented water-deficit samples relative to water-deficit treatment. After 25 days water stress, however, CAT activity was significantly decreased by drought stress, but SA application caused a significant increase in the activity of this enzyme. In canola leaves, continuation of the water stress with or without SA, Se and Si application caused significant accumulation of H<sub>2</sub>O<sub>2</sub> content (Fig. 4). Drought stress without SA and Si application caused significant accumulation of malondialdehyde (MDA). However, the malondialdehyde level was decreased by

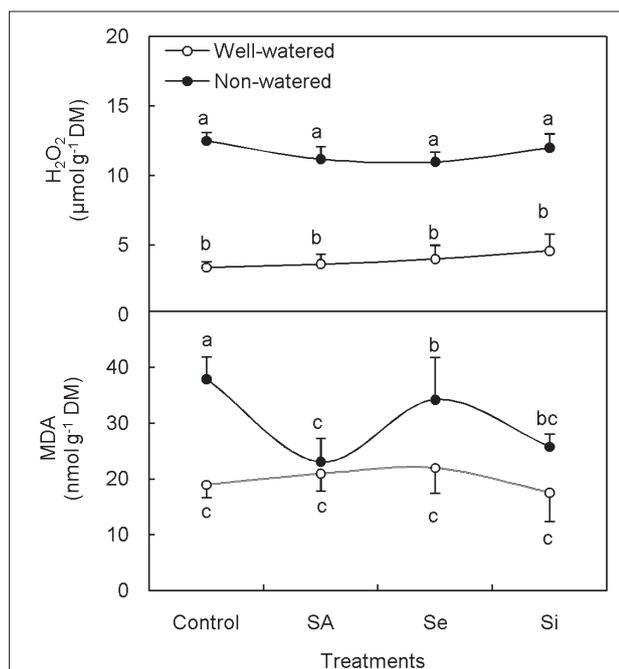


**Fig. 3.** Effects of SA, Se and Si application on the specific activity of antioxidant enzymes. Bars indicated with the same letter are not significantly different ( $P < 0.05$ ). Values are the mean  $\pm$  SD ( $n=4$ ).

soil application of Si and foliar application of SA under water stress conditions. At the end of the experiment after 25 days, in comparison to plants maintained under well-watered conditions, water stress caused a decrease of the leaf protein content (Fig. 5). However, exogenous SA and Si ameliorated the protein reduction of drought-stressed canola plants.

## DISCUSSION

Silicon significantly improved the growth of canola roots that were grown under drought conditions in this work, similar to that observed for soybean and sorghum seedlings grown under drought conditions (Gao et al., 2011, Ahmed et al., 2013). In the present work, shoot dry mass of canola plants was decreased by drought stress.



**Fig. 4.** Effects of SA, Se and Si application on the concentration of malondialdehyde (MDA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Bars indicated with the same letter are not significantly different ( $P < 0.05$ ). Values are the mean  $\pm$  SD ( $n=4$ ).

The silicon applied as well as SA-supplemented plants maintained higher shoot dry mass compared to those without application of these exogenous protectants under drought, which indicated that application of these exogenous protectants improved the growth of stressed plants (Kadioglu et al., 2011, Habibi, 2012). Under well-watered conditions, total chlorophyll content was not influenced significantly by Si application. However, exogenous Si ameliorated the total chlorophyll reduction of drought-stressed canola plants. This finding is consistent with other published reports suggesting that the silicon effects become manifest only when plants grow under abiotic and biotic stress (Gao et al., 2011).

Plants protect cell systems from the cytotoxic effects of drought-accumulated active oxygen species using enzymes such as SOD, APX and CAT (Verhagen et al., 2004). There is data supporting that SA increases the activity of SOD enzyme (Hayat et al., 2008, 2010), which in turn protects plants against ROS generation and lipid peroxidation. In previous studies, an increase in activities of SOD but an inhibition of CAT activity following SA treatment was reported (Shakirova, 2007). In this work, exogenous SA application improved CAT activity under drought conditions. This finding was in agreement with Farooq et al. (2009), who reported that CAT inhibition by SA could not be validated in all plants (Habibi et al., 2012). In this work, a significant rise in the activity of CAT and SOD revealed that SA exerts beneficial effects on the stress tolerance of canola by enhancing their antioxidative capacity. In addition, the amounts of MDA remained unchanged under SA-supplemented water-deficit conditions obviously because of an efficient scavenging following significant enhancement of SOD and CAT

activities. The effects of drought on the antioxidant enzyme activities of canola plants have been previously reported (Abedi and Pakniyat, 2010). Our results showed that the activity of SOD was increased by applying silicon under water stress, whereas the activity of CAT was not influenced. These findings were in agreement with Zhu et al. (2004), who reporting on cucumber leaves, also observed that the addition of silicon increased the activity of SOD, whereas CAT activity was not influenced by the addition of silicon.

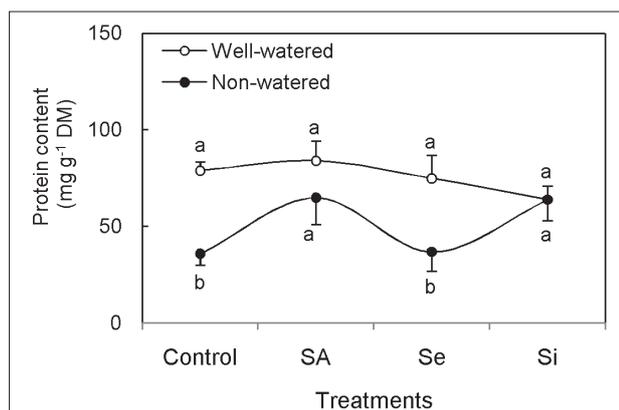
## CONCLUSIONS

In conclusion, both SA and Si applied plants maintained lower MDA content compared to those without application of these exogenous protectants under drought stress, which indicated that the application of these protectants could prevent lipid peroxidation of stressed canola plants because of an efficient scavenging of ROS following elevated activity of antioxidant enzymes. In this work, however, a significant rise in the activity of GSH-Px in the Se-supplemented

water-deficit samples was observed, but amounts of MDA and  $H_2O_2$  remained unchanged under Se-supplemented water-deficit conditions. This indicates that under water stress with Se spraying, an imbalance between the production and scavenging of ROS may cause stress because of barely sufficient scavenging by CAT and SOD, as could be judged by the accumulation of MDA. In conclusion, the results of the present work suggested that the improvement of SA on the drought tolerance of canola plants was associated with the increase of antioxidant defense abilities. In addition, silicon had ameliorative effects on canola growth and antioxidant parameters under drought. These results indicate that an application of SA and/or Si can be used to promote the induction in canola plants of the antioxidant system, thereby improving stress resistance.

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**Fig. 5.** Effects of SA, Se and Si application on protein content of canola under drought conditions. Bars indicated with the same letter are not significantly different ( $P < 0.05$ ). Values are the mean  $\pm$  SD ( $n=4$ ).

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