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RESEARCH ARTICLE

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PROTECTIVE ROLE OF SELENIUM IN CANOLA (*BRASSICA NAPUS* L.) PLANT SUBJECTED TO SALT STRESS

ABSTRACT:

The possible protective effect of selenium (Se) on canola plants (*Brassica napus* L.) subjected to salt stress was studied by investigating plant growth, yield and changes in photosynthetic pigments, carbohydrate, proline, certain mineral ions content, activities of some antioxidant enzymes of canola plants and fatty acid composition of the yielded seeds. For this purpose canola plants were irrigated with different levels of saline solution (0, 2000, 4000, and 6000 mg L^{-1}, prepared according to Stroganov equation, 1962), then the effect of different dosage of Se (0, 2.5, 5, or 10 mg L^{-1} selenium as sodium selenate) were examined as foliar spray on 50 and 65 – days old plants. Then samples were collected for analysis as 72 – days old plants. Salinity led to significant inhibition in plant growth, yield components, photosynthetic pigment contents, quantity and quality of seed oil. The detected inhibition was directly related to the applied concentrations of salt. Se applied alone or in combination with salt treatment significantly increased plant growth, yield, photosynthetic pigment content and improved the quality of canola oil. The most effective concentration of Se was 5 mg L^{-1}. In addition, Se-treated plants exhibited various defense mechanisms to cope with salt stress including increased endogenous proline content, enhanced catalase activity and increased magnesium and phosphorus ion contents.

KEY WORDS:

Brassica napus, Fatty acids; Growth, Reactive oxygen species (ROS), Selenium, Yield

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INTRODUCTION:

Selenium (Se) had long been considered as a toxic element until it was found also to be essential in 1957 (Schwarz and Foltz, 1957). The role of Se in mitigating environmental stress has been then extensively investigated in animals and human and, to a lesser extent, in plants (Feng *et al.*, 2013). In some areas of the world, e.g., Egypt, China and Thailand (Tapiero *et al.*, 2003), the inherent Se concentration in the soil is quite low, causing a Se deficiency in the human diet. A current technology to apply Se fertilizer as a foliar spray or base fertilizer has been used to increase Se content in the edible portion of crops (Pezzarossa *et al.*, 2012) and often to counteract the injuries generated by different environmental stresses.

A stimulatory effect of foliar application of Se on growth has been reported for ryegrass (Hartikainen *et al.*, 2000), lettuce (Xue *et al.*, 2001), soybean (Djanaguiraman *et al.*, 2005) and green tea leaves (Hu *et al.*, 2003). In addition, adding small amounts of Se to the soil increased tuber yield and starch concentration in young leaves of potato (*Solanum tuberosum* L.) (Turakainen *et al.*, 2004) and was associated with 43% increase in seed production of *Brassica* plants (Lyons *et al.*, 2009).

Salt stress impede vital physiological processes of plant growth and development such as seed germination, seedling growth, seed vigor, vegetative growth, flowering and fruiting set (Slathia *et al.*, 2012). Elevated levels of salt ions in soil solution surrounding plant roots induce an imbalance in water potential between plant root cells and ambient soil solution and result in cellular dehydration (Munns, 2002). In addition, elevated salts lead to a passive salt ion penetration via plasma membrane and to an accumulation of salt ions in cell cytoplasm which can lead to inhibition of intracellular enzyme activity (Munns and Tester, 2008). Salt stress can also result in the accumulation of reactive oxygen species (ROS) in plants. The enhanced production of ROS can pose a threat to plants, but they are also

believed to act as signals for the activation of the stress-response and defense pathways (Mittler, 2002). ROS mainly include superoxide anion (O2•−), hydrogen peroxide (H_2O_2), hydroxylic free radical (OH•), singlet oxygen ($^1O_2$), methyl radical (CH3•) and lipid peroxidation free radicals (LOO•, ROO•). Generally, two types of antioxidants are triggered in plants to balance the elevated ROS levels. One type is the low molecular weight substances, such as glutathione (GSH), ascorbate (AsA) and tocopherol, and the other type is the enzymes, such as, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GSH-Px), guaiacol peroxidase (GPOX) and glutathione reductase (GR) (Cao *et al.*, 2004; Asada, 2006). Either directly or indirectly via the regulation of antioxidants, Se can control the production and quenching of ROS (Feng *et al.*, 2013). The regulation of ROS levels by Se may be a key mechanism for counteracting environmental stress in plants. In this respect, three possible mechanisms have been proposed for the decrease in O_2•− levels when the appropriate doses of Se were added, including the spontaneous dismutation of $O_2^{\bullet-}$ into H_2O_2 (without catalysis by the SOD enzyme) (Hartikainen *et al.*, 2000; Cartes *et al.*, 2010), the direct quenching of $O_2^{\bullet-}$ and OH• by Se compounds (Xue *et al.*, 1993), and the regulation of antioxidative enzymes.

In spite of these beneficial effects of exogenous Se application on plants, the question is often raised: what is the optimal Se concentration? Se often exerts dual effect on plant growth. At low dosage, it can stimulate the growth of plants and counteract many types of environmental stresses, whereas at high dosages, it can also act as a pro-oxidant and cause damage to plants (Feng *et al.*, 2013).

Canola is the third oil plant in the world often cultivated in arid and semiarid regions of the world such as Egypt where salinity threatens to become, or already is, a problem. The present experiment was conducted as a trial to determine the appropriate concentration of Se to enhance canola growth and yield and to evaluate the possible protective role of exogenously applied Se on canola plants against salt stress.

MATERIAL AND METHODS:

Plant Material:

Brassica napus 'Pactole', a French cultivar, was obtained from Oil Crops Council, Ministry of Agriculture, Giza, Egypt.

Growth conditions:

The present study was conducted in two successive growth seasons (2009-2010) and (2010-2011) in green houses located in the Botany Department, the National Research Center, Dokki, Giza, Egypt. Canola seeds were surface-sterilized with 0.1% mercuric chloride for 5 min and washed thoroughly with several changes of sterile distilled water. Homogenous lots of canola seeds were then sown in clay pots (diameter, 50 cm; depth, 25 cm). Each pot contained 15 kg of clay soil. Phosphorus, nitrogen and potassium were added before and after sowing at a rate of 5.0, 6.0, and 5.0 g pot^{-1}, respectively, in the form of triple phosphate, urea and potassium sulphate. After emergence, seedlings (20- days old) were thinned to five uniform seedlings per pot. The plants were left to grow in a controlled growth chamber under the following growth conditions: 15 hours photoperiod, 70-75% relative humidity, with day/night temperature ranged between 18 and 25°C. Pots were irrigated with tap water for 40 days, the water holding capacity was maintained at 70%. Forty-days old plants were divided into four groups each was subjected to one of the desired salinity levels (0, 2000, 4000, and 6000 mg L^{-1}), the component of salt mixture was prepared according to Stroganov (1962) equation as shown in tables 1 & 2. The plants were irrigated twice every week with saline solution till the end of the experiment, each pot was given two liter of saline solution (70 % of the soil water holding capacity). The plants irrigated three times with equal amounts (2 liter pot^{-1}) of the saline solution followed by one with tap water to prevent the toxicity resulted from the accumulation of salts around the root system.

Table 1. The components of the salt mixture used for chloride salinization expressed as % of the total salt content.

$MgSO_4$	$CaSO_4$	NaCl	$MgCl_2$	$CaCO_3$
10	1	78	2	9

Table 2. The component of specific anions and cation in chloride mixture expressed as percentage of total mill equivalents.

Na^+	Mg^{2+}	Ca^{2+}	$(SO_4)^{2-}$	Cl^-	$(CO_3)^{2-}$
38	6	6	5	40	5

After 50 days from sowing, pots of each salt treatment group were divided into four subgroups sprayed with 0, 2.5, 5, or 10 mg selenium L^{-1} as sodium selenate (Na_2SeO_4). Second spray with selenium was carried out after 15 days of the first selenium treatment (65 days from sowing).

Shoot samples (including stem and leaves) from each treatment were collected at the vegetative stage, one week after the second selenium treatment, (72-days old plants) to measure several growth parameters including: number of leaves plant^{-1}; shoot length (cm plant^{-1}); number of branches plant^{-1} and fresh and dry weights of shoots (g plant^{-1}) and to determine selected biochemical compounds including: carbohydrates (total soluble sugars, polysaccharides and total carbohydrates), proline and mineral ion contents (potassium, calcium, magnesium, phosphorus and selenium). In addition, 1 gram fresh leaves were collected to measure photosynthetic pigment

contents (chlorophyll a, chlorophyll b, carotenoids, chl a/chl b, chl a+b/carotenoids and total photosynthetic pigments). Moreover, 1 gram fresh leaves were used for estimating the activity of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). At harvest (140 -days old plants), samples were collected to determine oil content and fatty acids composition of canola seeds and the following yield components: shoot length, number of branches $plant^{-1}$, number of pods $plant^{-1}$, weight of pods $plant^{-1}$, number of seeds pod^{-1}, dry weight of seeds $plant^{-1}$ and seed index (dry weight of 1000 seeds). Ten replicates were used to determine growth parameters and yield components and three replicates were used for the biochemical analysis.

Estimation of Photosynthetic pigments:

The contents of the photosynthetic pigments chlorophyll *a* (chl a), chlorophyll *b* (chl b) and carotenoids in fresh leaves were determined as described by Metzner *et al.* (1965).

The pigment contents were expressed as mg g^{-1} fresh weight (FW) of leaves.

Estimation of carbohydrate:

Soluble sugar was extracted according to Prud'homme *et al*. (1992). The soluble sugars were determined by the anthrone sulfuric acid method described by Scott and Melvin (1956). Polysaccharide content was determined in the dry residue left after extraction of soluble sugars according to Hodge and Hofreiter (1962). Total carbohydrates content was calculated as the sum of the amounts of soluble sugars and polysaccharides in the same sample. All data were calculated as mg 100 g^{-1} dry weight (DW) of leaves.

Estimation of proline:

Free proline was extracted and determined in fresh leaves in accordance with the method of Bates *et al.* (1973). The absorbance was read at 520 nm using toluene as a blank. Proline concentration was determined and calculated as µmol g^{-1} FW of leaves.

Antioxidant enzymes activities:

Samples were prepared for enzyme extraction following the method described by Mukherjee and Choudhury (1983). SOD (EC 1.15.1.1) activity was measured according to the method of Dhindsa *et al.* (1981). One unit of SOD was defined as the amount of enzyme that caused half the maximum inhibition of nitro blue tetrazolium (NBT) reduction to blue formazan at 560 nm under the experimental conditions. CAT (EC 1.11.1.6) activity was measured according to the method of Chen *et al.* (2000). One unit of enzyme activity was defined as the amount of the enzyme that reduced half of the H_2O_2 in 60 second at 25°C (Weems *et al.*, 1999). POD (EC 1.11.1.7) activity was determined by monitored the increase in absorbance (470 nm) resulting from dehydrogenation of guaiacol (Malik and Singh, 1980). One unit of enzyme activity was defined as the amount of the enzyme that catalyzed the conversion of one micromole of H_2O_2 per minute at 25°C (Weems *et al.*, 1999).

The activities of SOD, CAT and POD were expressed as enzyme units per gram FW (Ug^{-1} FW) of leaves.

Quantification of inorganic cations:

The dried matter digested according to the method of Chapman and Pratt (1982). The obtained solutions were used for potassium, calcium, magnesium and phosphorus determinations. Potassium was estimated by flame emission technique as adopted by Ranganna (1977). Magnesium, phosphorus and calcium were determined simultaneously by ICP Spectroscopy according to the method of Soltanpour (1985). Data were calculated as mg kg^{-1} DW.

Estimation of seed oil content:

The oil content of canola yielded seeds was extracted and estimated according to the official methods of analysis (AOAC, 1990).

Fatty acids determination:

Fatty acids from total lipids and lipid classes were methylated in anhydrous methanol (w/w) by the method of Fedak and De La Roche (1977).

Statistical analysis

The experimental design followed a complete random block design. According to Snedecor and Cochran (1990), the averages of data were statistically analyzed using two-way analysis of variance (ANOVA-2). Significant values determined according the Least Significant Difference (L.S.D; $p \leq 0.05$) by using the STAT-ITCF program (Foucart, 1982).

RESULTS:

Growth parameters:

All the measured growth parameters (shoot length, number of leaves $plant^{-1}$, leaves area $plant^{-1}$ and fresh and dry weights of shoots) decreased dramatically as salinity increased (Table 3). Plant response to selenium treatment is concentration-dependent; low concentrations of selenium (2.5 and 5.0 mg L^{-1}) have a stimulatory effect on canola growth as compared with untreated control plants, while higher concentration of Se (10 mg L^{-1}) significantly decreased shoot length, fresh and dry weights of shoot system by 6.15%, 19.7%, and 17.64%, respectively as compared with untreated control plants. The maximum growth was obtained in plants treated with 5 mg L^{-1} Se which also increased all the measured growth parameters in salt stressed plants (up to 4000 mg L^{-1}) over the level obtained in control.

Table 3. Effect of different dosages of selenium (0, 2.5, 5, and 10 mg L^{-1}) on growth parameters of canola plants (72-days from sowing) grown under different levels of salinity. The data shown are mean of ten replicates.

Selenium (mg L^{-1})	Salinity (mg L^{-1})	Plant height (cm)	Number of leaves $Plant^{-1}$	Leaves area $Plant^{-1}$ (cm^2)	Shoot system FW (g)	Shoot system DW (g)
0	0	32.500±0.500	8.000±0.500	321.0±11.5	7.520±0.128	63.050±0.550
	2000	30.200±0.610	7.500±0.500	285.2±4.6	7.040±0.079	60.670±0.350
	4000	27.500±0.500	6.667±0.577	246.0±8.4	6.300±0.044	53.960±0.246
	6000	23.830±0.290	6.200±0.819	231.8±22.6	5.200±0.044	45.917±0.208
2.5	0	35.500±0.500	9.167±0.764	386.1±8.1	8.730±0.070	71.850±0.236
	2000	33.500±0.500	8.333±0.577	333.2±8.1	8.100±0.105	68.110±0.340
	4000	29.567±0.513	7.667±0.764	303.9±2.3	7.500±0.105	63.420±0.234
	6000	26.500±0.500	6.943±0.418	257.8±6.8	6.400±0.062	54.700±0.450
5	0	40.333±0.764	9.933±1.007	436.9±11.2	10.560±0.070	86.500±0.563
	2000	38.333±0.764	9.500±0.500	403.8±2.6	9.640±0.079	80.280±0.303
	4000	35.500±0.500	8.167±1.041	326.8±10.3	8.200±0.070	68.500±0.607
	6000	31.000±0.707	7.663±0.335	302.3±9.6	7.165±0.078	60.440±0.438
10	0	30.500±1.291	8.500±1.000	392.5±9.6	6.032±0.747	51.925±5.853
	2000	33.333±3.686	8.167±1.258	353.4±7.7	8.900±0.085	73.700±0.427
	4000	37.500±0.500	7.400±0.656	313.2±15.8	8.000±0.090	67.300±0.464
	6000	34.167±0.764	6.277±0.254	251.3±11.7	7.050±0.111	59.500± 0.550
L.S.D ($p \leq 0.05$)						
Salinity		0.21	0.14	7.096	0.04	0.35
Selenium		0.21	0.14	7.096	0.04	0.35
Salinity * Selenium		0.87	N.S	14.19	0.18	1.42

Yield components at harvest:

Increased salt concentrations applied to the soil resulted in a significant reduction in the plant height, number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds pod^{-1} and seed index of canola plants estimated by 21.6%, 33.3%, 16.7%, 5.9% and 12.5%, respectively in plants treated with 6000 mg L^{-1} saline solution as compared with untreated plants (Table 4).

Table 4. Effect of different dosages of selenium (0, 2.5, 5, and 10 mg L^{-1}) on the yield of canola plants (140-days from sowing) grown under different levels of salinity. The data shown are mean of ten replicates.

Selenium (mg L^{-1})	Salinity (mg L^{-1})	Plant height(cm)	Number of Branches $plant^{-1}$	Number of pods $plant^{-1}$	Weight. of pods $plant^{-1}$ (g)	Number of seeds Pod^{-1}	Weight of seeds $plant^{-1}$ (g)	Seed index (g)	Seed oil content (%)
0	0	90.9±0.8	5.7±0.1	132.5±0.2	20.3±0.1	20.1±0.3	8.6± 0.1	3.20±0.1	38.00±0.5
	2000	85.7±1.6	5.5±0.1	125.3±2.0	17.8±0.1	19.6±0.04	7.2±0.1	2.90±0.1	37.20±0.1
	4000	77.8±2.1	4.5±0.1	118.8±0.3	15.5±0.1	19.3±0.1	6 .4±0.1	2.80±0.03	36.40±3.2
	6000	71.2±2.1	3.8±2.0	110.4±0.9	13.4±0.4	18.9±0.1	5 .1±0.1	2.40±0.04	34.70±0.9
2.5	0	96.4±1.3	6.5±0.1	139.3±1.3	24.8±0.2	20.7±0.7	10.6 ±0.1	3.70±0.1	38.90±0.3
	2000	88.4±1.2	5.8±0.2	132.8±0.8	22.9±0.2	20.0±0.3	9 .4±0.1	3.60±0.01	38.10±0.1
	4000	82.0±1.3	5.3±0.3	126.4±0.7	20.2±0.1	20.4±0.5	8 .4±0.1	3.30±0.1	37.70±0.2
	6000	75.2±1.5	4.4±0.2	119.9±0.8	17.0±0.6	19.4±0.4	6 .9±0.2	3.00±0.1	36.10±0.8
5	0	103.5±2.1	7.8±0.2	146.4±1.0	27.8±0.3	21.2±0.5	12. 9±0.2	4.20±0.1	40.70±0.3
	2000	93.8±1.7	7.2±0.2	140.4±0.7	25.8±0.4	20.9±0.3	1 1.3±0.2	3.90±0.04	39.60±0.1
	4000	89.3±0.7	6.5±0.1	134.2±1.1	22.8±0.3	20.5±0.4	1 0.1±0.2	3.70±0.1	38.60±0.2
	6000	82.0±1.4	5.3±0.3	129.8±3.6	22.1±3.2	20.3±0.4	8 .6±0.1	3.40±0.04	37.10±0.8
10	0	93.6±1.6	6.1±0.3	133.1±5.6	22.7±0.2	20.3±0.3	9.7± 0.1	3.50±0.1	39.40±0.5
	2000	86.0±1.6	5.7±0.1	131.6±4.3	19.9±0.3	19.8±0.6	8 .5±0.2	3.30±0.1	38.40±1.0
	4000	79.5±1.0	5.3±0.1	125.4±4.1	16.1±0.1	19.7±0.3	7 .1±0.1	3.00±0.04	37.90±0.3
	6000	72.6±2.7	4.0±0.2	118.2±3.0	12.8±0.3	17.7±0.6	5 .6±0.1	2.70±0.04	35.00±0.8
L.S.D ($p \leq 0.05$)									
Salinity		1.1	0.13	1.7	0.56	0.27	0.1	0.04	0.62
Selenium		1.1	0.13	1.7	0.56	0.27	0.1	0.04	0.62
Salinity * Selenium		N. S.	0.26	N. S.	1.1	0.54	0.19	N.S	N.S

The application of Se (2.5, 5, and 10 mg L^{-1}) has a positive effect on canola yield whether in plants grown under normal conditions or in plants subjected to different salinity levels as compared with their corresponding control. The maximum increase in yield was observed in response to treatment with 5 mg L^{-1} of Se and was estimated by 50%, 56.9%, 57.8% and 68.6% increase in weight of seeds $plant^{-1}$ in plants treated with 5 mg L^{-1} Se alone or in combination with 2000, 4000, and 6000 mg L^{-1} saline solution, respectively compared to plants treated with the different concentrations of saline solution alone.

Photosynthetic pigments:

Chlorophyll a, chlorophyll b and carotenoid contents were decreased in plants grown under salt stress in comparison to plants under normal conditions. The decrease was positively related to the applied salt concentration and estimated by 40.4%, 46.34%, and 41.93%, respectively in plant treated with 6000 mg L^{-1} saline solution, with Chl b the most affected pigment (Table 5). The application of Se alone increased photosynthetic pigments (chl a, chl b, and carotenoids) compared to control. In combination with salt treatment, all the tried concentrations of Se (2.5, 5, and 10 mg L^{-1}) significantly increased photosynthetic pigments as compared with untreated plants grown under salt stress. The stimulatory effect of Se was more pronounced, with its highest concentration (10 mg L^{-1}), where the inhibitory effect of salt treatment up to 4000 mg L^{-1} on photosynthetic pigments content was totally alleviated.

Table 5. Changes in the photosynthetic pigments and carbohydrates content of leaves of canola plants (72-days from sowing) treated with different concentrations of selenium (0, 2.5, 5, and 10 mg L^{-1}) and grown under different levels of salinity. The data shown are mean of three replicates.

Selenium (mg L^{-1})	Salinity (mg L^{-1})	Chl a (mg g^{-1}FW)	Chl b (mg g^{-1}FW)	Carotenoids (mg g^{-1}FW)	Chl a+Chl b (mg g^{-1}FW)	Chl a/ Chl b	Chl a+b/ carotenoids	Soluble sugar (mg 100g^{-1} DW)	Polysaccharides (mg 100g^{-1} DW)	Total carbohydrates (mg 100g^{-1} DW)
0	0	9.4±0.03	4.1± 0.08	3.1± 0.03	13.48±0.1	2.3± 0.04	4.3± 0.03	2583.2± 83.3	15739.3±121.8	18322.5± 205.0
	2000	8.5±0.03	3.7±0.1	2.8±0.02	12.2±0.09	2.3± 0.06	4.4±0.05	3010.99±34.7	17170.0±144.0	20181.0± 127.3
	4000	6.8±0.02	2.9±0.02	2.1±0.01	9.8± 0.04	2.3±0.01	4.6±0.02	3583.19± 83.3	16354.8±72.8	24129.0±903.7
	6000	5.6± 0.04	2.2±0.02	1.8±0.02	7.8±0.02	2.6± 0.04	4.3± 0.04	44 33.16±50.0	21134.5±145.3	26964.0±189.0
2.5	0	10.3± 0.04	4.6±0.05	3.4±0.02	14.9±0.01	2.3±0.03	4.4±0.03	2383. 2±33.3	13891.8±99.6	16275.0± 131.2
	2000	9.3± 0.02	4.2±0.03	3.1±0.02	13.4± 0.04	2.2±0.01	4.3±0.03	2599.9±50.0	16016.6± 162.6	18616.5±113.6
	4000	7.4±0.03	3.2±0.03	2.4±0.02	10.6± 0.0	2.3±0.03	4.4±0.03	3244.32±69.4	15138.3±163.7	21336.0±79.3
	6000	6.7±0.06	2.6± 0.04	2.1±0.02	9.3±0.05	2.6±0.05	4.4±0.07	3905.4± 85.5	19545.0± 81.6	25704.0± 144.4
5	0	11.3±0.03	5.1± 0.04	3.9±0.03	16.5± 0.04	2.2±0.02	4.2±0.03	2199.9± 33.3	11376.6± 221.4	13576.5± 206.6
	2000	10.4± 0.02	4.9± 0.02	3.4± 0.02	15.4±0.01	2.1±0.01	4.5± 0.02	23 99.90±88.2	20545.8±979.8	18322.5± 205.0
	4000	8.8± 0.04	3.8± 0.04	2.9± 0.02	12.7±0.06	2.3± 0.02	4.5±0.01	31 33.21± 33.3	22530.8±239.0	19488.0±79.3
	6000	7.3± 0.04	2.8± 0.02	2.5± 0.02	10.1± 0.02	2.6±0.03	4.0± 0.04	3666.52± 83.3	18996.5± 2838.3	24801.0±79.3
10	0	11.7± 0.04	5.9± 0.04	4.2±0.01	17.6±0.01	2.0±0.02	4.2±0.01	1983 .25±33.3	8884.2±238.9	10867.5± 220.5
	2000	11.0±0.03	5.3± 0.04	3.9±0.03	16.2±0.07	2.1±0.01	4.1±0.01	2166 .58±50.0	18091.7±85.1	13503.0± 127.3
	4000	9.2± 0.02	4.1± 0.04	3.1±0.03	13.3±0.04	2.2± 0.02	4.3±0.03	253 3.23± 66.7	21798.6±128.8	17671.5± 175.4
	6000	8.2± 0.04	3.3±0.03	2.9± 0.02	11.5±0.06	2.5±0.01	3.9±0.01	3166 .54±33.3	17449.7± 3048.6	22711.5±113.6
L.S.D ($p \leq 0.05$)										
Salinity		0.02	0.03	0.0	0.037	0.02	0.02	40.93	194.60	180.54
Selenium		0.02	0.03	0.0	0.037	0.02	0.02	40.93	194.60	180.54
Salinity * Selenium		0.04	0.06	0.0	0.074	0.04	0.04	81.87	389.20	361.07

Carbohydrates content:

The data presented in table 5 reveal that salt treatment had a stimulatory effect on soluble sugars, polysaccharides content and hence total carbohydrate contents of canola leaves. Highest total carbohydrate contents (147.16% relative to control) was observed in leaves of canola plants treated with 6000 mg L^{-1} saline solution. On the other hand, Se treatment was found to decrease carbohydrate fractions in canola leaves compared to controls, not treated with Se except in plants treated with 5 and 10 mg L^{-1} Se and subjected to 2000 and 4000 mg L^{-1} saline solutions where Se-treated plants had a higher polysaccharide contents relative to plants treated with salt only. The Se- induced reduction in soluble sugars and total carbohydrate contents was positively related to the used Se concentration, the greatest decrease was detected in plant received the highest Se dosage (10 mg L^{-1}) and was estimated by 23.22% and 40.7% in soluble sugars and total carbohydrates, respectively in plants grown under normal conditions compared to control plants.

Proline:

The results revealed that proline content of canola leaves was significantly increased with increasing amounts of salt as compared with control (Fig. 1). The greatest value (252.6% relative to control) was observed in plants treated with 6000 mg L^{-1} saline solution. Spraying salt stressed canola plants with Se resulted in the accumulation of additional amounts of proline compared to untreated plants. The Se – induced increase in proline content was calculated by 10.14%, 20.8% and 44.8% in plants treated with 6000 mg L^{-1} salt and sprayed with 2.5, 5 and 10 mg L^{-1}, respectively as compared with control.

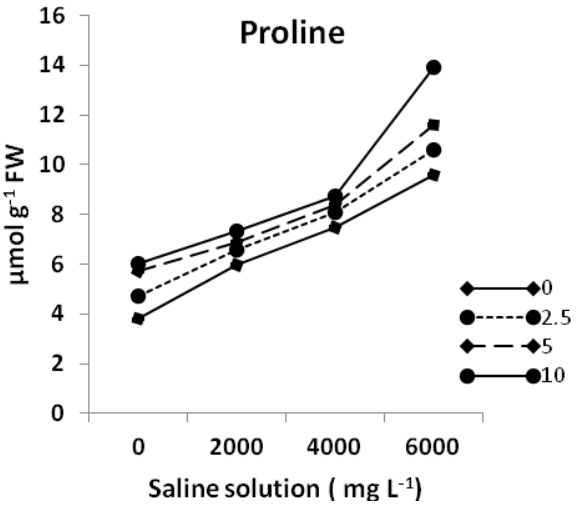


Fig. 1. Effect of different dosages of selenium (0, 2.5, 5, and 10 mg L^{-1}) on antioxidant enzymes activities and proline content of canola leaves (72-days from sowing) grown under different levels of salinity. The data shown are mean of three replicates (Data presented as mean ± SD).

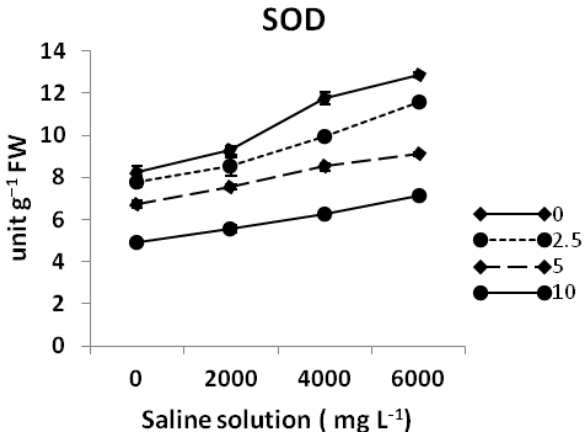
Antioxidant enzymes activities:

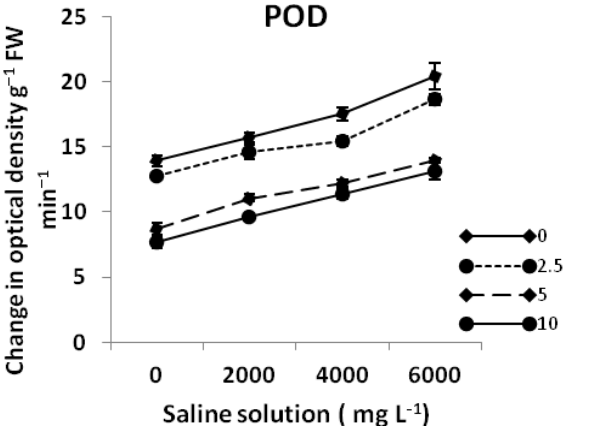
SOD and POD activities were significantly enhanced in canola leaves subjected to salt stress. The maximum activities (155.5% in case of SOD and 146.48% in case of POD relative to control) were observed in plants treated with 600 mg L^{-1} saline solution. In contrast, CAT activity was significantly and progressively decreased in response to salt stress to reach only 37.6% of its activity in plants treated with 600 mg L^{-1} salt as compared to control (Fig. 1).

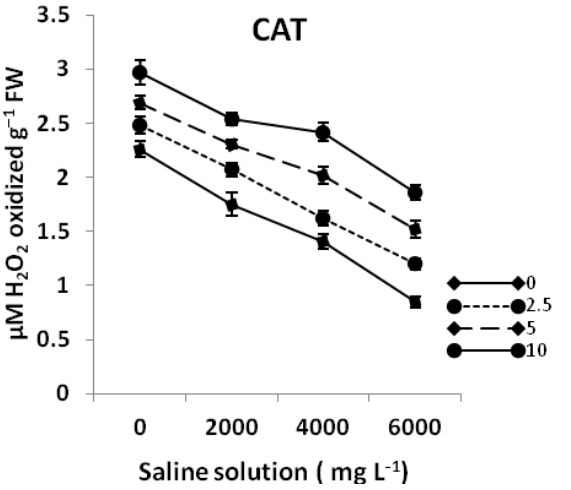
The effect of Se was opposite to that of salinity, by causing a significant decrease in the activities of SOD and POD and a significant increase in CAT activities as compared with plants receiving the same salinity level and not treated with Se. The effect of Se was much more pronounced in plants treated with 6000 mg L^{-1} of salt.

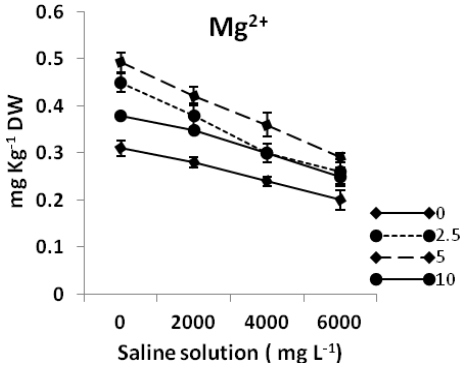
Inorganic cations:

The amounts of Mg^{2+}, Ca^{2+}, K^{+}, and P^{3+} were significantly decreased by increasing the level of salt treatment to reach 64.5%, 68%, 65.6% and 35.5% of the control value, respectively in plants treated with 6000 mg L^{-1} , P^{3+} is the most affected ion by salt treatment (Fig. 2).









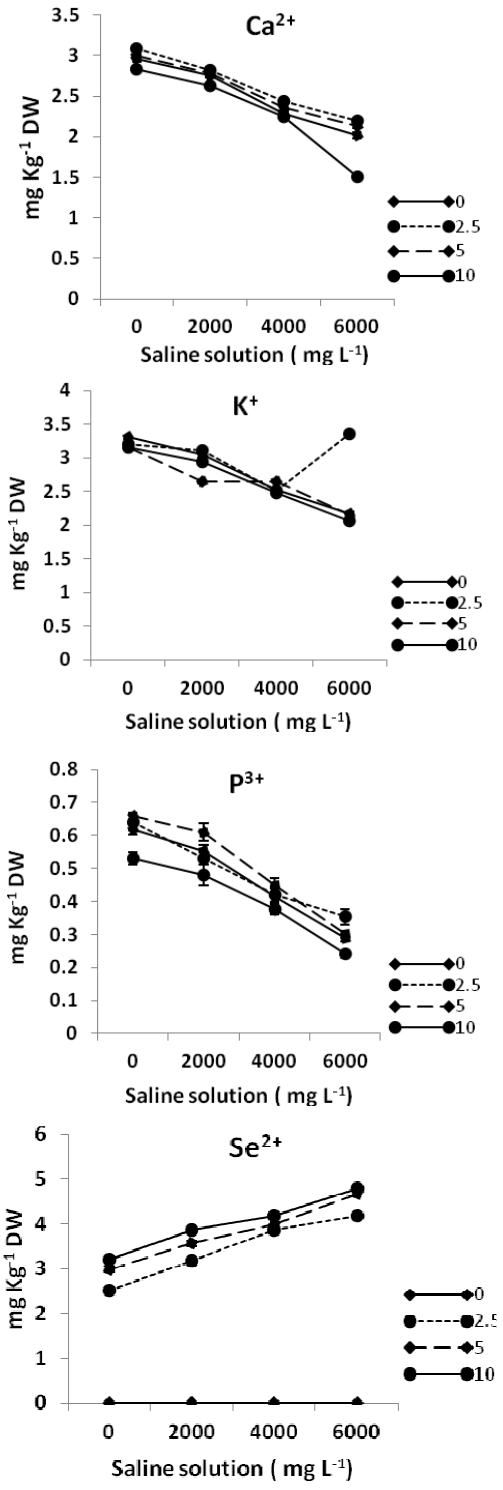


Fig. 2. Changes in inorganic cations content of leaves of canola plants (72-days from sowing) treated with different concentrations of selenium (0, 2.5, 5 and 10 mg L^{-1}) and grown under different levels of salinity. The data shown are mean of three replicates (Data presented as mean ± SD).

Se treatment had no significant influence on Ca^{2+} and K^{+} content in comparison to the corresponding controls, except in plants treated with 6000 mg L^{-1} salt where the 2.5 mg L^{-1} Se caused a significant increase (54.4%) in K^{+} content compared to plants treated with the same salt concentration alone and the 10 mg L^{-1} Se which caused a significant reduction (25%) in Ca^{2+} content compared to plants not treated with Se and grown under the same salt level.

Se treatment affected P^{3+} content in canola plant, such effect varied depending on the applied concentration of Se. Lower concentration of Se (2.5 and 5 mg L^{-1}) significantly increased P^{3+} content in plants grown under normal condition or subjected to different levels of salinity. This stimulatory effect was more pronounced in plants sprayed with 5 mg L^{-1}. On the other hand, higher level of Se (10 mg L^{-1}) significantly reduced P^{3+} content by 14%, 12.7%, 8.7% and 17.2% in plants treated with 0, 2000, 4000 and 6000 mg L^{-1} salt, respectively compared to untreated plants grown under the same levels of salinity.

For Mg^{2+} content measured in canola leaves, there was a significant reduction in plants subjected to salt stress compared to control plants, the lowest Mg^{2+} content (64.5% of the control value) was recorded in plants treated with 6000 mg L^{-1} salt. In addition, all the tested Se concentrations significantly increased the amounts of Mg^{2+} in canola leaves grown under normal condition or treated with different levels of salinity compared to corresponding control plants untreated with Se.

Considerable amounts of Se were accumulated in canola leaves sprayed with Se, the endogenous Se concentration was positively related to the applied concentration of Se and the used level of salt stress to reach its maximum value (4.8 mg kg^{-1} DW) in plants sprayed with 10 mg L^{-1} Se and treated with 6000 mg L^{-1} saline solution.

Oil content and fatty acids composition of Seeds:

The results showed that salt stress significantly decreased canola seed oil content, the reduction was directly proportional to the applied concentration of salt (Table 4). On the other hand, Se treatment has a positive impact on seed oil content, where, 5 mg L^{-1} Se is the most efficient treatment.

Fatty acids composition of canola seeds supplemented with salt, Se and their combination are presented in table 6. Salt treatment significantly increased the total saturated fatty acids, including palmitic acid, stearic acid, arachidic acid and behenic acid. The increase was directly proportional to the applied concentration of salt. On the other hand, the unsaturated fatty acids (including oleic acid, gadoleic acid, linoleic acid and linolenic acid) were significantly decreased in salt stressed canola seeds as compared with control seeds. Of the unsaturated fatty acids detected in canola seeds, erucic acid is the only one which showed a significant increase in response to salt treatment. The reduction in oleic, linoleic and linolenic acid in seeds of

salt stressed plants was evaluated by 7%, 4.8%, and 9.3%, respectively in response to 6000 mg L^{-1} saline solution. The effect of salt stress on saturated and unsaturated fatty acid contents resulted in declines in total unsaturated / total saturated fatty acids evaluated by 14.5%, 20.47% and 34.68% in seeds subjected to 2000, 4000, and 6000 mg L^{-1} saline solution, respectively compared to the seed of the control. In contrast to salt treatment, seeds of canola plants grown under normal conditions or treated with salt and sprayed with different doses of Se have a significantly higher total unsaturated / total saturated fatty acids value compared to reference controls. The highest content of oleic, linoleic and linolenic acid was detected in seeds of canola plants treated with 5 mg L^{-1} Se and was evaluated by 107.7%, 106.8%, 105.6%, and 101.2% in case of oleic acid, 114%, 112%, and 110.8% and 109.5% in case of linoleic acid and 116.5%, 108%, 105.9%, and 102.4% in case of linolenic acid in seeds of plants received 5 mg L^{-1} Se and treated with 0, 2000, 4000, and 6000 mg L^{-1} salt, respectively compared to their values in control. This dose of Se (5 mg L^{-1}) also resulted in the maximum decline in erucic acid content as compared with untreated plants or plants sprayed with other concentrations of Se.

Table 6. Effect of different dosages of selenium (0, 2.5, 5, and 10 mg L^{-1}) on fatty acids composition (mol%) of seeds of canola plants grown under different levels of salinity (140-days from sowing). The data shown are mean of three replicates.

		Saturated fatty acids				Unsaturated fatty acids									
Selenium (mg L^{-1})	Salinity (mg L^{-1})	Palmitic Acid C16:0	Stearic Acid C18:0	Arachidic Acid C20:0	Behenic Acid C22:0	Oleic Acid C18:1	Erucic Acid C22:1	Gadoleic Acid C20:1	Linoleic Acid C18:2	Linolenic Acid C18:3	Total known fatty acids	Total unknown fatty acids	Total sat. fatty acids	Total unsat. fatty acids	Total unsat. / total sat. fatty acids
0	0	5.10 ±0.13	2.85 ±0.05	1.70 ±0.03	0.91 ±0.03	57.70 ±0.24	0.60 ±0.03	4.25 ±0.05	15.66 ±0.04	7.98 ±0.18	96.75 ±0.58	3.25 ±0.58	10.56 ±0.17	86.19 ±0.43	8.16 ±0.10
	2000	5.55 ±0.18	3.13 ±0.06	2.23 ±0.03	1.13 ±0.03	56.43 ±0.10	0.68 ±0.02	3.97 ±0.06	15.48 ±0.03	7.51 ±0.10	96.11 ±0.26	3.89 ±0.26	12.04 ±0.20	84.07 ±0.09	6.98 ±0.11
	4000	5.72 ±0.10	3.42 ±0.03	2.55 ±0.05	1.07 ±0.59	55.51 ±0.26	0.75 ±0.02	3.81 ±0.05	15.22 ±0.04	7.41 ±0.11	95.45 ±0.23	4.55 ±0.23	12.76 ±0.47	82.70 ±0.28	6.49 ±0.27
	6000	6.51 ±0.15	3.92 ±0.04	2.87 ±0.06	1.75 ±0.05	53.65 ±0.31	0.88 ±0.02	3.65 ±0.06	14.90 ±0.06	7.15 ±0.15	95.28 ±0.44	4.72 ±0.44	15.05 ±0.0	80.23 ±0.44	5.33 ±0.03
2.5	0	4.55 ±0.10	2.62 ±0.09	1.51 ±0.05	1.10 ±0.59	60.53 ±0.12	0.39 ±0.01	3.33 ±0.05	16.18 ±0.07	8.54 ±0.06	98.75 ±0.69	1.25 ±0.69	9.78 ±0.72	88.97 ±0.09	9.13 ±0.65
	2000	4.77 ±0.07	2.90 ±0.03	1.75 ±0.04	0.95 ±0.04	59.12 ±0.15	0.50 ±0.02	3.11 ±0.04	15.83 ±0.04	8.28 ±0.18	97.21 ±0.43	2.79 ±0.43	10.37 ±0.07	86.84 ±0.36	8.37 ±0.03
	4000	4.91 ±0.09	3.10 ±0.04	2.33 ±0.55	1.17 ±0.02	58.66 ±0.16	0.61 ±0.03	3.00 ±0.05	15.60 ±0.07	8.02 ±0.13	97.40 ±0.78	2.60 ±0.78	11.51 ±0.54	85.89 ±0.27	7.47 ±0.32
	6000	5.05 ±0.20	3.34 ±0.05	2.17 ±0.07	1.37 ±0.05	56.25 ±0.22	0.78 ±0.03	2.89 ±0.02	15.27 ±0.04	7.87 ±0.17	95.99 ±0.61	4.01 ±0.61	11.93 ±0.27	84.06 ±0.38	7.05 ±0.13
5	0	3.95 ±0.05	2.35 ±0.07	1.30 ±0.03	0.56 ±0.04	62.17 ±0.08	0.18 ±0.01	1.50 ±0.04	17.92 ±0.07	8.91 ±0.11	98.84 ±0.05	1.16 ±0.05	8.16 ±0.03	90.68 ±0.07	11.11 ±0.04
	2000	4.15 ±0.15	2.57 ±0.06	1.62 ±0.03	0.78 ±0.04	61.65 ±0.13	0.22 ±0.03	1.33 ±0.03	17.59 ±0.10	8.63 ±0.10	98.54 ±0.22	1.46 ±0.22	9.12 ±0.16	89.42 ±0.17	9.81 ±0.18
	4000	4.33 ±0.04	2.84 ±0.06	1.81 ±0.04	0.90 ±0.02	60.93 ±0.21	0.35 ±0.02	1.15 ±0.04	17.36 ±0.05	8.45 ±0.15	98.12 ±0.20	1.88 ±0.20	9.88 ±0.03	88.24 ±0.19	8.93 ±0.03
	6000	4.60 ±0.06	3.00 ±0.04	2.11 ±0.04	1.20 ±0.03	58.41 ±0.22	0.43 ±0.01	0.83 ±0.06	17.15 ±0.10	8.17 ±0.17	95.90 ±0.21	4.10 ±0.21	10.91 ±0.09	84.99 ±0.22	7.79 ±0.07
10	0	4.22 ±0.09	2.23 ±0.04	1.63 ±0.04	0.83 ±0.02	59.81 ±0.27	0.49 ±0.02	3.65 ±0.04	16.55 ±0.10	8.33 ±0.13	97.74 ±0.43	2.26 ±0.43	8.91 ±0.14	88.83 ±0.30	9.97 ±0.13
	2000	4.46 ±0.06	2.40 ±0.05	1.85 ±0.03	1.84 ±0.04	59.15 ±0.13	0.56 ±0.03	3.22 ±0.04	16.30 ±0.15	8.13 ±0.12	97.91 ±0.50	2.09 ±0.50	10.55 ±0.07	87.36 ±0.44	8.28 ±0.02
	4000	4.80 ±0.07	2.65 ±0.04	2.03 ±0.08	1.18 ±0.03	57.83 ±0.19	0.63 ±0.03	3.10 ±0.05	15.92 ±0.12	7.85±0.10	95.99 ±0.17	4.01 ±0.17	10.66 ±0.15	85.33 ±0.04	8.00 ±0.11
	6000	4.96 ±0.11	2.88 ±0.05	2.21 ±0.06	1.30 ±0.03	56.42 ±0.39	0.70 ±0.03	2.77 ±0.06	15.40 ±0.20	7.53 ±0.06	94.25 ±0.65	5.75±0.65	11.35 ±0.21	82.90 ±0.44	7.31 ±0.10
L.S.D ($p \leq 0.05$)															
Salinity		0.061	0.122	0.086	0.106	0.137	0.061	0.086	0.061	0.102	0.280	0.280	0.173	0.183	0.106
Selenium		0.061	0.122	0.086	0.106	0.137	0.061	0.086	0.061	0.102	0.280	0.280	0.173	0.183	0.106
Salinity*Selenium		0.245	0.490	0.346	0.424	0.548	0.245	0.346	0.245	0.410	1.123	1.123	0.693	0.735	0.424

DISCUSSION:

High salt concentrations decrease the osmotic potential of soil solution creating a water stress in plants. In addition, they cause severe ion toxicity, since Na^+ is not readily sequestered into vacuoles as in halophytes. Finally, the interactions of salts with mineral nutrition may result in nutrient imbalances and deficiencies. The consequence of all these can ultimately lead to plant death as a result of growth arrest and molecular damage (McCue and Hanson, 1990). The present study revealed that salt treatment (2000, 4000, and 6000 mg L^{-1}) has a significant inhibitory effect on plant growth (including shoot length, number of leaves $plant^{-1}$, leaves area $plant^{-1}$ and fresh and dry weights of shoots), yield components (including number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds pod^{-1}, weight of seeds $plant^{-1}$ and seed index), photosynthetic pigments content, quantity and quality (as indicated by decreased oleic acid; omega 9 fatty acid , linoleic acid; omega 6 fatty acid and linolenic acid; omega 3 fatty acid and increased erucic acid content) of seeds oil content. Such inhibition was directly related to the applied concentration of salt, with maximum reduction in plants received the highest level of salt (6000 mg L^{-1}).

Similar results were obtained by Zamani *et al*. (2010) who found that, the most common adverse effect of salinity on *Brassica* species is the reduction in plant height, size and yield as well as deterioration of the product quality. The salinity may reduce the crop yield by upsetting water and nutritional balance of plant (Francois, 1994; Islam *et al.*, 2001). Water availability and nutrient uptake by plant roots is limited because of high osmotic potential and toxicity of Na and chlorine (Cl) ions (Kumar, 1995).

The reduction of chlorophyll a and chlorophyll b with NaCl application was reported in many plants such as *Zea mays*, *Carthamus tinctorius*, bean and *Paulownia imperiallis* and this due to increasing the destructive enzymes chlorophyllase (Rahdari *et al.*, 2012). Reduction in the pigments system is attributed to the induced weakening of protein-pigment-lipid complex by salt or to the increase in the chlorophyllase activity (Turan *et al.*, 2007). The changes in pigment system were affected by exposure time and salt concentration (Doganlar *et al.*, 2010).

On the other hand, application of saline solution had a significant stimulatory effect on proline and carbohydrates (including soluble sugars and polysaccharides) accumulation as compared with control. The detected increase in these compounds in canola plants was found to be directly proportional to the applied concentration of salt. The accumulation of these compatible solutes (proline and sugars) is proportional to the change of external osmolarity accommodates the ionic balance in the vacuoles and cytoplasm (Hasegawa *et al.*, 2000) protects cell structures and osmotic balance supporting continued water influx (or reduced efflux) (Parida and Das, 2005). The arrested growth rate accompanied by accumulation in carbohydrates content can be attributed to the decreased rate of degradative metabolism particularly at the high concentration of salinity used.

Activities of SOD and POD were also significantly increased by increasing salt stress level in canola plants. Tuteja (2007) stated that the major ROS-scavenging mechanisms of plants include superoxide dismutase, ascorbate peroxidase, catalase, and GSH reductase, which help in the deactivation of active oxygen species in multiple redox reactions, thereby contributing to the protective system against oxidative stress. Increase in activity of SOD and POD in response to salinity stress, as well as higher antioxidant activity in tolerant species/varieties have also been reported by various workers (Gomez *et al.*, 1999; Sreenivasulu *et al.*, 2000).

The present work proved that application of 2.5 and 5.0 mg L^{-1} Se significantly enhanced canola growth under normal and salt stress conditions as compared with untreated plants grown under the same conditions. Probably the first positive effect of Se on plant growth was reported by Singh *et al.* (1980), who showed that the application of 0.5 mg kg^{-1} Se as selenite stimulated growth and dry-matter yield of Indian mustard (*Brassica juncea* L.). More recently, it was revealed that Se, applied at low concentrations, enhanced growth and antioxidative capacity of both mono- and dicotyledonous plants (Hasanuzzaman *et al.*, 2010). On the other hand, the present results showed that relatively higher concentration of Se (10 mg L^{-1}) has an inhibitory effect on canola growth indicating that the effect of Se on canola plants growth is concentration dependent. According to the current results 5 mg L^{-1} Se is the optimum stimulatory concentration for canola growth.

The growth-promoting function of low dosages of Se was associated with a significant increase in photosynthetic pigments content. The addition of appropriate levels of Se can restore the damage of the chloroplasts and increase the chlorophyll contents (Chu *et al.*, 2010; Wang, 2011; Yao *et al.*, 2011; Malik *et al.*, 2012). In addition, through proteomic analysis, Wang *et al.* (2012) revealed that in rice (*Oryza sativa* L.) seedlings, low doses of Se enhanced photosynthesis. Moreover, in sorghum, Se application significantly increased the photosynthetic rate, stomatal conductance and transpiration rate (Djanaguiraman *et al.*,

2010). The restoration of photosynthesis in stressed plants after Se application may be closely related to the decreased ROS levels, reactivation of antioxidants, restored structure of the damaged chloroplasts and enhanced production of other vital metabolites. Despite the observed increase in canola growth and photosynthetic pigments content, it was found in the present results that carbohydrate contents including soluble sugars and polysaccharides were, in most cases, significantly decreased in response to Se treatment. Further investigations should be done to detect the efficiency of photosynthesis in Se-treated plants and the fate of the produced carbohydrates.

Positive correlation between accumulation of endogenous proline and improved salt tolerance has been found (Tyagi *et al.*, 1999). In the present work foliar spray of canola plants with different concentrations of Se caused a significant increase in proline content compared to untreated plants. This could be due to the effect of Se on proline metabolism enzymes. High proline synthesis in stressed plants could be a possible defense mechanism for survival under salt stress condition.

The present results indicated that Se treatment resulted in a significant decrease in the activities of SOD and POD and a significant increase in CAT activities as compared with untreated plants, this effect of Se was detected in plants growing under normal or salt stress conditions. In this respect, Habibi (2013) working on barley postulated that the significant rise in the activity of CAT and GSH-Px in Se supplemented water – deficit samples relative to water – deficit treatment revealed that Se exerts beneficial effects on stress tolerance of barley by enhancing their antioxidant capacity. Many other authors observed that Se significantly increased the reactivity of CAT (Yao *et al.*, 2011; Malik *et al.*, 2012). Decreases in the ROS levels upon Se addition in plants subjected to salt and drought stresses have been reported in rape seed seedlings (Hasanuzzaman *et al.*, 2011; Hasanuzzaman and Fujita, 2011) and in *Trifolium repens* L. (Wang, 2011). Three possible mechanisms have been proposed for the decrease in O2•− levels when the appropriate doses of Se were added, including the spontaneous dismutation of O2•− into H_2O_2 (without catalysis by the SOD enzyme) (Hartikainen *et al.*, 2000**;** Cartes *et al.*, 2010), the direct quenching of O2•− and OH• by Se compounds (Xue *et al.*, 1993), and the regulation of antioxidative enzymes (Habibi, 2013). However, in their *in vitro* experiment Xue *et al.* (1993) found that Se compounds failed to directly scavenge H_2O_2. In the light of the present results it could be proposed that the Se – ROS detoxification mechanism in rape plants subjected to salt stress takes place by direct dismutation of O2•− and OH• into H_2O_2 by Se compounds followed by H_2O_2 scavenging by CAT enzyme.

The present data revealed that Se treatment had a positive effect on P^{3+} and Mg^{2+} content of canola plants growing under normal or salt stress condition with 5 mg L^{-1} Se the most effective concentration. The regulation of the uptake and redistribution of some essential elements by Se is believed to be an important mechanism to reactivate associated antioxidants, reduce the ROS levels and improve plant tolerance to stress (Feng *et al.*, 2013). However, the information concerning the effects of Se on the uptake of essential elements in stressed plants is insufficient.

Interestingly, according to the present results, Se was found to have a positive impact on the quality of canola seed oil. Significant increase was detected in oleic, linoleic and linolenic acid contents of seeds oil produced by Se-treated plants. Linoleic and linolenic acid are among the most important components of the oil, because they are dietary essential fatty acids, preventing nutritional deficiency symptoms, and are not produced by humans (Omidi *et al.*, 2010). In addition, Se treatment resulted in lower eruric acid contents in canola oil. Lowering of erucic acid in response to exogenously applied Se make canola oil more appropriate for the market standards and safer for human consumption. Despite the finding of increased vegetative growth and reproductive capacity, in terms of seed production and viability in different *Brassica* species associated with exogenous application of Se (Hajiboland and Amjad, 2007; Lyons *et al.*, 2009; Hajiboland and Keivanfar, 2012), the present study is the first definitive evidence to date of the improvement of canola oil quality in response to Se treatment.

CONCLUSION:

The present study indicated that saline irrigation presents potential hazards to canola plants. Salinity led to significant inhibition in canola growth, yield components, photosynthetic pigments content, quantity and quality of seeds oil. The detected inhibition was directly related to the applied concentration of salt. The protective role of Se on salt-stressed canola plants which was observed in the increase in plant growth, yield and photosynthetic pigments content is not unambiguous. It was found to be attributed to the defense mechanisms induced upon Se treatment including increased endogenous proline content, enhanced catalase activity and increased magnesium and phosphorus contents. The results also revealed that Se improves canola oil quality. This is an important finding as in recent years, due to the adaptation of canola to different climatic

conditions; their seed oil has been the center of attention for its use for nutritional, industrial and pharmaceutical usages. The most effective concentration of Se was 5 mg L^{-1} that is recommend to be used to achieve the best growth and yield for canola crop under normal or salt stress conditions.

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REFERENCES:

AOAC. 1990. Official methods of analysis. 20th edition. Association of official analytical chemists, Arlington, Virginia, USA.

Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol., 141 (2): 391–396.

Bates LS, Wladren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39(1): 205-207.

Cao XD, Ma LQ, Tu C. 2004. Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). Environ. Pollu., 12(3): 317–325.

Cartes P, Jara AA, Pinilla L, Rosas A, Mora ML. 2010. Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. Ann. Appl. Biol., 156(2): 297–307.

Chapman HD, Pratt FP. 1982. Method and of analysis of soil, plant and water. 2nd Edn. California: California University Agricultural Division, pp: 170.

Chen Y, Cao XD, Lu Y, Wang XR. 2000. Effects of rare earth metal ions and their EDTA complexes on antioxidant enzymes of fish liver. B. Environ. Contam. Tox., 65(3): 357–365.

Chu JZ, Yao XQ, Zhang ZN. 2010. Responses of wheat seedlings to exogenous selenium supply under cold stress. Biol. Trace Elem. Res., 136(3): 355–363.

Dhindsa RS, Plumb-Dhinsda P, Thorne PA. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32(1): 93-101.

Djanaguiraman M, Durga Devi D, Shanker AK, Sheeba JA, Bangarusamy U. 2005. Selenium an antioxidative protectant in soybean during senescence. Plant Soil, 272(1-2): 77– 86.

Djanaguiraman M, Prasad PVV, Seppänen M. 2010. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhanc-ing antioxidant defense system. Plant Physiol. Biochem., 48(12): 999-1007.

Doganlar ZB, Demir K, Basak H, Gul I. 2010. Effects of salt stress on pigment and total soluble protein contents of the three different Tomato cultivars. Afr. J. Agri., 5(15): 2056-2065.

Fedak G, De La Roche I. 1977. Lipid and fatty acid composition of barley kernels. Can. J. Plant Sci., 57(1): 257-260.

Feng R, Wei C, Tu S. 2013. The roles of selenium in protecting plants against abiotic stresses. Environ. Exp. Bot., 87: 58-68.

Foucart T. 1982. Analyse factorielle, programmatiol sur micro-ordinateur. Masson, ITCF, Paris.

Francois LE. 1994. Growth, seed yield, and oil content of canola grown under saline environments. Agron. J., 86(2): 233-237.

Gomez JM, Hernandez JA, Jimenez A, del Rio LA, Sevilla F. 1999. Differential response of antioxidative systems of chloroplasts and mitochondria to long term NaCl stress of pea plant. Free Radical Res., 31 (Suppl): 11–18.

Habibi G. 2013. Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley. Acta Agri. Slov., 101(1): 31-39.

Hajiboland R, Amjad L. 2007. Does antioxidant capacity of leaves play a role in growth response to selenium at different sulfur nutritional status? Plant Soil Environ., 53(5): 207-215.

Hajiboland R, Keivanfar N. 2012. Selenium supplementation stimulates vegetative and reproductive growth in canola (*Brassica napus* L.) plants. Acta Agri. Slov., 99(1): 13-19.

Hartikainen H, Xue T, Piironen V. 2000. Selenium as an antioxidant and pro-oxidant in ryegrass. Plant Soil, 225(1-2): 193-200.

Hasanuzzaman M, Fujita M. 2011. Selenium pretreatment upregulates the antiox-idant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol. Trace Elem. Res., 143(3): 1758–1776.

Hasanuzzaman M, Hossain A, Fujita M. 2010. Selenium in Higher Plants: Physiological Role, Antioxidant Metabolism and Abiotic Stress Tolerance. J. Plant Sci., 5(4): 354-375.

Hasanuzzaman M, Hossain MA, Fujita M. 2011. Selenium-induced up-regulation of the antioxidant defense and methylglyoxal detoxification system reduces salinity-induced damage in rapeseed seedlings. Biol. Trace Elem. Res., 143(3): 1704–1721.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol., 51: 463–499.

Hodge JE, Hofreiter BT. 1962. Determination of reducing sugars and carbohydrates. In: "Methods in carbohydrate chemistry. (Whistler RL, Walfrom ML. Eds)". Academic Press, New York, London, pp. 380-394.

Hu Q, Xu J, Pang G. 2003. Effect of selenium on the yield and quality of green tea leaves harvested in early spring. J. Agric. Food Chem. 51(11): 3379–3381.

Islam MR, Bhuiyan MAR, Prasad B, Quddus MA. 2001. Salinity effect on yield and component

characters in rapeseed and mustard varieties. J. Biol. Sci., 1(9): 840-842.

Kumar D. 1995. Salt tolerance in oilseed brassicas-present status and future prospects. Plant Breed. Abstr., 65(10): 1439-1477.

Lyons GH, Genc Y, Soole K, Stangoulis JCR, Liu F, Graham RD. 2009. Selenium increases seed production in *Brassica*. Plant Soil, 318(1-2): 73-80.

Malik CP, Singh MB. 1980. Plant Enzymology and Histoenzymology. Kalyani Publishers, New Delhi, pp. 53.

Malik JA, Goel S, Kaur N, Sharma S, Singh I, Nayyar H. 2012. Selenium antag-onises the toxic effects of arsenic on mungbean (*Phaseolus aureus* Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. Environ. Exp. Bot., 77: 242–248.

McCue KF, Hanson AD. 1990. Salt-inducible betaine aldehyde dehydrogenase from sugar beet: cDNA cloning and expression. Plant Mol. Biol., 8(1): 1-11

Metzner H, Rau H, Senger H. 1965. Untersuchungen Zur Synchronisier barkeit einzelner Pigmentmangel-Mutanten *Von Chlorella*. Planta, 65(2): 186-194.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7(9): 405–410.

Mukherjee SP, Choudhury MA. 1983. Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plantarum, 58(2): 166-170.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59: 651–681.

Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25(2): 239–250.

Omidi H, Tahmasebi Z, Naghdi Badi HA, Torabi H, Miransari M. 2010. Fatty acid composition of canola (*Brassica napus* L.), as affected by agronomical, genotypic and environmental parameters. C. R. Biol., 333(3): 248–254.

Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicol. Environ. Saf. 60(3): 324-349.

Pezzarossa B, Remorini D, Gentile ML, Massai R. 2012. Effects of foliar and fruit addition of sodium selenate on selenium accumulation and fruit quality. J. Sci. Food Agric., 92(4): 781–786.

Prud'homme MP, Gonzalez B, Billard JP, Boucaud J. 1992. Carbohydrate content, fructan and sucrose enzyme activities in roots, stubble and leaves of ryegrass (Lolium perenne L.) as affected by source/sink modification after cutting. J. Plant Physiol., 140(3): 282-291.

Rahdari P, Tavakoli S, Hosseini SM. 2012. Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in Purslane (*Portulaca oleraceae* L.) leaves. Stress Physiol. Bio. J., 8(1): 182-193.

Ranganna S.1977. Manual Analysis of Fruit and Vegetable Products. Tata MC craw-Hill publishing company Limited New York.

Schwarz K, Foltz CM. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. 1951. Nutrition, 15(3): 255.

Scott TA, Melvin EH. 1956. Anthrone colorimetric method. In: Whistler RL, Walfrom ML (ed.) Methods in Carbohydrate Chemistry. Academic Press, New York, London, Vol 1, pp. 384.

Singh M, Singh H, Bhandari DK. 1980. Interaction of selenium and sulphur on the growth and chemical composition of raya. Soil Sci., 129(4): 238-244.

Slathia S, Sharma A, Choudhary SP. 2012. Influence of exogenously applied epibrassinolide and putrescine on protein content, antioxidant enzymes and lipid peroxidation in *Lycopersicon esculentum* under salinity stress. Am. J. Plant Sci., 3(6): 714-720.

Snedecor GW, Cochran WG. 1990. Statistical Methods, 11th ed. Iowa State Univ. Press, Ames, Iowa, USA.

Soltanpour PN. 1985. Use of ammonium bicarbonate DTPA soil test to evaluate elemental availability and toxicity soil. Commun. Sci. Plan., 16(3): 333-338.

Sreenivasulu N, Grimm B, Wobus U, Weschke W. 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of fox-tail millet (*Setaria italica*). Physiol. Plantarum, 109(4): 435–442.

Stroganov BP. 1962. Physiological basis of salt tolerance of plants as affected by various types of salinity. Izadatel 'Stvo Akademii Nauk, USSR, pp. 279.

Tapiero H, Townsend DM, Tew KD. 2003. The antioxidant role of selenium and seleno-compounds. Biomed. Pharmacother, 57(3-4): 134-144.

Turakainen M, Hartikainen H, Seppanen MM. 2004. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J. Agric. Food Chem., 52(17): 5378–5382.

Turan MA, Katkat V, Taban S. 2007. Variations in proline, chlorophyll and mineral elements contents of Wheat plants grown under salinity stress. Agron. J., 6(1): 137-141.

Tuteja N. 2007. Mechanisms of high salinity tolerance in in plants. Method. Enzymo., 428: 419-438.

Tyagi A, Santha IM, Mehta SL. 1999. Effect of water stress on proline content and transcript levels in *Lathyrus sativus*. Indian J. Biochem. Biophys., 36(3): 207–210.

Wang CQ. 2011. Water-stress mitigation by selenium in *Trifolium repens* L. J. Plant Nut. Soil Sci., 174(2): 276–282.

Wang YD, Wang X, Wong YS. 2012. Proteomics analysis reveals multiple reg-ulatory mechanisms in response to selenium in rice. J. Proteomics, 75(6): 184–1866.

Weems YS, Bridges PJ, LeaMaster BR, Sasser RG, Vincent DL, Weems CW, Kong FX, Hu W, Chao SY, Sang WL, Wang LS. 1999. Physiology responses of the lichem Xanthoparmelia mexicana to oxidative stress of SO2. Environ. Exp. Bot., 42(3): 201–209.

Xue TL, Hartikainen H, Piironen V. 2001. Antioxidative and growth-promoting effect of selenium on senescing lettuce. Plant Soil, 237(1): 55–61.

Xue TL, Hou SF, Tan JA, Liu GL. 1993. The antioxidative function of selenium in higher plants: II. Non-enzymatic mechanisms. Chinese Sci. Bull., 38: 356–358 (in Chinese).

Yao X, Chu J, He X, Ba C. 2011. Protective role of selenium in wheat seedlings subjected to enhanced UV-B radiation. Russ. J. Plant Physiol., 58(2): 283-289.

Zamani Z, Nezami MT, Habibi D, Khorshidi MB. 2010. Effect of quantitative and qualitative performance of four canola cultivars (*Brassica napus* L.) to salinity conditions. Adv. Environ. Biol., 4(3): 422-427.

الدور الواقي لعنصر السيلينوم في نباتات الكانولا (براسيكا نابا) المعرضة للاجهاد الملحي

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تهدف هذه الدراسة الي تحديد التأثير الواقي المحتمل لعنصر السيلينيوم علي نبات الكانولا (براسيكا نابا) المتعرض للاجهاد الملحي عن طريق قياس دالات النمو، مكونات المحصول والتغيير في محتوي أصباغ البناء الضوئي، البرولين، بعض المعادن، نشاط بعض الانزيمات المضادة للأكسدة وتركيب الأحماض الدهنيه في البذور الناتجة من النباتات المعاملة. لهذا الغرض تم معامله نباتات الكانولا بتركيزات مختلفه من محلول ملحي تم تحضيره وفقا لمعادله ستراجانوف (0، 2000، 4000 و 6000 ملجم / لتر) ثم تم رش النباتات مرتين عند عمر 50 ، 65 يوما من الزراعة بجرعات مختلفة من السيلينوم (0 ، 2.5 ، 5 و 10 ملجم /لتر في صوره سيلينات الصوديوم). وأخذت العينات عند عمر 72 يوما من الزراعة. أدت الملوحة إلي تثبيط واضح في نمو النباتات ونقص في المحصول، محتوى أصباغ البناء الضوئي، كمية وجودة الزيت المستخلص من البذور.وقد كان التثبيط يتناسب طرديا مع مستوي الأجهاد الملحي الذي تعرضت له النباتات. كما أثبتت النتائج ان عنصر السيلينيوم يعمل علي زيادة نمو النباتات ،المحصول الناتج ، محتوي أصباغ البناء الضوئي ويعمل علي تحسين جوده زيت الكانولا الناتج سواء في النباتات المتعرضه للاجهاد الملحي أو التي تنمو في الظروف العادية. وقد وجدنا أن اكثر تركيزات السيلينيوم فاعلية هو تركيز 5 مليجم / لتر. بالأضافة الي ذلك فأن النباتات المعاملة بالسيلينيوم أظهرت العديد من اليات الدفاع لمواجهة تأثير اجهاد الملوحة تتمثل في زياده محتوي البرولين، نشاط انزيم الكاتاليز وزيادة محتوي أيونات الماغنسيوم والفسفور.

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