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Selenium supplementation stimulates vegetative and reproductive growth in canola (*Brassica napus* L.) plants

Roghieh HAJIBOLAND¹ and Nasrin KEIVANFAR²

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ABSTRACT

Selenium (Se) is a beneficial element for higher plants and its positive effect on plants growth and performance has been reported. Effect of Se under non-stress conditions especially during reproductive phase has not been attracted enough attention. In this work effect of Se supplementation at 0, 10 and 20 μg Se plant⁻¹ was studied in canola (*Brassica napus* 'RGS') plants during vegetative and reproductive phase of growth under greenhouse conditions. Selenium addition resulted in a significant enhancement of dry matter production of vegetative parts as well as pod and seed dry weight. In addition, Se supplementation caused a considerable acceleration of reproductive events. In vegetative plants, higher photosynthesis rate, carbohydrates and protein content in the leaves was observed in Se treated plants compared with control. Our results suggested beneficial effect of Se on canola seed yield that may also contribute in improving nutritional value of canola for livestock and human.

Key words: flowering, pod dry weight, reproductive phase, seed yield

IZVLEČEK

DODATEK SELENA POSPEŠUJE RAST IN REPRODUKCIJO PRI RASTLINAH KANOLE (*Brassica napus* L.)

Selen (Se) je za višje rastline koristen element, znani so njegovi ugodni vplivi na rast rastlin. Pri rastlinah, ki rastejo v nestresnih razmerah, zlasti v reproduktivni fazi razvoja, učinki Se še niso bili ustrezno raziskani. V tem delu so avtorji raziskovali dodatek Se (0, 10 in 20 μg Se na rastlino) pri kanoli (*Brassica napus* L., cv. RGS) tekom vegetativne in reproduktivne faze. Dodatek Se je povzročil značilno povečanje sušine vegetativnih delov, luskov in semen. Dodatek Se je vplival tudi na pospešitev poteka reprodukcije. Pri rastlinah je bila ugotovljena tudi povečana fotosinteza, večja vsebnost ogljikovih hidratov in beljakovin v listih tretiranih rastlin v primerjavi s kontrolo. Rezultati kažejo na ugoden vpliv Se na pridelek kanole, kar lahko vpliva tudi na izboljšano hranilno vrednost te poljščine za živali in ljudi.

Ključne besede: cvetenje, sušina luskov, reproduktivna faza, pridelek semen

1 INTRODUCTION

Selenium (Se) has long been recognized as an essential micronutrient for animal and human nutrition, but the essentiality of Se to higher plants is still under debate (Terry *et al.*, 2000; Germ *et al.*, 2007). Growth stimulating effect of trace amounts of Se has been frequently reported in some plant species such as ryegrass (Hartikainen *et al.*, 2000), lettuce (Xue *et al.*, 2001), potato (Seppänen *et al.*, 2003) and different varieties of *Brassica oleracea* (Hajiboland and Amjad 2007). At proper levels it also delays some of the effects of senescence (Djanaguiraman *et al.*, 2005). The

growth-promoting response of Se is mainly accompanied with the enhanced antioxidative capacity manifested in decreasing lipid peroxidation, marked increase in the activity of antioxidant enzymes and a peak concentration of antioxidant metabolites (Xue *et al.*, 2001). Selenium stimulates plants growth even under non-stress conditions. A considerable growth promotion up to 59% has been reported for cabbage plants in response to Se supplementation at 20 μM (Hajiboland and Amjad 2007).

¹ Associate Professor of Plant Physiology, Plant Science Department, University of Tabriz, 51666-14779 Tabriz, Iran

² M.Sc. Student of Plant Physiology, Plant Science Department, University of Tabriz, 51666-14779 Tabriz, Iran

While much is known about the effect of Se on vegetative growth particularly under stressful conditions, published works on the effects of Se on reproductive events are rare. Effect of Se on seed yield was studied in soybean (Djanaguiraman *et al.*, 2004) plants. Spraying leaves with Se increased seed yield in soybean likely due to a better partitioning efficiency, as evidenced by greater number of pods per plant, seeds per pod and seed weight (Djanaguiraman *et al.*, 2004). However, effect of Se on the time of flowering and other characteristics of reproductive growth has not been investigated so far.

Species of Brassicaceae are able to take up more sulfur from medium and also need higher sulfur on dry weight basis compared with Graminae and Leguminosae species (Marschner 1995). Since Se (SeO_4^{2-}) is taken up and assimilated through uptake system and biochemical reduction pathway of sulfur (SO_4^{2-}) respectively (Terry *et al.*, 2000, White *et al.*, 2004; Germ *et al.*, 2007), it is likely to be more taken up and assimilated in the

members of Brassicaceae compared with other species. It may also result in higher responsiveness to Se treatment in Brassicaceae compared with other plant families.

Canola (*Brassica napus*) (Brassicaceae) is considered a secondary accumulator of Se with concentrations of several hundred mg Se/kg dry weight when grown in soils with moderate levels of Se (Terry *et al.*, 2000). Agronomic biofortification of food and feed crops with Se can improve their nutritive quality (Vogrinic *et al.*, 2009; Seppänen *et al.*, 2010; Stibilj *et al.*, 2011).

This study was aimed to investigate the effects of selenium on vegetative and reproductive growth in canola plants. Some physiological parameters during vegetative growth as well as timing of reproductive events and seed yield were studied using a spring canola cultivar grown hydroponically under greenhouse conditions.

2 MATERIALS AND METHODS

2.1 Plants culture and treatment

Seeds of canola (*Brassica napus* 'RGS', a spring cultivar) provided by Seed and Plant Improvement Institute (Karaj, Iran) were surface-sterilized with 1% active hypochlorite and germinated on perlite in the dark and moistened by distilled water. After germination, young seedlings were transferred to the light. One week-old seedlings were transferred to 10 L flat plastic container filled with washed perlite, 8 plants were cultivated in each container. Irrigation of plants was carried out with nutrient solution (Hoagland and Arnon 1945) or water at field capacity after daily weighing. The volume of nutrient solution was 500 ml per week in the first 4 weeks and 700 ml in the following growth period. Plants were grown in greenhouse conditions with a temperature regime of 25/18 °C day/night, a relative humidity of 70/80% and at a photon flux density of about 200-300 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Two separate groups of plants were cultivated in parallel in this work. The first group were cultivated for 19 weeks and foliarly treated with selenate with three levels of Se (0, 10 and 20 $\mu\text{g plant}^{-1}$ Se) in order to examine Se effect on reproductive growth of plants. Reproductive phase was demonstrated as four distinct stages and the time course of reproductive phase was divided into time intervals of 10 days. Reproductive

events were monitored by visual daily inspection throughout 15 weeks growth in this group. Number of plants (% over total) at each stage and over the four divided time intervals was calculated. Thereafter, plants were irrigated daily with distilled water and let to grow for further 4 weeks for development of seeds. Nineteen weeks after sowing, plants were harvested. In addition of dry matter production of vegetative parts, pods weight and length and seed dry weight (DW) were determined. Results of this experiment were presented in Figures 1, 2 and Table 1.

The second group of plants was treated with two levels of Se including control (no Se addition) and 10 $\mu\text{g plant}^{-1}$ Se. This group was harvested 9 weeks after sowing (shortly before flowering) and samples were used for measurement of various physiological parameters at vegetative stage. Before harvest, chlorophyll (Chl) fluorescence and gas exchange parameters were determined in the attached leaves. Results of this experiment were presented in the Tables 2 and 3.

Both groups were cultured with four independent replications (four containers) per treatment. Selenium was added gradually between the 3rd and 7th weeks after sowing as sodium selenate (Na_2SeO_4 , Fluka) dissolved in the nutrient solution.

Table 1. Effect of Se supplementation on shoot DW, pod number, length and DW, and seed DW in canola (*Brassica napus* L.) plants grown for 19 weeks in greenhouse. Data of each parameter followed by the same letter are not significantly different ($P < 0.05$).

Treatments	Shoot DW (mg plant ⁻¹)	Pod number (plant ⁻¹)	Pod length (cm plant ⁻¹)	Pod DW (mg plant ⁻¹)	Seed DW (mg plant ⁻¹)
-Se	2.09±0.75 ^b	55±12 ^a	5.19±1.08 ^a	2.44±0.92 ^b	1.05±0.29 ^b
10 $\mu\text{g plant}^{-1}$ Se	4.44±0.91 ^a	41±17 ^a	5.58±1.17 ^a	5.41±1.13 ^a	1.59±0.19 ^a
20 $\mu\text{g plant}^{-1}$ Se	3.17±0.60 ^{ab}	36±15 ^a	5.81±1.35 ^a	3.49±1.10 ^{ab}	1.20±0.16 ^{ab}

Table 2. Effect of Se supplementation (10 µg plant⁻¹) on the concentration of chlorophyll a, b, carotenoids (mg g⁻¹ FW) and anthocyanins (mg cyanidin⁻³-glucoside g⁻¹ FW), chlorophyll fluorescence parameters including F_v/F_0 (the ratio of variable to initial fluorescence), F_v/F_m (photochemical efficiency of PSII), F'_v/F'_m (excitation capture efficiency of open PSII), qP (photochemical quenching) and qN (non-photochemical quenching) and leaf gas exchange parameters including net photosynthetic rate (A , µmol CO₂ m⁻² s⁻¹), transpiration rate (E , mmol H₂O m⁻² s⁻¹) and stomatal conductance to water vapor (g_s , mol m⁻² s⁻¹), in canola (*Brassica napus* L.) plants grown for 9 weeks in greenhouse. Data of each parameter followed by the same letter are not significantly different ($P < 0.05$).

	Chl a	Chl b	Carotenoids	Anthocyanins
-Se	2.07±0.19 ^a	0.65±0.06 ^b	150±9 ^b	2.44±0.94 ^a
+Se	2.18±0.25 ^a	0.78±0.08 ^a	167±2 ^a	2.69±0.47 ^a
	F_v/F_0	F_v/F_m	F'_v/F'_m	qP
-Se	4.82±0.37 ^a	0.82±0.01 ^b	0.63±0.05 ^b	0.82±0.14 ^a
+Se	5.23±0.19 ^a	0.85±0.01 ^a	0.72±0.03 ^a	0.98±0.22 ^a
	qN	A	E	g_s
-Se	0.37±0.07 ^a	7.98±0.14 ^b	0.62±0.09 ^b	0.96±0.08 ^a
+Se	0.42±0.09 ^a	9.27±0.24 ^a	0.77±0.04 ^a	0.99±0.01 ^a

Table 3. Effect of Se supplementation (10 µg plant⁻¹) on the total soluble sugars and starch (mg g⁻¹ FW), soluble proteins (mg g⁻¹ FW) and total free α -amino acids (µmol g⁻¹ FW) in canola (*Brassica napus* L.) plants grown for 9 weeks in greenhouse. Data of each parameter followed by the same letter are not significantly different ($P < 0.05$).

	Soluble sugars		Starch		Protein		Amino acids	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
-Se	8.0±0.97 ^b	3.4±0.02 ^a	2.4±0.25 ^a	0.25±0.04 ^a	11.4±1.3 ^b	1.2±0.11 ^a	5.2±0.3 ^b	0.98±0.1 ^b
+Se	11.5±0.85 ^a	3.6±0.34 ^a	1.6±0.06 ^b	0.03±0.01 ^b	15.4±1.2 ^a	1.1±0.06 ^a	7.2±0.1 ^a	1.23±0.1 ^a

2.2 Determination of chlorophyll fluorescence and gas exchange parameters

Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark adapted and light adapted leaves. Measurements were carried out on the 3 youngest, fully-expanded leaves. An average of 4 records from different parts of each individual leaf was considered for each replicates. Leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. Initial (F_0), maximum (F_m), variable ($F_v = F_m - F_0$) fluorescence, the ratio of variable to initial fluorescence (F_v/F_0) as well as maximum quantum yield of PSII (F_v/F_m) were recorded. Light adapted leaves (300 µmol m⁻²s⁻¹) were used for measurement of initial (F_i) and maximum (F'_m) fluorescence. Calculations were made for F'_0 ($F'_0 = F_0 / [(F_v/F_m) + (F_0/F_m)]$), excitation capture efficiency of open PSII (F'_v/F'_m), photochemical quenching, qP [$(F'_m - F_i) / (F'_m - F'_0)$] and non-photochemical quenching, qN [$1 - [(F'_m - F'_0) / (F_m - F_0)]$] (Oxborough 2004).

CO₂ assimilation and transpiration rates were measured in parallel with Chl fluorescence measurements in the same leaf with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 and 13:00 at harvest. The measurements were conducted with photosynthetically active radiation intensity at the leaf surface of 300 µmol m⁻² s⁻¹

¹. measured by a quantum sensor attached to the leaf chamber of the gas exchange unit. The net photosynthesis rate by unit of leaf area (A , µmol CO₂ m⁻² s⁻¹), transpiration rate (E , mmol H₂O m⁻² s⁻¹) and the stomatal conductance to water vapor (g_s , mol m⁻² s⁻¹) were calculated using the values of CO₂ and humidity variation inside the chamber, both measured by the infrared gas analyzer of the portable photosynthesis system.

2.5 Determination of chlorophyll, carotenoids, anthocyanins and carbohydrates

Leaf concentration of Chl a, b and carotenoids were determined after extraction of pigments in the cold acetone and allowing the samples to stand for 24 h in the dark at 4 °C (Lichtenthaler and Wellburn 1985). Determination of anthocyanins was performed using a pH differential method at pH 1 and pH 4.5 in the methanol/HCl (98:2, v/v) extract (Giusti and Wrolstad 2001). Concentration of total anthocyanins was expressed as g of cyanidine-3-glucoside g⁻¹ FW. For determination of carbohydrates, leaves were homogenized in 100 mM phosphate buffer (pH 7.5) at 4°C, after centrifugation at 12000 g for 15 min, supernatant was used for determination of total soluble sugars whereas the pellets were kept for starch analysis (Magné *et al.*, 2006).

2.6 Determination of soluble proteins and total free aminoacids

Soluble proteins were determined according to the method of Bradford (1976) using a commercial reagent (Sigma) and BSA (Merck) as standard. Content of total free α -amino acids was assayed using ninhydrin colorimetric method. Glycine was

used for production of standard curve (Hwang and Ederer 1975).

Experiments were undertaken in complete randomized block design with 4 replications. Statistical analyses were carried out using Sigma Stat (3.02) with Tukey test ($p < 0.05$).

3 RESULTS AND DISCUSSION

Dry matter production of shoot increased by Se supplementation, this effect was significant for the lower Se level (10 μ M). Pod DW (but not pod length) and seed DW were also significantly higher in Se supplemented plants. In contrast to other parameters, Se treatment influenced negatively the number of pods, though this effect was not statistically significant (Table 1). According to daily inspection of flowering plants, lower pod number was not due to reduction in the number of flowers or seed abortion. There is a known adverse relationship between the number of fruits and their size in plants, thus, an increase in pod and seed DW can reasonably be expected from reduction in the number of pods in this work. However, pod DW at harvest could be also the result of interaction of many other factors.

Number of leaves was slightly higher in the presence of 10 μ M Se, while at higher Se concentration (20 μ M) it did not differ from control plants. In contrast, in the reproductive growth phase, the length of inflorescence was higher in Se treated plants and 20 μ M Se was more effective than 10 μ M Se (Figure 1). Selenium accelerated all four stages of reproductive growth and the two applied Se concentrations did not differ considerably in this respect. The most responsive parameter was podding, that was mainly influenced by Se application in last three intervals (Figure 2). Other reproductive characters such as timing of side branches

formation and total number of side branches were not influenced by Se addition. Difference between side and main branches in the reproductive characters as influenced by Se was not also detected, thus, data of side and main branches were combined.

In vegetative plants, leaf Chl b and carotenoids content increased by Se treatment, while Chl a and anthocyanins contents were not affected by Se supplementation. Maximum quantum yield of PSII (F_v/F_m) and excitation capture efficiency of open PSII (F'_v/F'_m) increased significantly by Se treatment. Net assimilation rate was enhanced by about 16% while transpiration and stomatal conductance were influenced only slightly by Se treatment (Table 2). Accordingly, significant enhancement of the net CO_2 assimilation rate appeared to be mainly caused by increase of non-stomatal parameters such as improved leaf photochemistry as was reflected in the significant rise of F_v/F_m and F'_v/F'_m . It is also likely that Se activates photosynthetic carbon metabolizing enzymes (see below). Regarding increase in the number of leaves upon Se treatment, a considerable enhancement in the photosynthesis of whole canola plants is expected in this work. Higher Chl b and β -carotene in the leaves of Se treated plants may support photochemistry of leaves via improved protection of reaction centers against active oxygen species produced inevitably by light (Demmig-Adams and Adams 1992).

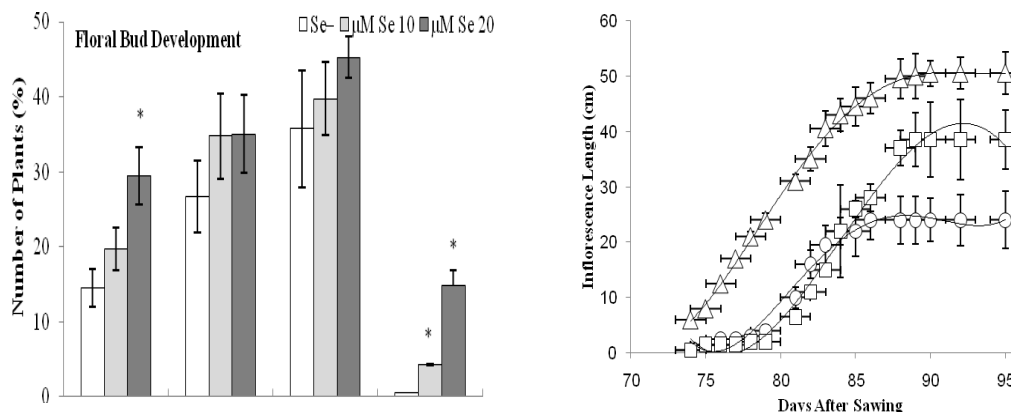


Figure 1. Effect of Se supplementation on the number of leaves (a) and length of stem (b) in canola (*Brassica napus*) plants grown in greenhouse.

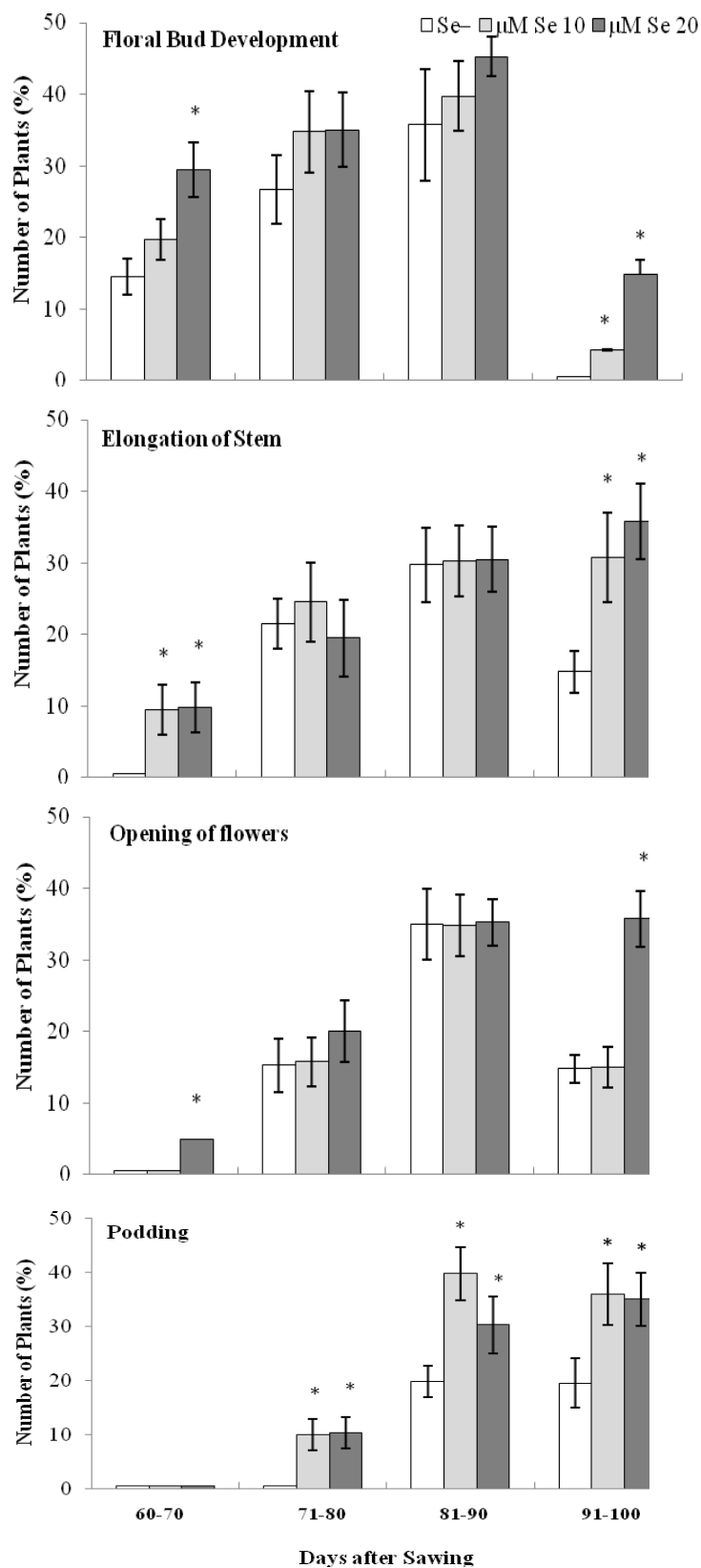


Figure 2. Effect of Se supplementation on the timing of reproductive events in canola (*Brassica napus*) plants grown in greenhouse.

In vegetative plants, leaf Chl b and carotenoids content increased by Se treatment, while Chl a and anthocyanins contents were not affected by Se supplementation. Maximum quantum yield of PSII (F_v/F_m) and excitation capture efficiency of open PSII (F'_v/F'_m) increased significantly by Se treatment. Net assimilation rate was enhanced by about 16% while transpiration and stomatal conductance were influenced only slightly by Se treatment (Table 2). Accordingly, significant enhancement of the net CO₂ assimilation rate appeared to be mainly caused by increase of non-stomatal parameters such as improved leaf photochemistry as was reflected in the significant rise of F_v/F_m and F'_v/F'_m . It is also likely that Se activates photosynthetic carbon metabolizing enzymes (see below). Regarding increase in the number of leaves upon Se treatment, a considerable enhancement in the photosynthesis of whole canola plants is expected in this work. Higher Chl b and β -carotene in the leaves of Se treated plants may support photochemistry of leaves via improved protection of reaction centers against active oxygen species produced inevitably by light (Demmig-Adams and Adams 1992).

Improved reproductive characters, acceleration of reproductive stages and seed yield increase of canola plants in this study appeared to be the result of an increased vegetative growth of plants following improved photosynthetic capacity of whole plant and enhanced carbohydrates and protein synthesis. However, effect of Se on phytohormones balance and/or polyamine content could not be excluded. Selenium treated potato plants had higher putrescine content (Turakainen *et al.*, 2008). Polyamines have been implicated in various plant growth and developmental processes including stimulation of cell division,

embryogenesis, senescence, floral development, and fruit ripening (Kakkar and Sawhney 2002).

Effect of Se on the stimulation of canola flowering in this work was not accompanied by accelerating the aging process as could be judged by the higher leaf Chl concentration measured shortly before flowering. In addition, during the 4-weeks period of seed development, Se treated plants showed a considerable delay in the aging of pods. The pods had been remaining green for a longer time in Se treated plants while seed development appeared not to be influenced. It implies likely the effect of Se on delaying senescence without affecting seed development. Se addition delayed monocarpic senescence in soybean plants (Djanaguiraman *et al.*, 2004).

Timing of flowering is a critical factor in canola production. Cultivars are preferred that flower at a time before the onset of severe drought stress and high temperatures because it enables plants to complete seed development (Robertson *et al.*, 2002). In addition, Se enrichment of canola not only could accelerate flowering and improve its yield, but also contributes in the improvement of nutritional quality of canola seed and oil for livestock and human, respectively.

Although a considerable effect of Se enrichment on reproductive growth of canola was demonstrated in this work, more detailed studies are needed on the effect of Se on carbohydrates partitioning in vegetative and reproductive plants, polyamines metabolism, seed development and fruit ripening. In addition, studies are needed for finding optimum concentrations and application methods in the field in order to improve canola seed yield by Se supplementation.

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