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Interaction of salicylic acid and ethylene and their effects on some physiological and biochemical parameters in canola plants (*Brassica napus* L.)

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Abstract

Environmental stresses, such as cold, heat, salinity, and drought, induce ethylene production and oxidative stress and cause damage in plants. On the other hand, studies have shown that salicylic acid (SA) induced resistance to environmental stresses in plants. In this research, the effects of ethylene on chlorophyll (Chl), carotenoid (Car), anthocyanin, flavonoids, ascorbic acid, dehydroascorbic acid, total ascorbate, lipid peroxidation, and ethylene production in leaves of canola pretreated with SA were studied. The plants were grown in pots until they have four leaves. Leaves were sprayed for two days with three different concentrations of SA (0, 0.5, and 1 mM). The plants were treated for three days with three concentrations of ethylene (0, 50, and 100 ppm). At the end of the ethylene treatments, all examined parameters were measured. The results showed that the ethylene treatments induced lipid peroxidation, while SA mitigated this effect. The ethylene treatment lowered significantly Chl and Car contents and anthocyanin accumulation, but SA alleviated these effects. SA induced an increase in ascorbic acid content in canola plants after the ethylene treatments. Therefore, we concluded that SA played an important role in the alleviation of damages caused by stress conditions.

Additional key words: lipid peroxidation; oxidative stress; phenolic compound; photosynthetic pigments.

Introduction

Ethylene is produced either chemically through the incomplete combustion of hydrocarbons or biologically by almost all living organisms (Wang *et al*. 2002, Pech *et al.* 2005). There is a lot of evidence showing that ethylene is an essential component of a wide range of responses to biotic and abiotic environmental stresses Shinozaki *et al*. 1999, Wang *et al*. 2002, Guo and Ecker 2004, El-Tayeb 2005). Further, many of these stress responses integrate ethylene signaling into more complex circuitry involving salicylate and jasmonate signaling (Wang *et al*. 2002). The effects of ethylene on plants are regulated both at the level of its synthesis and perception of the hormone (Caren *et al.* 2007, Wang *et al*. 2002). It has been shown that water stress and ethylene causes generation of H_2O_2 (Shinozaki *et al*. 1999, Zafar *et al*. 2011). Therefore, lipid peroxidation expressed by malon-dialdehyde (MDA) production is expected in plant cells (Kao and Yang 1983). Thus, ethylene, such as many environmental stresses, could induce the oxidative damage in plant cells.

Salicylic acid (SA) or ortho-hydroxybenzoic acid and related compounds belong to a diverse group of plant phenolics (Raskin 1992, Popova *et al*. 1997). It is well documented that many phenolic compounds play an essential role in the regulation of different physiological processes, including plant growth and development, ion uptake, and photosynthesis (Harper and Balke 1981, Raskin 1992). The established effects of SA on stomata function, Chl content, transpiration rate, and respiratory pathways lead to the assumption that SA might possess a physiological function, it is involved most probably in a regulation of some photosynthetic reactions (Raskin 1992, Popova *et al*. 1997, Singh and Usha 2003, Horváth *et al*. 2007a,b).

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Abbreviations: ACC – 1-aminocyclopropane-1-carboxylic acid; ASA – ascorbic acid; DHAS – dehydroascorbic acid; ET_{50} – 50 ppm ethylene-treated plants; ET_{100} – 100 ppm ethylene-treated plants; FM – fresh mass; Car – carotenoids; Chl – chlorophyll; GC – gas chromatography; LSD – least significant difference; MDA – malondialdehyde content; SA – salicylic acid; SAM – *s*-adenosylmethionine; TBA – thiobarbituric acid; TCA – trichloroacetic acid.

It has also been reported that SA is an effective inhibitor of ethylene biosynthesis (Srivastava and Dwivedi 2000), and the SA effect is pH-dependent (Ganesan and Thomas 2001). Aharoni *et al*. (1979) suggested that SA acted by blocking the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene, when it was applied to tobacco leaf discs.

Studies have shown that SA is the cause of plant resistance to environmental stresses, such as cold, heat, Cd, salinity, and drought (Dat *et al*.1998, Senaranta *et al*. 2002, Sakabutdinova *et al*. 2003, Choudhury and Panda 2004, Agrawal *et al*. 2005, El-Tayeb 2005, Horvath *et al*. 2007, Simone *et al*. 2007). SA may inhibit the ethylene biosynthesis induced by environmental stresses. Thus, in this experiment, the effects of SA treatments on some physiological and biochemical parameters were studied in leaves of canola under ethylene treatments.

Materials and methods

Canola plants (*Brassica napus* cv. Hayola 401) were grown from seeds in pots with sand, loam, and peat mixture (2:1:1) in a greenhouse with 16 h day/8 h night photoperiod, temperature of 22ºC day/18ºC night, and with relative humidity of 50% and luminosity of 10,000 lux. The seedlings were irrigated with water once a day and with a half-strength Long Ashton solution once a week (pH about 6.7) (Meidner 1994). After one month, the plants were pretreated with SA. For this purpose, SA was dissolved in distilled water and pH was adjusted to *ca*. 5.5 with KOH (El-Tayeb 2005). Triton X-100 (0.01%) was added as a surfactant. Leaves of the plants were sprayed with solutions of 0.5 and 1 mM SA concentration for 2 d (about 20 ml of SA solution sprayed on leaves of each plant). After 2 d, the plants were transferred to boxes made of PMMA (polymethyl methacrylate) for the ethylene treatment. Three levels of ethylene (0, 50, and 100 ppm) were applied through Suba-Seal fixed on the chambers. The plants were treated with ethylene for three days. Every day, the boxes were opened and flashed with fresh air, then sealed again and the particular concentrations of ethylene were applied to boxes. Three replicates were used for each treatment. After the treatment with ethylene, the plants were transferred from boxes to the greenhouse. After 7 d, the plants were harvested, divided into shoots and roots, frozen in liquid nitrogen, and stored at –20ºC for subsequent biochemical analysis.

Chl content was determined using the methods of Lichtenthaler (1987). In this method, Chl was extracted in 80% acetone. Extracts were centrifuged at 3,000 × *g* and the absorbance of supernatant was measured at 663.2, 646.8, and 470 nm with *UV-VIS* spectrophotometer (*Cary 50*, *Varian*, Germany).

Chl *a*, Chl *b*, total Chl, and Car contents were calculated using following formulas:

Chl a = $(12.25A_{663.2} - 2.79A_{646.8})$ × volume of supernatant [ml] ×dilution factor/sample mass [g]

Chl b = $(21.21A_{646.8} - 5.1\ A_{663.2})$ × volume of supernatant [ml] × dilution factor/sample mass [g]

Car = $[(1000A_{470} - 1.8\text{Chl } a - 85.02\text{Chl } b)/198]$ × volume of supernatant [ml] × dilution factor/sample mass

Anthocyanins: Frozen tissue samples (100 mg) were soaked immediately in 10 ml of acidified methanol [methanol: HCl = 99:1 (v/v)]. Tissues were crushed using a glass pestle and kept at 25ºC for 24 h in the dark. The extract was then centrifuged at 4,000 × *g* for 5 min at room temperature and absorption at 550 nm was read by a *UV-VIS* spectrophotometer (*Cary 50*, Germany) in the supernatant. The nonspecific absorption at 640 nm was subtracted. For the calculation of the amount of anthocyanins, the extinction coefficient of 33,000 M^{-1} cm^{-1} was used (Wagner 1979).

Lipid peroxidation: The level of lipid peroxidation in plant tissues was measured by determination of MDA, which is known to be a breakdown product of lipid peroxidation. The MDA content was determined with the thiobarbituric acid (TBA) reaction. Briefly, 0.2 g of a tissue sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10,000 × *g* for 5 min. To 1 ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA were added. The mixture was heated at 95ºC for 15 min and cooled immediately in ice. The absorbance was measured at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted. The level of lipid peroxidation was expressed as μM of MDA formed using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer 1968).

Ethylene released was determined from detached leaves in three replicates. In each treatment, all leaves detached from a plant were placed in a 120 ml flask, capped with a rubber stopper for 2 h. 1 ml of the gas-phase samples was removed from the head space using an air-tight syringe. The 1-ml gas samples were injected into a gas chromatograph (GC) (*115-34H*, *Agilent*, USA) fitted with a flame ionization detector and a glass column packed with (30–100 mesh) activated alumina (180 cm × 0.34 cm outside diameter, o.d.). The GC was operated at the injector, detector, and oven temperatures of 90, 200, and 250ºC, respectively. Nitrogen was used as the mobile phase. Pure ethylene (99.9%) was used as a standard (Kalantari *et al*. 2000). Ethylene production was calculated per plant.

Total soluble proteins were determined according to the method of Bradford (1976) using bovine serum albumin as a standard (Bradford 1976).

Ascorbate and dehydroascorbate: 200 mg of leaf tissue was homogenized in 10 ml of 5% (w/v) metaphosphoric acid and centrifuged for 15 min at 10,000 × *g*. The concentrations of ascorbate (ASA) and dehydroascorbate (DHAS) were determined spectrophotometrically according to the method of de Pinto *et al*. (1999).

Statistical analysis: The data were statistically analyzed by two-way analysis of variance (*ANOVA*) using *SPSS* (version 10) software and the least significant difference (LSD) were used to test the significant differences between treatments.

Results

Photosynthetic pigments (Chl *a*, *b*, total, and Car) were measured in this experiment (Table 1). Chl *a* content decreased in ethylene-treated plants in comparison with the control plants. SA treatment decreased the amounts of Chl *a, b*, and *a*+*b* in plants, while the pretreatment with SA following exposure to 50 ppm ethylene caused the significant increase in Chl *a* (Table 1). The total Chl content showed the same pattern as Chl *a* ($\alpha \leq 0.05$). Car decreased significantly in ethylene-treated plants in comparison with the control plants. SA treatment increased significantly Car content in the plants treated with 50 ppm ethylene (ET_{50} plants) (Table 1)**.**

Table 1. Effects of SA (0.5 and 1 mM) and ethylene treatments (0, 50, and 100 ppm) on photosynthetic pigment content of *Brassica napus*. The mean comparisons of treatments were done using LSD method at $p<0.05$ significant level. Means followed by *the same letter(s)* in each column are not significantly different at the 5% level.

Treatment	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Total Chl [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]
Control	15.04 ± 1.027^{a}	6.58 ± 0.208^{a}	21.62 ± 1.12^{a}	3.53 ± 0.361^{ab}
0.5 mM SA	9.01 ± 0.925^{bc}	4.33 ± 0.589^{cd}	13.34 ± 1.48^{bcd}	3.07 ± 0.380^{b}
1 mM SA	6.13 ± 1.344^{cd}	2.84 ± 0.323^{d}	8.97 ± 1.66^{d}	1.33 ± 0.156^{d}
50 ppm ethylene	5.34 ± 0.598^{d}	3.89 ± 0.574^{cd}	9.23 ± 0.604^{d}	0.95 ± 0.114^{d}
100 ppm ethylene	6.39 ± 0.461^{cd}	$4.9 \pm 0.750a^{bc}$	11.29 ± 1.01^{cd}	1.65 ± 0.184^{cd}
0.5 mM SA + 50 ppm ethylene	12.30 ± 0.378^{ab}	5.95 ± 0.173^{ab}	18.25 ± 0.55^{ab}	4.21 ± 0.230^{a}
0.5 mM SA + 100 ppm ethylene	4.56 ± 0.516^{cd}	4.04 ± 0.881^{bc}	8.6 ± 1.30^{cd}	0.92 ± 0.003^{d}
1 mM SA + 50 ppm ethylene	9.87 ± 1.78^{bc}	5.05 ± 0.515^{abc}	14.92 ± 1.68^{bc}	2.34 ± 0.401^{c}
1 mM SA + 100 ppm ethylene	7.05 ± 1.16^{cd}	3.92 ± 0.278^{cd}	10.98 ± 1.44^{cd}	1.53 ± 0.571^{cd}

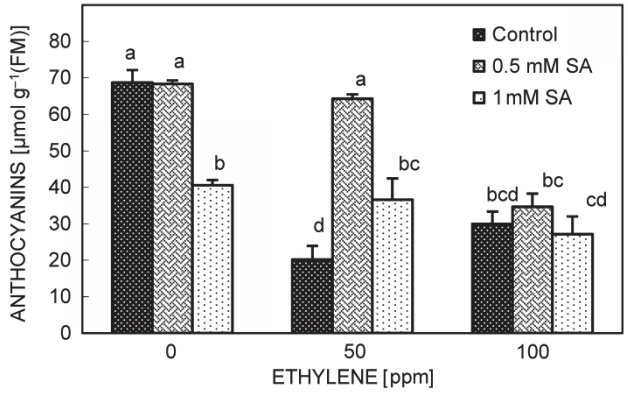

Fig. 1. Effects of SA (0.5 and 1 mM) and ethylene treatments (0, 50, and 100 ppm) on anthocyanins content of *Brassica napus*. Values are the means of three replicate, and bars indicate SEM significant difference at $P<0.05$ according to LSD test.

Anthocyanin content: Ethylene treatment decreased significantly the content of anthocyanins, while SA increased significantly anthocyanin content in ET_{50} plants ($\alpha \leq 0.05$) (Fig. 1). In those plants, which were not treated with ethylene, 1mM SA significantly decreased the amount of anthocyanins, while 0.5 mM SA had no effect on anthocyanin content (Fig.1).

Proteins: Our studies showed that the content of proteins increased in plants, which were pretreated with 1 mM SA (Fig. 2). The protein content decreased significantly in ET_{50} plants when compared with the untreated plants. SA pretreatments induced the increase in the protein content in ET_{50} plants. Proteins decreased in SA-treated plants treated with 100 ppm ethylene (ET_{100} plants), when compared with the ET_{50} plants ($\alpha \leq 0.05$).

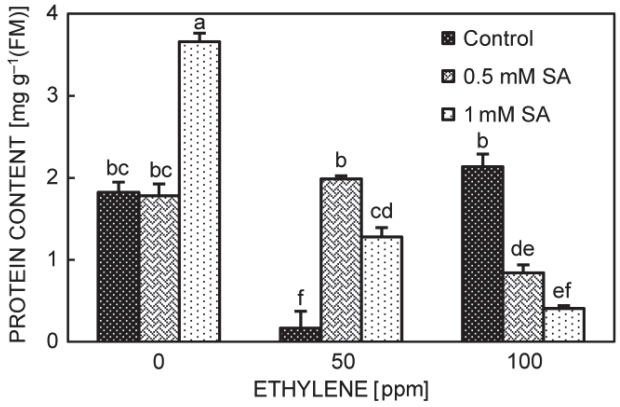

Fig. 2. Effect of SA (0.5 and 1 mM) and ethylene treatments (0, 50, and 100 ppm) on protein content of *Brassica napus*. Values are the means of three replicate, and bars indicate SEM significant difference at P<0.05 according to LSD test.

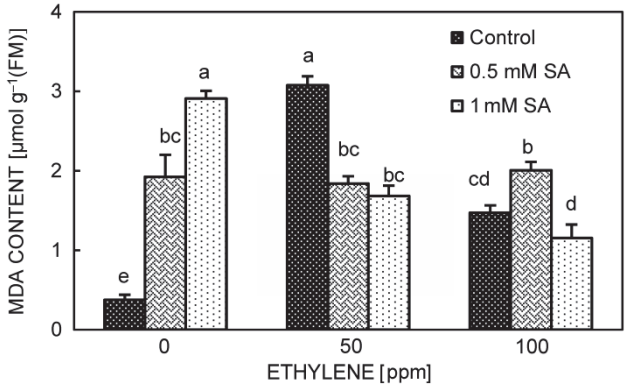

Fig. 3. Effect of SA (0.5 and 1 mM) and ethylene treatments (0, 50, and 100 ppm) on MDA content of *Brassica napus*. Values are the means of three replicate, and bars indicate SEM significant difference at P<0.05 according to LSD test.

Lipid peroxidation: MDA content increased significantly in the ethylene-treated plants (Fig. 3). The treatment with SA increased also the MDA content. The plants pretreated with SA had much lower MDA content when exposed to 50 ppm ethylene. However, in those plants, which were pretreated with 0.5m M SA and exposed to 100 ppm ethylene, MDA content significantly increased in comparison with the SA non-pretreated plants ($\alpha \leq 0.05$) (Fig. 3).

Ascorbate and dehydroascorbate contents: The content of ASA increased in plants pretreated with 1 mM SA. ASA content increased following SA pretreatment in ET_{50} plants (Table 2). DHAS increased following 100 ppm ethylene treatment. Treatment of plants with 0.5 mM SA and 50 ppm ethylene increased the content of DHAS, when compared with the control plants. The total ASA content showed the same pattern as DHAS ($\alpha \leq 0.05$) (Table 2).

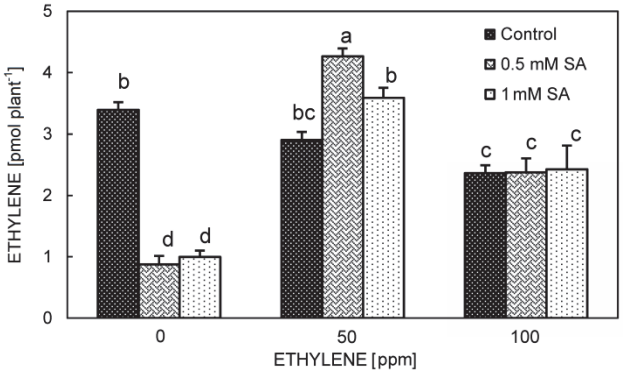

Fig. 4. Effect of SA (0.5 and 1 mM) and ethylene treatments (0, 50, and 100 ppm) on ethylene production content of *Brassica napus*. Values are the means of three replicate, and bar indicate SEM significant difference at P<0.05 according to LSD test.

Table2. Effects of SA (0.5 and 1 mM) and ethylene treatments (0, 50, and 100 ppm) on ASA, DHAS, and total ascorbate content of *Brassica napus*. The mean comparisons of treatments were done using LSD method at p<0.05 significant level. Means followed by *the same letter(s)* in each column are not significantly different at the 5% level.

Treatment	ASA [mg g^{-1}(FM)]	DHASA [mg g^{-1}(FM)]	Total ASA [mg g^{-1}(FM)]
Control	0.92 ± 0.057^{d}	1.19 ± 0.088^{cd}	2.1 ± 0139^{c}
0.5 mM SA	1.19 ± 0.087^{cd}	1.34 ± 0.12^{cd}	2.54 ± 0.158^{bc}
1 mM SA	1.42 ± 0.130^{bc}	1.56 ± 0.045^{cd}	2.98 ± 0.139^{b}
50 ppm ethylene	1.23 ± 0.11^{c}	1.18 ± 0.088^{cd}	2.41 ± 0.04^{bc}
100 ppm ethylene	1.13 ± 0.008^{cd}	3.57 ± 0.0714^{a}	4.7 ± 0.13^{a}
0.5 mM SA+50 ppm ethylene	1.97 ± 0.19^{a}	2.74 ± 0.062^{b}	4.72 ± 0.13^{a}
0.5 mM SA+100 ppm ethylene	1.63 ± 0.062^{c}	1.51 ± 0.140^{c}	2.75 ± 0166^{b}
1 mM SA+50 ppm ethylene	1.24 ± 0.024^{b}	1.27 ± 0.21^{cd}	2.9 ± 0.271^{b}
1 mM SA+100 ppm ethylene	1.39 ± 0.062^{bc}	1.12 ± 0.063^{d}	2.51 ± 0.097

Ethylene: Our results showed that SA caused a decrease in ethylene production in the plants (Fig. 4). Ethylene treatment (50 ppm) increased ethylene production in either 0.5 mM or 1 mM SA-pretreated plants but 100 ppm ethylene had no significant effect on ethylene production in the plants pretreated with SA ($\alpha \leq 0.05$).

Discussion

The effects of plant growth regulator, ethylene, on photosynthetic pigments were reported by some researchers, *e.g*. it was found that Chl content decreased significantly in ethylene-treated plants, such as rice (Kao and Yang 1983), radish (Adachi *et al*.1996), citrus (Jacob-Wilk and Holland 1999), maize (Nemeth *et al.* 2002), and broccoli (Costa *et al*. 2005). In this research, the determination of photosynthetic pigments showed that Chl *a, b*, and *a+b* contents were reduced in the ethylene-treated plants (Table 1). Car content also decreased significantly after the ethylene treatment when compared with the control plants (Table 1). An induction of the Chl breakdown in seedlings of cucumber treated with ethylene has been reported by Nilsson (2005). However, in this study, when SA was used as pretreatment, the pigment contents significantly increased in ET_{50} plants ($\alpha \leq 0.05$). It has been reported that exogenous ethylene induced probably a change in the gene expression of Chl-degrading enzymes under drought stress (Trebitsh *et al*. 1993). Popova *et al*. (1997) suggested that the ethylene-induced increase in membrane permeability during senescence is enzyme-mediated. This could be valid also for Chl loss in our study. Another reason of Chl degradation might be related to induction of ROS production and oxidative stress in the ethylene-treated plants in our study, which caused the oxidation of photosynthetic pigments. In our experiments, the treatment of plants with SA increased significantly the pigment contents in ET_{50} plants. SA has a dual role in plants; it is dependent on its concentration, treatment duration, and the plant species. In high concentrations, SA could increase the amount of ROS productions, induce the thylakoid lipid peroxidation and chloroplast protein degradation, and so decline contents of photosynthetic pigments. Popova *et al*. (2003) showed that SA in a favourable concentration increased the antioxidant ability of cell and induced new protein synthesis in the photosynthetic apparatus.These results are also in agreement with those of El-Tayeb (2005), who reported that SA treatment reduced the stress-induced loss in Chl content and photosynthetic rate under salinity stress. It was also reported that Chl and Car content of water-stressed plants increased significantly following SA application (Simone *et al*. 2007). Our results showed that SA increased Car content after 50 ppm ethylene treatments. Induction of Car synthesis by SA could be related to the protective role of these compounds in photosynthetic machinery (Koyro 2006). Car could scavenge or quench the singlet oxygen and protect the chloroplast from lipid peroxidation and oxidative damages (Loggini *et al*. 1999). SA also affected the growth rate, anthocyanins, and Chl contents in *Spirodella polyrriza* (Popova *et al*. 1997), *Triticum aestivum* (Horvath *et al*. 2007), and *Hordeum vulgare* (Deef 2007). Our experiments showed that anthocyanins of ET_{50} plants were significantly lower than in the control plants (Fig. 1). SA pretreatment increased the anthocyanin content in 50 ppm ethylene, while in ET_{100} plants, SA had no significant effect. Accumulation of anthocyanins and phenolic compounds has been reported under different stress conditions (Kang *et al*. 1973, Shinozaki *et al*. 1999, Ganesan and Thomas 2001, Sakihama *et al*. 2002, Simone *et al*. 2007). In addition, it has been reported that ethylene treatment-induced oxidative stress (Kendrick and Chang 2008). Thus, it seemed that ethylene treatment-induced oxidative stress and the anthocyanin accumulation in this study is related to antioxidant characteristics of these compounds. In strawberry cultivars, the amount of anthocyanins and total phenolic compounds increases substantially under long-term salt stress (Keutgen and Pawelizik 2008). Several reports have shown that soluble phenols, anthocyanins, and flavones accumulate in cells of plants subjected to environmental stresses, and they are thought to have an antioxidant role in plant cells (Kang *et al*. 1973, Shinozaki *et al*. 1999, Ganesan and Thomas 2001, Sakihama *et al*. 2002, Simone *et al*. 2007, Wahid and Ghazanfar 2006). These compounds can protect the cell *via* scavenging the ROS, breakdown of peroxidation chain, hydrogen donation, quenching of singlet oxygen, and peroxidase enzyme (Chu *et al*. 2000). Ethylene treatments induced H_2O_2 production (Shinozaki *et al*. 1999) and oxidative stress (Kendrick and Chang 2008). Therefore it is likely that accumulation of anthocyanins in this study was the reflection of stress caused by ethylene. In addition, some researchers showed that exogenous SA could induce the expression of many genes, including the enzyme phenylalanine ammonia lyase (PAL), in plants (Wen *et al*. 2005). Thus, we assumed that SA caused the accumulation of anthocyanins by the induction of PAL under the ethylene treatment.

Decrease in protein content is a common phenomenon after the ethylene treatment (Zhu 2001). The reason for this is that amino acids in proteins react with active radicals, which may be induced by ethylene, and are degraded (El-Tayeb 2005). In this study, the decline in the protein content was observed in ET_{50} plants (Fig. 2). Protein degradation might occur under these conditions, because of the induction of oxidative stress by ethylene. It has been reported that cells exhibit increased rates of proteolysis following the exposure to oxidation-inducing agents (Inze and Montagu 2000, Yazdanpanah *et al*. 2011). Vera and Conejero (1990) also showed that ethephon (ethylene releasing substance) caused a degradation of proteins in tomato leaf discs. SA application increased the protein content in ET_{50} plants. In ET_{100} plants, the protein content did not decrease, while SA pretreatment caused the reduction of the protein amount. The increase in amount of proteins could be related to the synthesis of antioxidant enzymes or other stress proteins. SA pretreatment alleviated the oxidative stress under such

conditions and the synthesis of stress proteins probably decreased. MDA formation in plants exposed to stress and ethylene, is a reliable indicator of free radical formation in the tissue, and it is currently used as an indicator of lipid peroxidation (Meirs *et al*. 1992, Borsanio *et al*. 2001). The level of the lipid peroxidation (MDA content) increased significantly in the ethylene treated plants. The plants pretreated with SA had much lower MDA content than plants treated with ethylene alone (Fig. 3). Similarly, in barley seedlings, foliar application of SA decreased MDA content as well as electrolyte leakage under salt stress (El-Tayeb 2005). Metwally *et al*. (2003) also found that treatment with SA decreased significantly the MDA content in Cd-treated plants. Yazadanpanah *et al.* (2011) reported that SA significantly decreased the MDA content in drought-stressed plants. Further investigation showed that SA could decline the lipid peroxidation through the inhibition of lipoxygenase activity, decline in H_2O_2 content, and thus maintaining the integrity of cellular membranes under stress conditions (Janda *et al.* 2007).

Plant cells are normally protected against the detrimental effects of active oxygen by a complex antioxidant system. Active oxygen species can be scavenged by both enzymatic and nonenzymatic detoxification mechanisms (Ingram and Bartels 1996, Shinozaki *et al*. 1999). ASA is an essential metabolite implicated in vital cell functions, and plays a key role in quenching active oxygen (Shinozaki *et al*. 1999). ASA content increased following SA pretreatment in ET_{50} plants (Table 2). DHAS did not show any increase following SA pretreatment. Pretreatment of plants with SA and following exposure to 50 ppm ethylene caused the significant increase in DHAS. Total ASA content showed the same pattern as DHAS. A marked increase in ASA, DHAS, and total ASA contents have been reported in other plants and discussed as an adaptive response against oxidative damage induced by ethylene (Vera *et al*. 1990). Oxidation of ASA by $O_2^{-\cdot}$ can result in the production of monodehydroascorbate, which is converted then, both enzymatically and nonenzymatically, to ASA and DHAS (Kang *et al*. 1973, Inze and Montagu 2000, Borsanio *et al*. 2001). Free oxygen radicals are highly toxic and can damage many important cellular components, such as lipids and proteins (Harper and Balke 1981, Shinozaki *et al*. 1999). The decrease of MDA content in ET_{100} plants in comparison with ET_{50} plants might be related to ASA converted to DHAS. It is possible that the increase in ASA content, which was observed in the SA treated plants, was due to SA affecting pathways of ASA biosynthesis; the possibility that SA affected the pathways of DHAS biosynthesis could not be also ignored. It has been reported that plants with higher foliar ASA content can better cope with oxidative stress (Shinozaki *et al*. 1999, Inze and Montagu 2000).

Ethylene production in ET_{100} plants decreased significantly ($\alpha \leq 0.05$). SA pretreatment also significantly decreased ethylene production in the control plants (Fig. 4). The mechanism of SA action in plant ethylene emanation has not been clarified yet, although some published data suggested that SA has an association with ethylene production (Kao and Yang 1983, Metwally *et al.* 2003). Zhang *et al.* (2003) showed that SA inhibits the ethylene biosynthesis. This suppression was believed to be due to its inhibitory effect on the conversion of ACC into ethylene (Beltrano *et al*. 1997, Borsanio *et al*. 2001, Leslie and Romani 1988, Zhang *et al*. 2003). However, although we did not measure ACC content in this experiment, it could be also the cause. It has been reported that s-adenosylmethionine (SAM) is involved in ethylene and polyamine biosynthesis, and also used as a methyl donor in many cellular metabolic reactions. Therefore, another reason for the effects of SA on ethylene biosynthesis inhibition might be related to polyamine biosynthesis and reduced amount of SAM, which is the common precursor of both ethylene and polyamines.

Data presented in this study indicated that ethylene could cause oxidative stress and exogenous SA application could improve stress tolerance in canola plant. Plants pretreated with SA exhibited mild injury symptoms, whereas those, which were not pretreated with SA, showed moderate damage and they lost considerable portions of their foliage. SA decreased very profoundly the severity of stress caused by 50 ppm ethylene, which was observed in parameters indicating oxidative stress.

Based on our results, the application of exogenous SA could protect plants from many environmental stresses, which cause higher ethylene emanation and ethylene-caused damage. However, the application dose of SA needs further investigation in different plant species and at different growth stages.

References

Adachi, M., Tsuzuki, E., Shimokawa, K.: Effect of ethylene on de greening of intact radish (*Raphanus sativus* L.) cotyledons. – Sci. Horti. **65**: 1-9, 1996.

Aharoni, N., Anderson, J.D., Lieberman, M.: Production and action of ethylene in senescing leaf discs. – Plant Physiol**. 64**: 805-809, 1979.

Agarwal, S., Sairam, R.K., Srivasta, G.C., Meena, R.C.: Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and SA in wheat genotypes. – Biol. Plant. **49**: 541-550, 2005.

Beltrano, J., Montaldi, E., Bartoli C., Carbone, A.: Emission of water stress ethylene in wheat (*Triticum aestivum*L.) ears: effects of re watering. – Plant Growth Regul. **21**: 121-126, 1997.

Borsanio, Valpuesta. V., Botella M.A.: Evidence for a role of SA in the oxidative damage generated by NaCl and osmotic

stress in *Arabidopsis* seedlings. – Plant Physiol. **126**: 1024-1030, 2001.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. **72**: 248-254, 1976.

Caren, C.: Ethylene biosynthesis, perception, and response. – J. Plant Growth Regul. **26**: 89-91, 2007.

Choudhury, S., Panda S.K.: Role of SA in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. – Plant Physiol. **30**: 95-110, 2004.

Chu, Y.H., Chang C.L., Hsu H.F.: Flavonoid content of several vegetable and their antioxidant activity. – J. Sci. Food Agr. **80**: 561-566, 2000.

Costa, M., Lcivello. P.M., Chaves, A.R., Martinez, G.A.: Effect of ethephon and 6-benzylaminopurine on Chl degrading enzymes and peroxidase-linked Chl bleaching during post-harvest senescence of broccoli (*Brassica oleracea* L.) at 20°C. – Postharvest Biol. Technol. **35**: 191-199, 2005.

Dat, J.F., Foyer, C.H., Scott, I.M.: Changes in SA and antioxidants during induced thermotolerance in Mustard seedlings. – Plant Physiol. **118**: 1455-1461, 1998.

Deef H.E.: Influence of SA on Stress tolerance during seed germination of *Triticum aestivum* and *Hordeum vulgare*. – Adv. Biol. **1**: 40-48, 2007.

de Pinto, D., Francis, L., Gara, D.: The redox state of the ascorbate-dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. – Protoplasma **209**: 90-95, 1999.

El-Tayeb, M.A.: Response of barley gains to the interactive effect of salinity and salicylic. – Plant Growth Regul. **45**: 215-225, 2005.

Ganesan V., Thomas G.: SA response in rice: influence of SA on H_2O_2 accumulation and oxidative stress. – Plant Sci. **160**: 1095-1106, 2001.

Guo, V., Ecker, J.R.: The ethylene signaling pathway: new insights. – Cur. Opinion Plant Biol. **7**: 40-49, 2004.

Harper, J.P., Balke N.E.: Characterization of the inhibition of K^+ absorption in oat roots by salicylic acid. – Plant Physiol. **68**: 1349-1353, 1981.

Heath, R.L., Packer. L.: Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. – Arch. Biochem. Biophys. **125**: 189-198, 1968.

Horváth, E., Szalai, G., Janda, T.: Induction of abiotic stress tolerance by SA signaling. – J. Plant Growth Regul. **26**: 290-300, 2007a.

Horváth, E., Pál, M., Szalai G., Páldi, E.: Exogenous 4-hydroxy-benzoic acid and SA modulate the effect of short-term drought and freezing stress on wheat plants. – Biol. Plant. **51**: 480-487, 2007b.

Ingram, J., Bartels, D.: The molecular basis of dehydration tolerance in plants. – Plant Physiol. **47**: 377-403, 1996.

Inze, D., Montagu, M.V.: Oxidative Stress in Plants. – Tylor & Francis Group Publ., London – New York 2002.

Jacob-Wilk, D., Holland, D.: Chlorophyll breakdown by chlorophyllase: isolation and function expression of *Chlase 1* gene from ethylene-treated *Citrus* fruit and its regulation during development. – Plant J. **20**: 653-661, 1999.

Janda, T., Horváth, E., Szalai, G.: Role of salicylic acid in the induction of abiotic stress tolerance. – In: Hayat, S., Ahmad A. (ed.): Salicylic Acid: a Plant Hormone. Pp. 91-150. Springer, Dordrecht 2007.

Kalantari, K. M., Smith, R.A., Hall, M.A.: The effect of water stress on 1-(malonylamino)cyclopropane-1-carboxylic acid concentration in plant tissues. – Plant Growth Regul. **31**: 183-193, 2000.

Kang, G., Wang, C., Sun, G., Wang, Z.: SA changes activities of H_2O_2-metabolizing enzymes and increases the chilling tolerance of banana seedlings. – Environ. Exp. Bot. **50**: 9-15, 1973.

Kao, C.H., Yang, S.F.: Role of ethylene in the senescence of detached rice leaves. – Plant Physiol. **73**: 881-885, 1983.

Kendrick, M.D., Chang, C.: Ethylene signaling: new levels of complexity and regulation. – Curr. Opin. Plant Biol. **11**: 479-485, 2008.

Keutgen, A.J., Pawelizik, E.: Quality and nutritional value of strawberry fruit under long term salt stress. – Food Chem. **107**: 1413-1420, 2008.

Koyro, H.W.: Effect of salinity on growth, photosynthesis, water relations and solute composition of potential cash crop halophyte *Plantago coronopus* (L.). – Environ. Exp. Bot. **56**: 136-149, 2006.

Leslie, C.A., Romani, R.J.: Inhibition of ethylene biosynthesis by salicylic acid. – Plant Physiol. **88**: 833-837, 1988.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. – Meth. Enzym. **148**: 350-382, 1987.

Loggini, B., Scartazza, A., Brugonli, E., Navari-izzo, F.: Ant oxidative defense system, pigment composition, and photosynthesis efficiency in two wheat cultivars subjected to drought. – Plant Physiol. **119**: 1091-1099, 1999.

Meidner, H.: Long Ashtone nutrition solution.– In: Reiss, C. (ed.): Class Experiments in Plant Physiology. Pp. 156-156. Prentice Hall Publ., London 1994.

Meirs, S., Philosophhadas, S., Aharoni, N.: Ethylene-increased accumulation of fluorescent lipid-peroxidation products detected during senescence of Parsley by a newly developed method. – J. Amer. Soc. Hort. Sci. **117**: 128-132, 1992.

Metwally, A., Finkemeier, I., Georgi, M., Dietz, K.J.: SA alleviates the Cadmium Toxicity in Barley seedlings. – Plant. Physiol.: **132**: 272-281, 2003.

Németh, M., Janda, T., Horváth, *et al*.: Exogenous SA increase polyamine content but may decrease drought tolerance in maize. – Plant Sci. **162**: 569-574, 2002.

Nilsson, T.: Effects of ethylene and 1-MCP on ripening and senescence of European seedless cucumbers. – Postharvest Biol. Technol. **36**: 113-125, 2005.

Pech, J., Bouzayen, M., Latche, A.: Ethylene biosynthesis. – In: Davies, P.J. (ed.): Plant Hormones. Biosynthesis, Signal Transduction, Action. Pp 115-136. Springer, Dordrecht 2004

Popova, L., Ananieva, E., Hristova, V. *et al*.: Salicylic acid-and metyl jasmonate-induced protection on photosynthesis to paraquat oxidative stress. – Bul. J. Plant Physiol. **30**: 133-152, 2003.

Popova, L., Pancheva, T., Uzunova, A.: Salicylic acid: Properties, biosynthesis and physiological role. – Plant Physiol. **23**: 85-93, 1997.

Raskin, I.: Role of SA in plants. – Ann. Rev. Plant Physiol. Plant Mol. Biol. **43**: 439-446, 1992.

Sakabutdinova A.R., Fatkhutdinova M.V., Bezrukova M.V., Shakirova, F.M.: SA prevents the damaging factors on wheat plants. – Bul. J. Plant Physiol. **30**: 314-319, 2003.

Sakihama, Y., Cohen, M.F., Grace, S.C., Yamasaki, H.: Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. – Toxicology **177**: 67-80, 2002.

Senaranta, T., Touchell, D., Bummol, E., Dixon, K.: Acetyl aslicylic (aspirin) and SA induce multiple stress tolerance in bean and tomato plants. – Plant Growth Regul. **30**: 157-161, 2002.

Shinozaki, K., Yamaguchi-shinozaki, K., Liu, Q. *et al*.: Molecular responses to drought stress in plants: regulation of gene expression and signal transduction. – In: Smallwood, M.F., Calvert, C.M., Bowles, D.J.: Plant Responses to Environmental Stress. Pp133-180. Oxford BIOS Scientific Publ., Oxford 1999.

Singh, B., Usha, K.: SA induced physiological and biochemical changes in wheat seedlings under water stress. – Plant Growth Regul. **39**: 137-141, 2003.

Srivastava, M.K., Dwivedi, U.N.: Delayed ripening of banana fruit by salicylic acid. – Plant Sci. **158**: 87-96, 2000.

Trebitsh, T., Goldschmidt, E., Riov, J.: Ethylene induces de novo synthesis of Chlase, a Chl degrading enzyme, in Citrus fruit peel. – Proc. Nat. Acad. Sci. USA **9**: 9441-9445, 1993.

Vera, P., Conejero, V.: Effect of ethephon on protein degradation and the accumulation of pathogenesis-related (PR) proteins in tomato leaf discs. – Plant Physiol. **92**: 227-233, 1990.

Wahid, A., Ghazanfar, A.: Possible involvement of some secondary metabolites in salt tolerance of sugarcane. – Plant Physiol. **163**: 723-30, 2006.

Wagner, G.J.: Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids, and anthocyanins in protoplast. – Plant Physiol. **64**: 88-93, 1979.

Wang, K., Li, H., Ecker, J.: Ethylene biosynthesis and signaling networks. – Plant Cell **14**: 131 -151, 2002.

Wen, P.F., Chen, J.Y., Kong, W.F. *et al*.: Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. – Plant Sci. **169**: 928-934, 2005.

Yazdanpanah, S., Baghizadeh, A., Abbassi, F.: The interaction between drought stress and salicylic and ascorbic acids on some biochemical characteristics of *Satureja hortensis*. – Afr. J. Agr. **6**: 798-807, 2011.

Zafar, A., Basra, S., Hassan, M., et al.: Mitigation of drought stress in maize by natural and synthetic growth promoters. – J. Agr. Social Sci. **7**: 56-62, 2011.

Zhang, Y., Chen, K., Zhang, Sh., Fergusen, I.: The role of SA in postharvest ripening of kiwifruit. – Postharvest Biol. Technol. **28**: 67-74, 2003.

Zhu, J.K.: Cell signaling under salt, water and cold stresses. – Curr. Opin. Plant Biol. **4**: 401-406, 2001.