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How Salicylic Acid Modulate Photosynthetic Pigments, Yield and Yield Components of Canola Plant

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Abstract

In this experiment, the possibility of growth promoting of exogenous salicylic acid during two growth stages on chlorophyll content, yield and yield components of canola plant was investigated. For the purpose of improving above traits, salicylic acid was applied in four concentrations (0, 100, 200 and 400 µM) during two different growing stages (first time; when temperature was close to 7-10 °C and second time; when temperature was close to 7-10 °C at the end of winter) on two canola cultivars (Licord and RGS). The total chlorophyll content was higher when 100 µM was applied at the second stage of the application on Licord cultivar. Salicylic acid foliar application led to increase in seed yield of both cultivars. The highest plant height, seed weight, and plant biomass were obtained from Licord cultivar. The greatest pod number per plants was observed in Licord which was treated with 100 µM salicylic acid at the first time while the highest seed number per pod was observed in the former cultivar which was treated with 200 µM salicylic acid at the first time. In general, our results revealed that salicylic application increased canola growth and productivity of grain yield in both cultivars.

Key words: Canola; Photosynthetic pigments; Salicylic acid; Seed yield

Introduction

The results of studies carried out under field or laboratory strongly suggest that phenolic compounds and salicylates [Salicylic acid (SA) especially] play an important role in many physiological responses in different plants. The effect of these substances on the physiology activity of the plant is variable and promotes some processes or inhibiting others (Raskin, 1992).

One of the main oil production crop in Iran is Canola growing in the north of the country. The major factor affecting canola production is the short frost period. Incidences of early or late forest during the growing season have serious detrimental effect on canola yield and seed quality.

Photosynthesis plays an important role in plant productivity. Working with modern and obsolete plants cultivars suggested that improvement in plant yield may be achieved through enhanced assimilatory process in modern cultivars (Faver *et al*., 1997) and previous authors found that rate of photosynthesis had a positive association with the crop yield (Faville *et al*., 1999). Thus, the biological or economic yield can be increased by increasing the rate of photosynthesis. The site of the photosynthesis in the plant is predominantly the green leaf and its productivity directly depends on the chlorophyll (Chl) content, photosynthesis enzymes, and its potential to utilize CO_2. Leaves are the major contributors to net productivity during vegetative and reproductive growth stage.

Salicylic acid is a common plant produced phenolic compound that can function as a plant growth regulator and various biochemical functions of SA in plants have been reported (Raskin, 1992). Exogenous applications of SA to different species of crops have been shown to elicit effects on yield and yield components. An increase in the number of pods and yield has

been found in mung bean (Singh and Kaur, 1980), common bean (Lang, 1986; Rendon, 1983) and wheat (Lopez, 1989) by SA application. Salicylic acid may also influence a range of transpiration rate (Larque Saavedra, 1979), stomatal closure (Rai et al., 1986), membrane permeability (Barkosky and Einhellig, 1993), growth and photosynthesis (El-Tayeb, 2005; Khan et al., 2003; Khodary, 2004). Similarly, SA has received much attention due to its role in plant responses to abiotic stress and a lot of data exist on the protective effect of SA against ultraviolet light (Yalpani *et al*., 1994), salinity (Shakirova *et al*., 2003), drought (Senaratna *et al*., 2000), heavy metal (Pal *et al*., 2005), low temperature (Wang *et al*., 2006) and paraquat (Kusumi *et al*., 2006). It was shown that exogenous treatment of maize plants with SA provided protection against low temperature stress (Horváth *et al*., 2002). Further studies proved that other salicylates compounds also might have protective role against low temperature stress (Horváth *et al*., 2002; Janda *et al*., 2000). The observation suggests that the role of SA in low temperature is related to its effect on the antioxidant system and antioxidative enzymes activity. In view of all aforementioned studies, this experiment was carried out to studied induced changes in photosynthetic pigments by different SA concentrations and this plant regulator could modulate growth and grain and compound yield of two canola cultivars by differently cold resistant.

Materials and Methods

Experiment location and general methodology

A field experiment was carried out at Faculty of Agriculture, Tarbiat Modares University (35º41′ N, 51º19′ E and 1215 m a.s.l.), Tehran, Iran during growing season of 2009-2010. The region is characterized as semi-arid, with mean annual precipitation of 298 mm which is mostly occurred during the autumn and winter months. Daily meteorological data including precipitation and air temperature were obtained from the nearest weather station (500 m apart) (Figs 1 and 2, Table 1). Before planting, several soil samples were taken at depths of 0-30 (Table 2). Soil texture was sandy loam based on the textural triangle classification.

Table 1. Daily temperature and precipitation during the leaf sampling.

Date	Minimum (°C)	Maximum (°C)	Total precipitation (mm)
2010.3.18	13	26	0
2010.3.19	8	17	0
2010.3.20	-4	12	0
2010.3.21	-3	4	0
2010.3.22	-3	13	2.2

Land preparation and treatments

The field was prepared by shallow plough followed by a disk in the fall. In order to pre-plant weed control, Trifloralin (3.5 Liter.ha^{-1}) was sprayed and then incorporated into the soil by disk. The experimental design was a randomized complete block arranged in 2×2×4 factorial with three replications (48 treatments). Seeds of canola (*Brassica napus* L.) cv. Licord and RGS (cold-tolerant and cold-sensitive, respectively) were sown on 2nd October 2009 in 5 meters rows long, the distance between hills along the row were 35 cm apart, plot area was approximately 10 m^2 (2.0 m in width and 5.0 m in Length). The recommended agricultural practices of growing canola were applied. Irrigation was regularly carried out at intervals according to weather conditions to keep the moisture content of the soil to field capacity. Canola plants were foliar sprayed with SA at the concentration of 0.0, 100, 200 and 400 µM on 20th November 2009 and on 6th March 2010, respectively (Figs 1 and 2). First time; when the temperature was close to 7-10 °C and the second time; when the temperature was close to 7-10 °C at the end of winter. The volume of the spraying solution was maintained just to cover completely the plant foliage till dripping. Leaf samples for biological assay were collected on 20th March 2010 (Table 1). The samples were washed and

then frozen in liquid nitrogen and then stored at -80 °C pending biochemical analysis.

Chlorophyll assay

Chlorophyll was extracted in 80% acetone from the leaf samples, according to the method of Arnon, 1949. Extracts were filtrated and content of Chl a, b and total Chl were determined by spectrophotometry at 645 and 663 nm. The content of Chl was expressed as mg g^{-1} FW.

Plant measurements

In order to determine dry matter and seed yield of canola, four square meters of each plot was hand-harvested on 25th June 2010, when 30 to 40 % of seeds were discolored from green to brown or black (Ozer, 2003). In order to evaluate the dry matter, harvested plants were separated into seed and straw. They were oven-dried (Unitherm oven, Vismara, HVL 1000, Italy) at 60 °C for 72 h to constant weight. Yield and yield components including pod number per plant, seed number per pod and 100 seed weight at 10% moisture (Ozer, 2003) were measured at the physiological maturity stage. The oil percentage of the seeds was determined using Inframatic 8620 Percor, Germany.

Results

The results of the present study (Table 3) showed that most growth traits as plant height, the number of pods/plant, the number of seed/ pod, 100 seed weight, seed yield and plant biomass of canola plant (like photosynthetic pigment) were remarkably affected by applied treatments. However, number of branches and seed oil did not change due to cultivars, time or concentration of SA treatment.

In general, the pigments content were significantly elevated at the second time of salicylic application and the highest of chl a, and total chl was observed in Licord cultivar with 100 µM at second time of salicylic treatment. On the contrary, the content of chl b was more pronounced with 200 µM SA in RGS cultivar. However, this rise was not in the linear relationship with concentration and 400 µM SA led to decrease in chl b content (Table 6).

A Significant response was found in all growth and physiology characters of two rape cultivars, although Licord cultivar exhibited the highest mean value of most traits, i.e. plant height, plant biomass and 100 seed weight (Table 4). It's evident from the present data that all traits (except chl a, and total chl), did not change by the time of application (Table 3). Also, all growth traits and photosynthetic pigments significantly changed with SA concentration except plant height, number of branches, seed oil and biomass yield. However, 100 µM SA recorded the highest mean values for 100 seed weight and seed yield (Table 5).

Seed yield and 100 seed weight of both cultivars were increased by salicylic application and 100 µM SA caused an increase in aforementioned characters. Although, the plant did not show greater alternation in seed yield by SA treatment relative to non-salicylic treatment and beyond of 100 µM SA (200 and 400 µM) did not show significant alteration and this parameter remained almost unaltered by increasing SA concentration (Table 5). In contrast, 200 and 400 µM SA application led to a reduction in seed yield and it was lower than control (Table 5). The greatest plant height, seed weight, and plant biomass were observed in Licord cv. (Table 4), furthermore exogenous application of SA did not change plant height and plant biomass. The no. of pod and seed were significant in interaction between cultivar, time and concentration and the highest content of these traits were showed in Licord cv. with 200 µM SA at first time of application (Table 6).

Discussion

Table 7 shows the values for photosynthetic pigments in two cultivars treated by SA at two-time application that caused to an increase in chl a, b and total chl. However, plant biomass not affected by SA concentration. These results agree with those of Zhou et al., 1999 who reported that photosynthetic pigments and an enhancement of leaf enzymes activity by SA increased. The content of chl a, was higher in Licord cultivar than RGS. It is possible that

Licord has better mechanisms against low temperature stress. Many data have proved that capacity of antioxidant enzymes is a markedly responsive mechanism for plants to overcome low-temperature stress (Bowler *et al*., 1992; Fadzillah *et al*., 1996; Guo *et al*., 2006). Also, it has been recognized that the level of antioxidant enzyme activities has a close relationship with resistance of plant species (Li *et al*., 2000; Foyer *et al*., 1994). Similarly, Song and Guo, 1995 reported that for rape varieties with high cold tolerant, their anti-oxidative system was relatively higher. Moreover, SA plays a role in alleviated the harmful effect of stress in canola cultivars. It is possible that SA application as foliar spraying was effective in increasing level of pigments in leaves of canola plant. High levels of these pigments might be explained by the fact that SA had preventing chloroplast degradation. It has been reported that SA caused an increase in photosynthetic rate and carboxylating enzymes (Khodary, 2004; Singh and Usha, 2003). These results are in agreement with those obtained by Gunes *et al*. 2007 who reported that SA acts as an endogenous signal molecule responsible for inducing abiotic stress in the plant. Exogenous application of SA had an amelioration as well as growth promoting effect. These results can be related to earlier studies which observed that exogenous application of SA promotes growth that might be ascribed to its effect on stimulation of cell division, increasing chlorophyll biosynthesis and photosynthesis output (Taiz and Zeiger, 1998). It is possible that SA could be involved in the regulation of cell enlargement and division in synergy with other substance such as auxin (Kling and Meyer, 1983; Li and Li, 1995; Singh and Kaur, 1980). Growth and grain yield of both cultivars were increased by SA applied and this effect was more pronounced in Licord cv. by 200 µM SA. From these results, it can be concluded that beneficial effect of SA application depended on the type of species or cultivars. These results are similar to those of Kothule *et al*., 2003 who reported that SA pretreatment increased yield and compound yield of the soybean plant. In the same direction Zhou et al., 1999 reported that maize stem injected with SA produced %9 more grain weight than those with distilled water treatments. The beneficial of SA on yield and yield component is perhaps due to the translocation of more photo assimilates to grain during grains filling, thereby improving grain weight. Similar results were obtained by Shakirova *et al*., 2003 and Khan *et al*., 2003. However, others reported variations in response to SA applications (El-Mergawi *et al*., 2004; El-Hakem, 2008. Also, improving in yield by SA treatment might be an increase in the number of pods and number and weight of seeds, due to SA capacity in directly and indirectly yield control (Dolatabadian *et al*., 2009).

Moreover, SA application can improve stomatal closure; thereby the enhancement in net CO_2 assimilation rate is possible (Dolatabadian *et al*., 2009). So, an increase in photosynthesis rate of both cultivars due to SA application might have contributed more to yield and yield component. Although, exogenous application of SA increased photosynthesis rate, it did not in turn improve plant biomass. The results can be related to increase in photosynthetic pigments and photosynthesis rate due to salicylic acid application which promotes growth and increasing photo assimilation. Although, photosynthesis variation is not only due to increase photosynthetic pigment but also it can be due to other metabolic factors such as PEP carboxylase and Rubisco (Pancheva *et al*., 1996). However, the photosynthesis rate was not determined in the present study, but it is possible that SA causes an increase in photosynthesis approach led to increase in photosynthesis rate that it observed earlier studies in other plants (Singh and Usha , 2003; Fariduddin *et al*., 2003). The result of the current study can be compared with result of Fariduddin *et al*., 2003. They concluded that there was an increase in pod number *B. Juncea* in SA treatment. Similarly, Ghulam *et al*., 2007 showed that the number of pod/plant was increased with salicylic application (10^{-4} M) as compared to no SA in pea plant.

Table 2. Mechanical and chemical properties of experimental soil

Soil sample	EC	pH	TNV	OC	Total N	Clay	Silt	Sand	Texture	P (ava)	K (ava)	Fe	Zn	Mn
	ds/m	%								mg. kg^{-1}				
Average	1.23	7.54	17.73	1.58	0.15	7	13	80	sandy loam	119.2	908	17.48	38.8	9.07

Table 3. Effect of variety, time and different concentration of SA on growth and physiology parameters of canola plant

SOV	df	Chl a	Chl b	Chl T	Plant height	No. of Branches/plant	No. of Pods/plant	No. of Seed/pod	100 Seed weight	Seed Oil	Seed Yield	Biomass Yield
Rep	2	0.002	0.006	0.01	401.33	19.52**	1705.18**	3.11	0.001	17.89**	0.12	4.41
Variety	1	0.29**	0.46**	1.49**	3305.06**	2.42	9075.00**	710.71**	0.012*	7.00	0.34	29.56*
Time	1	0.49**	0.0006	0.52**	22.27	4.44	33.33	0.09	0.0000083	6.85	0.19	11.69
Concentration	3	0.17**	2.82**	4.02**	140.74	1.20	667.86*	0.47	0.016**	0.69	0.36*	4.07
Var×Time	1	0.01	0.1**	0.03	19.63	2.25	184.08	3.05	0.00067	0.07	0.09	2.37
Var×Con	3	0.02	0.01	0.02	200.20	2.59	1663.77**	1.59	0.00050	7.55	0.08	0.33
Time×Con	3	0.18**	0.05**	0.32**	159.50	1.59	154.77**	12.51*	0.000047	1.08	0.15	0.69
Var×Time×Con	3	0.05**	0.05**	0.16**	212.99	1.09	1310.75**	11.52*	0.00018	3.85	0.08	9.07
Error	30	0.008	0.007	0.01	125.24	1.82	157.89	3.12	0.0028	2.68	0.1	4.92
C.V %		6.06	8.06	4.84	10.97	23.32	20.98	9.55	13.78	3.72	19.16	23.77

* and ** significant at 0.05, 0.01 probability level, respectively.

Table 4. Effect of variety treatment on plant height, 100 seed weight and dry biomass yield of *canola cultiars*

Variety	Plant height (cm)	100 seed weight (g)	Dry biomass yield (t/ ha)
RGS	97.3 b	0.37 b	8.54 b
Licord	110.30 a	0.40 a	10.11 a

Values are means and differences between means were compared by multirange Duncan's significance test. Different letters indicate significant differences at $P < 0.05$.

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Table 6. Effect of variety, time and different concentrations of SA application on chl content and yield components of canola

Variety	Time	Conentartion (µM)	Chl a (mg/g FW)	Chl b (mg/g FW)	Chl Total (mg/g FW)	No. of Pods/plant	No. of seeds/pod
RGS	1	0	0.86 g	0.37 h	1.23 g	44.00ed	15.33 bcd
RGS	1	100	1.53 bcd	1.38 bc	2.91 bc	25.67 ef	13.43 cd
RGS	1	200	1.43 ed	1.36 c	2.8 c	67.67bc	13.30 cd
RGS	1	400	1.44 ed	0.56 g	1.97 ef	44.67 def	15.73 bc
RGS	2	0	1.56 bcd	0.70 ef	2.26 d	52.67 dc	13.90 bcd
RGS	2	100	1.64 abc	1.18 d	2.83 c	38.67 def	16.83 b
RGS	2	200	1.42 ed	1.65 a	3.07 ab	19.67 f	16.50 bc
RGS	2	400	1.29 ed	0.51 gh	1.8 f	78.00 b	12.23 d
Licord	1	0	1.22 f	0.82 e	2.05 e	42.33 def	22.66 a
Licord	1	100	1.48 cd	1.52 ab	3.00 bc	79.39 b	21.46 a
Licord	1	200	1.51 cd	1.62 a	3.13 ab	103.33 a	23.46.a
Licord	1	400	1.52 bcd	0.83 e	2.36 d	80.67 b	23.00 a
Licord	2	0	1.62 abc	0.72 ef	2.34 d	70.67 bc	23.03 a
Licord	2	100	1.76 a	1.52 ab	3.28 a	76.67 b	22.43 a
Licord	2	200	1.69 ab	1.58 a	3.27 a	83.67 ab	20.40 a
Licord	2	400	1.63 abc	0.64 fg	2.28 d	52.33 d	22.36 a

Values are means and differences between means were compared by multirange Duncan's significance test. Different letters indicate significant differences at $P < 0.05$.

Table 5. Effect of Salicylic treatment on 100 seed weight and seed yield of canola cultivars

Concentration (µM)	100 seed weight (g)	Seed Yield (t/ ha)
0	0.33 b	1.68 ab
100	0.40 a	1.93 a
200	0.40 a	1.63 b
400	0.40 a	1.52 b

Values are means and differences between means were compared by multirange Duncan's significance test. Different letters indicate significant differences at $P < 0.05$.

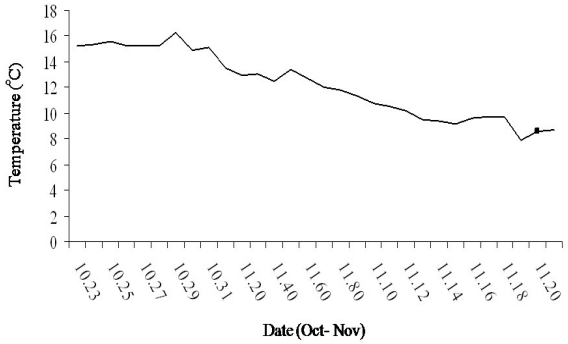

Fig. 1. Daily mean of temperature (°C) of Karaj in long term (Oct- Nov, 1997-2007)

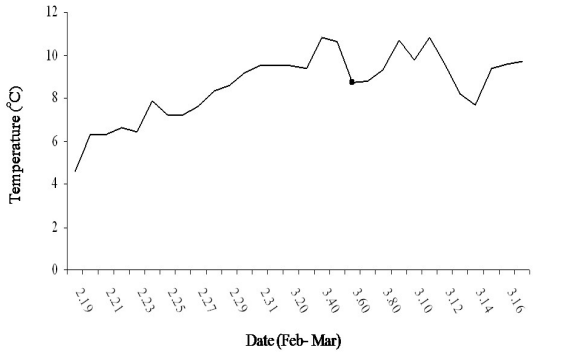

Fig. 2. Daily mean of temperature (°C) of Karaj in long term (Feb- Mar, 1997-2007)

In our research, the highest content of total chl was observed in Licord cultivar that maybe this cultivar had the better defense mechanisms against low temperature. An endogenous enzymes could be able to clear away extra active oxygen and eliminate or alleviate injure. The level of these enzymes has a close relationship with hardness of plant. In brief, we hypothesize that SA could induced expression of cold resistance of plant and could regulated defense mechanisms in cold tolerance cultivar and led to better growth and development. Similarly, since SA involved in cell division and cell expansion and has a synergy effect with other hormones it could able to an increase in pod and seed number. In addition, from the above discussion, it can be concluded that the improvement in growth and yield of canola plants due to salicylic application has associated with improvement in photosynthesis capacity. Changes in photosynthetic rate by salicylic application were due to photosynthetic pigments and other metabolic factors (efficiency of photosystem II and rubisco enzyme concentration and activity). Also, this effect of salicylic acid treatment on photosynthetic pigments shows that this increase depends on the type of species or cultivars.

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