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The negative effects of clethodim in photosynthesis and gas-exchange status of maize plants are ameliorated by salicylic acid pretreatment

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Abstract

In this work, the injuries caused by clethodim herbicide application as well as the use of exogenous salicylic acid (SA) as a protective agent against clethodim in *Zea mays* leaves were examined. Although the target for clethodim is the inhibition of acetyl coenzyme A carboxylase (ACCase) which is the key enzyme for fatty acid biosynthesis, it can indirectly affect the photosynthetic machinery, gaseous exchange and some biochemical parameters. Clethodim application caused chlorosis and yellowing of leaf-tip parts. Higher doses caused browning or reddening of leaves and sometimes dead parts of the leaf margins were observed. The rate of photosynthesis was significantly lowered and the pigments content was highly reduced as a response to clethodim spraying. Moreover, other gas-exchange properties were altered. Furthermore, accumulation of high amounts of carbohydrates, proteins and proline were detected. SA spraying three days prior clethodim application caused partially or totally disappearance of clethodim injuries and kept the leaves similar to those of control. Improved photosynthesis and enhanced pigments content were observed in leaves treated with SA. Other analyzed parameters showed values similar to those of the corresponding control. From the experimental work, an evidenced role of SA working against clethodim effects was suggested and discussed in this paper.

Additional key words: clethodim herbicide; gas exchange; photosynthesis; pigments; proteins; salicylic acid.

Introduction

Clethodim, a selective postemergence herbicide, is very effective for the control of wide spectrum of annual and perennial grass species (Vencill 2002). In maize fields, weeds can compete for moisture, nutrients, and light. Weeds drastically reduce yield and grain quality by seed contamination (Anderson and Geadelmann 1982). Jordan *et al.* 2001 suggested that clethodim would be a good alternative to some other herbicides such as glyphosate or paraquat for controlling ryegrass. Clethodim belongs to this cyclohexanedione family of herbicides. This herbicide interferes with fatty acid biosynthesis *via* inhibition of ACCase (Iwataki 1992). ACCase is considered the key enzyme in fatty acid biosynthesis which catalyses the carboxylation of acetyl-CoA to malonyl-CoA (Nikolskaya *et al.* 1999) which is required for the biosynthesis of fatty acids and secondary metabolites (Harwood 1988).

Clethodim affects all physiological processes and membrane formation within the cell by affecting ACCase activity. One of these processes is photosynthesis which plays an important role in plant productivity. Shuting *et al.* 1997 found that the maize cultivars with higher grain yield maintained higher rates of photosynthesis than low-yielding cultivars during plant development. Moreover, Faville *et al.* 1999 found that rate of photosynthesis had a positive association with the crop yield. Thus, final biological or economic yield can be increased by increasing the rate of photosynthesis.

Salicylic acid (SA) is one of the key components of defence signal transduction (Halim *et al.* 2006, Maleck *et al.* 2000). It can generally control both biotic and abiotic defence programs (Borsani *et al.* 2001). Thus, exogenous application of SA may affect many physiological processes such as growth and photosynthesis (Arfan *et al.* 2007, El-Tayeb 2005, Gunes *et al.* 2007), transpiration rate (Larque 1979), stomatal closure (Rai *et al.* 1986), membrane permeability (Barkosky and Einhellig 1993) and antioxidant capacity (Ananievaa *et al.* 2004). Moreover, SA provides protection in maize

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Abbreviations: ACCase – acetyl coenzyme A carboxylase; C_i – intercellular CO_2 concentration; Cars – carotenoids; Chl – chlorophyll; DM – dry mass; E – transpiration rate; g_s – stomatal conductance; P_N – photosynthetic rate; SA – salicylic acid; WUE – water-use efficiency.

against heat and other stresses (Janda *et al.* 1999). It alleviates the oxidative stress generated by paraquat in tobacco and cucumber (Strobel and Kuc 1995) (Ananievaa *et al.* 2004). Pretreatment of plants with SA before paraquat application caused a protection against paraquat induced chlorophyll (Chl) losses (Ananievaa *et al.* 2002). Hayat *et al*. (2010), found that SA, in lower concentrations, is beneficial in enhancing the photosynthesis, growth and various other physiological and biochemical characteristics of plants. Foliar application of SA can increase the pigment contents in *Brassica napus* (Ghai *et al.* 2002). Exogenous application of SA was found to enhance the net photosynthetic rate, internal CO_2 concentration, water-use efficiency, stomatal conductance and transpiration rate in *B. juncea* (Fariduddin *et al.* 2003). To our knowledge, this is the first study reporting the use of SA as a protecting agent against clethodim herbicide injuries in maize plants.

The objective of this study is to investigate the indirect effects of clethodim herbicide on photosynthesis and other biochemical parameters of maize leaves. Furthermore, this work aims to provide an insight about the role of SA treatment in protecting maize plants against the harmful effects caused by clethodim application.

Materials and methods

Experiment preparation and treatments: Grains of maize (*Zea mays*) were sown in a mixture of sand and clay soil in clean 3-l plastic pots (7 plants per pot); these pots were irrigated with water. Four weeks later, pots containing plants with similar growth were chosen and divided into nine groups with four pots per group. SA and/or doses of clethodim were applied by spraying to leaves until saturation. Treatments were done as the following:

Control, sprayed with water.

Cont SA, a positive control for SA, sprayed with 1 mM SA.

50, 100, 200, 500, and 1,000 ppm, sprayed with the corresponding concentration of clethodim.

200 ppm + 0.5 mM SA and 200 ppm + 1 mM SA treated with SA three days before spraying with 200 ppm clethodim.

In this experiment, clethodim doses were chosen to test the negative effects of clethodim over the large scale of concentrations. This was based on the recommended dose which was calculated as 100 ppm. Preliminary experiments were done with all clethodim doses and several SA concentrations. Double recommended dose of clethodim (200 ppm) was chosen as the medium dose to test the protective action of SA against clethodim effects.

Photosynthetic pigments content: Leaf samples (100 mg) were mashed in a mortar and pestle with 80% acetone (v/v) at 4ºC and dark conditions, the extract was filtrated through two layers of nylon and centrifuged in sealed tubes at 2,268 × *g* for 5 min. The supernatant was collected and read at 663 and 647 nm for Chl *a* and Chl *b*, respectively, and at 470 nm for carotenoids (Cars) content. The concentrations for Chl *a*, Chl *b*, and the sum of leaf Cars (xanthophylls and carotenes) were given in mg ml^{-1} extract solution according to the equations of Lichtenthaler and Buschmann (2001):

$$\text{Chl } a = 12.25\ A_{663} - 2.79\ A_{647}$$
$$\text{Chl } b = 21.50\ A_{647} - 5.10\ A_{663}$$
$$\text{Cars} = (1{,}000\ A_{470} - 1.82\ \text{Chl } a - 95.15\text{l Chl } b)/225$$

Gas-exchange parameters: Control and treated leaves were subjected to analyses of net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) using an infrared analyzer (*LI-6400 System, Li-COR*, Lincoln, NE, USA). The values are the means of 8 individuals. Water-use efficiency (WUE) was calculated as follows:

$$\text{WUE} = P_N/E$$

Analyses were conducted at midday between 9:00–11:00 h with light intensity of PAR range 1,400–1,800 µmol m^{-2} s^{-1}. Throughout the whole measurements, the atmospheric CO_2 concentration (C_{ref}) was 347.2 µmol mol^{-1} and the leaf temperature was 29–32°C. The relative air humidity was 65–70%. Over the experimental period, the conditions did not differ between treatments.

Carbohydrates content: 100 mg of dry tissue powder were boiled in distilled water in a water bath for 1 h and for extraction of total carbohydrates, 50 mg of dry tissue powder was boiled in 1 N HCl in water bath for 1.5 h. Then the extracts were cooled and filtrated and then subjected to analysis by using anthrone-sulphuric acid reagent (Fales 1951 and Schlegel 1956). A mixture of extract:anthrone (1:9) was then boiled in water bath for 7 min. After cooling, the developed blue-green color was measured against blank by using *Spekol 11* (*Carl-Zeiss*, Jena, Germany) at the wavelength of 620 nm. Soluble and total carbohydrate contents were finally calculated as mg g^{-1}(dry mass, DM). The water-insoluble carbohydrates were calculated as the difference between the amount of the total and water-soluble carbohydrates.

Protein content: According to Lowry *et al.* (1951) method, protein contents (soluble, insoluble and total) were determined. For analysis of soluble protein, tissue powders (100 mg DM) were extracted in distilled water by boiling in water bath for 1h. For total protein, 50 mg of DM was extracted in 10 ml NaOH (0.1 N) for 2 h at 90ºC. The extracts were centrifuged and the supernatants were collected. One ml of extract was mixed thoroughly with 5 ml of alkaline reagent and then allowed to stand for 10 min.

A total of 0.5 ml of Folin phenol reagent diluted 1:1 (v/v) was then added and mixed immediately. After 30 min, the absorbance against blank was measured at 700 nm. Results were expressed as mg g^{-1}(DM). Insoluble proteins were calculated as the difference between the amounts of total and water-soluble proteins.

Proline content was determined according to Bates *et al.* (1973). A 100 mg of dry powder of leaves was extracted in 10 ml of aqueous 3% sulfosalicylic acid overnight. The extract was centrifuged at 2,268 × *g* for 10 min. A total of 2 ml of the supernatant proline were mixed with 2 ml of fresh acid ninhydrin solution for reaction and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C. The reaction was terminated in an ice bath, and the mixture was extracted with 4 ml toluene. The extract was vigorously stirred for 20 s using a test tube stirrer. Therefore, the chromophore-containing toluene was aspirated from the aqueous phase, and its absorbance was measured at 520 nm. The proline contents were calculated using the standard curve of proline.

Statistical analyses: The obtained data were tested for significance by using *ANOVA* test (Duncan 1951). Means were compared by least significant differences (LSD) test at levels $P<0.05$ and $P<0.01$. All statistic tests were carried out using *SPSS* software (*Version 15, SPSS Inc.*, IL, USA).

Results

Morphological changes: Application of clethodim caused severe changes in leaf morphology (Fig. 1). The severity of changes was depending on the clethodim concentration. Lower concentrations of clethodim (50–200 ppm) caused chlorosis starting from the apical portion of the leaf. Leaves turned to yellow and sometimes took red coloration when treated with higher doses (500 and 1,000 ppm) of clethodim. Furthermore, spraying with clethodim (1,000 ppm) led to the appearance of some dead parts of the leaf margins. This phenomenon can be clearly detected 10 d after spraying (Fig. 1).

Plants treated with SA showed enhanced growth even if subjected to clethodim after three days. From Fig. 1 it is clear that SA-treated leaves were morphologically similar to those of the control.

Pigments: Among treatments, the control and SA- sprayed leaves recorded the highest contents of Chl *a*, Chl *b* and Cars (Table 1). In comparison with the untreated control, plants sprayed with SA had higher total pigments content and higher Chl *a/b* ratio. The higher Chl *a/b* ratio was due to presence of more Chl *a* than Chl *b* in SA-treated leaves. Obviously, clethodim treatments significantly reduced all pigment fractions. The reduction in pigments content was found to be dose-dependent. In response to spraying of the lowest clethodim dose, 50 ppm, a high reduction of Chl *a* was noticed but Chl *b* and Cars were slightly decreased. Application of higher doses of clethodim caused highly significant reduction in pigments content which led to yellow appearance of leaves. For example, leaves sprayed with 500 and 1,000 ppm clethodim showed reduction in total pigments of about 66% lower than control. Moreover, Chl *a/b* ratios were lower than control in response to the highest doses of clethodim. It seems that Chl *a* is more affected by clethodim application than Chl *b* in maize leaves. Interestingly, leaves pretreated with SA followed by clethodim showed recovered pigments content. SA

Fig. 1. Effect of clethodim and SA treatments on leaf morphology of *Zea mays*. (1) control; (2) SA-treated; (3, 4, 5, 6, and 7) expressing leaves treated with 50, 100, 200, 500, and 1,000 ppm clethodim, respectively. (8 and 9) expressing leaves pretreated with 0.5 mM SA and 1 mM SA prior clethodim application.

Table. 1. Effects of clethodim herbicide and salicylic acid (SA) treatments on pigments content of *Zea mays* leaves. The values are means of four replicates ± standard deviation. Statistical significance of differences compared to control: * – significant *at* $P<0.05$; ** – significant at $P<0.01$. Chl – chlorophyll; Cars – carotenoids.

Treatments	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Cars [mg g^{-1}(FM)]	Chl *a/b*	Total [mg g^{-1}(FM)]	[%]
Control	1.15 ± 0.27	0.36 ± 0.12	0.52 ± 0.15	3.18	2.04	100
Control + SA	1.32 ± 0.16	0.38 ± 0.05	0.58 ± 0.067	3.50	2.28	111.90
50 ppm	0.82 ± 0.13*	0.31 ± 0.15	0.41 ± 0.11	2.66	1.53	74.94
100 ppm	0.68 ± 0.21**	0.20 ± 0.08*	0.37 ± 0.08*	3.34	1.25	61.57
200 ppm	0.50 ± 0.045**	0.15 ± 0.02**	0.34 ± 0.08*	3.26	0.99	48.80
500 ppm	0.31 ± 0.16**	0.15 ± 0.13**	0.24 ± 0.089**	2.12	0.70	34.23
1,000 ppm	0.31 ± 0.14**	0.11 ± 0.06**	0.28 ± 0.06**	2.81	0.70	34.23
200 ppm + 0.5 mM SA	0.88 ± 0.11	0.24 ± 0.01	0.41 ± 0.05	3.74	1.52	74.48
200 ppm +1 mM SA	1.01 ± 0.19	0.28 ± 0.03	0.46 ± 0.09	3.60	1.74	85.45

applied three days prior spraying of 200 ppm clethodim recorded values nearly similar or insignificantly lower than those of untreated control leaves in all pigments fractions.

***P*$_N$ and gas-exchange parameters**: Except for C_i, it was clear that all gas-exchange parameters were highly reduced in response to clethodim application (Fig. 2). For example, P_N was significantly decreased ($P<0.05$). The decrease in P_N was concomitant with the clethodim concentration. In details, spraying of 500 and 1,000 ppm clethodim could reduce the P_N to 10.5% and 5.9% compared with the control, respectively (Fig. 2*A*). Similarly, *E* of leaves treated with high doses of clethodim showed pronouncedly reduced values in comparison with the control. A reduction of about 70.5 and 86.4% in transpiration rate was recorded with 500 and 1,000 ppm clethodim treatments, respectively. Contrary, the lower doses of clethodim (50 and 200 ppm) caused induction of *E* by 46% and 25% over control, respectively (Fig. 2*B*). Moreover, the stomatal conductance (g_s) was significantly reduced by application of high doses of clethodim. The highest reduction in g_s was reached with 500 and 1,000 ppm where the valued were reduced to be 28% and 20% of the control (Fig. 2*C*).

On the contrary, C_i followed different behaviour than other gas-exchange parameters. An increase in C_i was noticed with the increase of clethodim concentration. Application of 500 ppm clethodim caused 8 fold increases in C_i compared with the control (Fig. 2*D*). Clethodim herbicide, regardless its applied dose, caused highly significant decrease in WUE. About 80% reduction in WUE was recorded in leaves treated with 1,000 ppm clethodim (Fig. 2*E*).

Exogenous SA application resulted in average (*i.e.* more or less than control) values of gas-exchange parameters compared with the control. In most cases, the values were mostly higher than those of control. SA spraying, three days prior clethodim treatment caused improved P_N and g_s compared with those treated with the same dose of clethodim. Moreover, the (SA + clethodim) treated leaves showed WUE values higher than the control.

Carbohydrates: In all treatments, alterations in soluble and total carbohydrates content were noticed while insoluble carbohydrates remained without significant change (Table 2). It seemed that clethodim application induced accumulation of carbohydrates in maize leaves. Lower concentrations of clethodim caused accumulation of higher amounts of both soluble and total carbohydrates. It was noticed that the higher the clethodim dose the lower the carbohydrates content. Generally, in all treatments, the carbohydrate values analyzed were higher than those of the control. Treatment with the lowest clethodim dose, 50 ppm, recorded the highest increase in soluble carbohydrates that reached about 118% more than control. The total carbohydrates increased by 26% and 32% over control in leaves treated with 50 and 100 ppm, respectively.

SA treatment caused induction of higher amounts of carbohydrates whether the leaves were further sprayed with clethodim or not. A highly significant increase in soluble and total carbohydrates due to SA spraying prior clethodim application reached about 97.5% and 34% more than the corresponding control, respectively.

Proteins: Accumulation of soluble, insoluble, and total proteins was observed in maize plants as a response to clethodim and SA treatments (Table 3). With respect to the control, clethodim treatments increased soluble proteins to about 1.5 fold in most cases. Moreover, soluble proteins recorded the highest increase in 500 ppm clethodim treated samples where the increase reached 63.4% more than control. From data shown in Table 3, all values of insoluble or total proteins were found to be significantly higher than control. For example, 100 ppm clethodim caused a 66.5% and 59.8% increase in the insoluble and total proteins, respectively. Spraying SA to leaves affected only the soluble protein content by increasing 20% more than control while other protein fractions, insoluble and total, showed no clear change. On

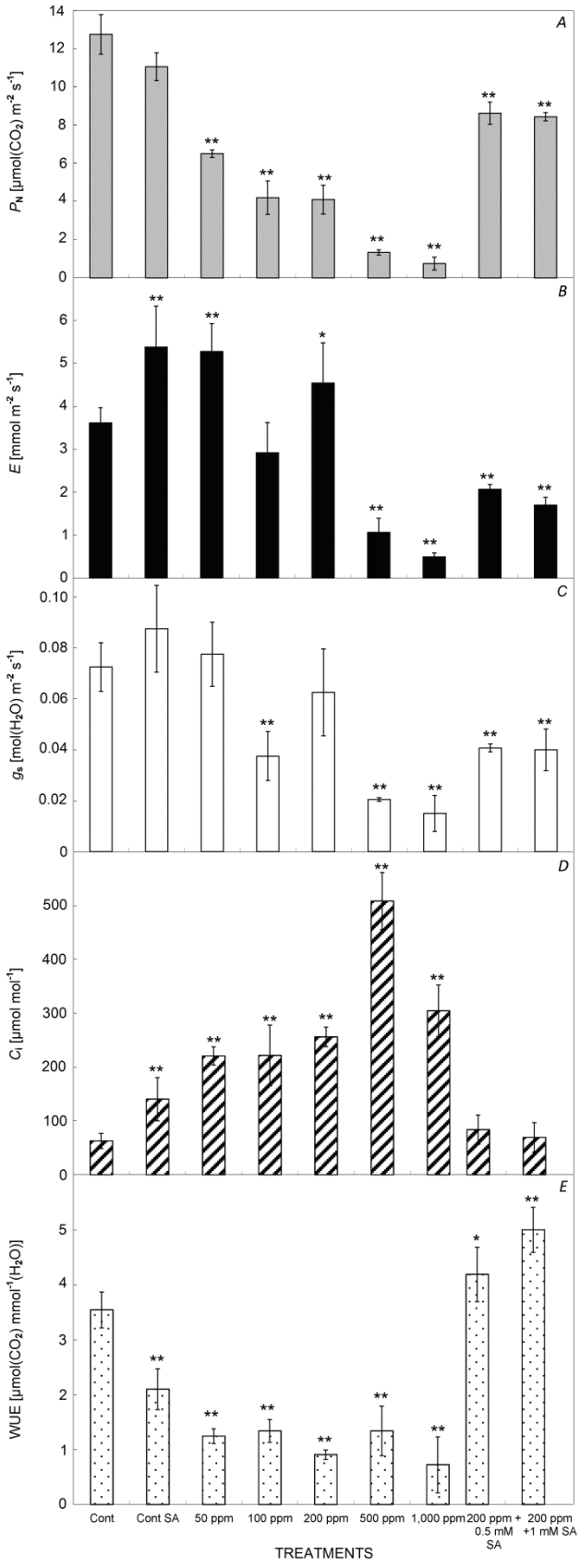

Fig. 2. Effect of clethodim and SA treatments on gas-exchange properties of *Zea mays* leaves. (*A*) photosynthetic rate (P_N); (*B*) transpiration rate (*E*); (*C*) stomatal conductance (g_s); (*D*) intercellular CO_2 concentration C_i); (*E*) water-use efficiency (WUE). Values are means of four replicates ± standard deviation. Statistical significance of differences compared to control: * – significant *at* $P<0.05$; ** – significant at $P<0.01$.

the other hand, treatment with SA prior clethodim spraying caused a highly significant increase in both soluble and total proteins. Among the treatments, 0.5 mM SA+clethodim induced 88.7% more soluble proteins than control and 1 mM SA+clethodim induced 25.7% more total proteins than its corresponding control. Furthermore, treatment with SA+clethodim had no significant effect on the insoluble proteins content.

Proline: Accumulation of proline amino acid was detected in all leaves treated with clethodim, regardless its applied dose (Table 4). Moreover, the amount of the accumulated proline due to clethodim was found to be dose-dependent. In other words, 50–1,000 ppm clethodim caused an increase ranged from 66% to 81.5% in proline content higher than control. Application of SA led to induction of amounts of proline amino acid. Without herbicide, SA increased the proline content by 25% above control while application of SA three days prior clethodim spraying caused higher increases.

Discussion

This work was to study the indirect effects of clethodim herbicide on photosynthetic apparatus of maize leaves and to discuss a protective role of salicylic acid against alterations caused by clethodim herbicide. In this experiment, maize leaves were highly affected by application of clethodim, these effects including chlorosis, yellowing of leaf-tip parts and high doses of clethodim caused browning or reddening of leaves and sometimes dead parts of the leaf margins were observed. On the other side, leaves treated with SA prior clethodim application showed no noticeable symptoms and normal leaf morphology. Lowered pigment contents are known to be responsible for the appearance of chlorosis and yellowing of leaves under unfavorable conditions such as spraying of certain herbicides (Barry *et al.* 1990, Fayez 2000).

Application of clethodim interferes with all cell metabolic processes, specifically their photosynthetic machinery. This clearly appeared in pigments content and photosynthesis rate analyses of leaves treated with clethodim (Table 1, Fig. 2). Highly significant reduction of all pigments fractions occurred due to clethodim application; in some cases the total pigment content reached 1/3 of the control. From Chl *a*/*b* ratio results, Chl *a* is more affected than Chl *b* in maize leaves subjected to high doses (1,000 ppm) of clethodim. In presence of herbicides, the pigment content was reduced either by direct pigment degradation or by inhibition of biosynthesis of either Chls or Cars (Sandmann and Böger 1986, Nakajima *et al*. 1996). Obviously, clethodim lowered the Cars content of leaves. ACCase enzyme is in part responsible for synthesizing precursors of Cars and the phytol group for Chl and thus inhibition of the enzyme by clethodim caused the decrease in pigments content. Moreover, Cars (known to be important quenchers of highly reactive triplet chlorophyll or singlet oxygen) protect Chls from photodamage (Barry *et al.* 1990, Munné-Bosch and Alegre 2000). Moreover, leaves sprayed with clethodim had lower P_N compared with the control. The severe alterations in the Chl level and subsequent leaf photochemistry are ultimately responsible for the destruction of photosynthesis caused by high doses of clethodim.

Clehtodim is known as ACCase inhibitor (Incledon and Hall 1999, Ohlrogge and Jaworski 1997) which is a key enzyme in fatty acid biosynthesis in plants (Webb and Hall 2000). Directly, clethodim affects molecules of photosynthetic pigments which are rich in lipids, especially Cars, they are predominantly hydrocarbons (lipid),

Table. 2. Effects of clethodim herbicide and salicylic acid (SA) treatments on carbohydrate content of *Zea mays* leaves. The values are means of four replicates ± standard deviation. Statistical significance of differences compared to control: $^{*-}$ significant *at* $P<0.05$; ** – significant at $P<0.01$.

Treatments	Soluble [mg g^{-1}(DM)]	[%]	Insoluble [mg g^{-1}(DM)]	[%]	Total [mg g^{-1}(DM)]	[%]
Control	33.2 ± 7.4	100	145.7 ± 1.3	100	169.7 ± 17.0	100
Control + SA	42.4 ± 6.4	127.7	132.4 ± 16.0	90.9	174.8 ± 20.8	103.0
50 ppm	72.3 ± 6.8**	217.9	143.2 ± 8.2	98.3	213.7 ± 16.8*	126.1
100 ppm	70.4 ± 0.9**	212.0	147.3 ± 6.0	101.1	225.5 ± 13.7**	132.9
200 ppm	69.2 ± 8.0**	208.5	133.5 ± 16.4	91.6	191.3 ± 26.4	112.8
500 ppm	59.1 ± 8.0**	178.1	133.7 ± 18.7	91.8	192.8 ± 11.3	113.6
1,000 ppm	58.3± 5.7**	175.7	128.0 ± 13.9	87.9	176.9 ± 15.4	104.3
200 ppm + 0.5 mM SA	60.5 ± 4.4**	182.3	167.1 ± 25.9	114.7	227.6 ± 22.4**	134.1
200 ppm +1 mM SA	65.6 ± 9.1**	197.5	161.5 ± 8.2	110.9	227.0 ± 13.4**	133.8

Table. 3. Effects of clethodim herbicide and salicylic acid (SA) treatments on protein content of *Zea mays* leaves. The values are means of four replicates ± standard deviation. Statistical significance of differences compared to control: $^{*-}$ significant *at* $P<0.05$; ** – significant at $P<0.01$.

Treatments	Soluble [mg g^{-1}(DM)]	[%]	Insoluble [mg g^{-1}(DM)]	[%]	Total [mg g^{-1}(DM)]	[%]
Control	48.0 ± 6.4	100	131.5 ± 18.9	100	179.5 ± 12.5	100
Control + SA	57.7 ± 3.3	120.2	114.7 ± 6.3	87.2	172.1 ± 11.0	95.9
50 ppm	76.3 ± 2.6**	159.0	211.2 ± 14.9**	160.6	262.1 ± 32.2**	146.0
100 ppm	74.7 ± 12.1**	155.8	219.0 ± 0.5**	166.5	286.8 ± 0.6**	159.8
200 ppm	72.4 ± 10.1**	151.0	167.6 ± 18.9*	127.5	240.1 ± 12.0**	133.7
500 ppm	78.4 ± 16.3**	163.4	154.4 ± 21.9	117.4	232.1 ± 4.2**	129.3
1,000 ppm	69.6 ± 1.8*	145.1	201.6 ± 2.9**	153.3	270.4 ± 4.4**	150.7
200 ppm + 0.5 mM SA	90.6 ± 0.6**	188.7	125.9 ± 9.9	95.7	216.0 ± 7.5*	120.3
200 ppm +1 mM SA	82.2 ± 1.8**	171.4	146.9 ± 7.4	111.7	225.7 ± 8.9**	125.7

Table. 4. Effects of clethodim herbicide and salicylic acid (SA) treatments on proline content of *Zea mays* leaves. The values are means of four replicates ± standard deviation. Statistical significance of differences compared to control: * – significant at $P<0.05$; ** – significant at $P<0.01$.

Treatments	Proline [μg g^{-1}(DM)]	[%]
Control	1.73 ± 0.76	100
Control + SA	2.19 ± 0.60	126.9
50 ppm	2.87 ± 0.50*	166.3
100 ppm	2.78 ± 0.62*	160.8
200 ppm	2.94 ± 0.37*	170.3
500 ppm	3.07 ± 0.85**	178.0
1,000 ppm	3.14 ± 0.97**	181.5
200 ppm + 0.5 mM SA	2.51 ± 0.41	145.1
200 ppm +1 mM SA	2.28 ± 0.56	131.9

and chloroplasts have an almost identical lipid composition. In this respect, ACCase inhibitors affect not only pigments but also membranes including thylakoid membranes. Target ACCase is present in rapidly dividing cells and in active chloroplasts (Croon *et al.* 1989). In addition to ACCase inhibition, cyclohexanedione herbicides are known to affect plasma membranes of treated plants due to perturbation of fatty acid synthesis (Tomaso 1994). Hence, clethodim can indirectly alter metabolic processes including the photosynthetic machinery.

In this work, SA-treated leaves had normal contents of each pigment fractions (similar to or over the control). These data suggest a protective role of SA to avoid clethodim negative effects. This role may be through the improvement of photosynthesis by activation of synthesis of pigment constituents especially Cars that protected the other pigment fractions from oxidation and damage. Similarly, Moharekar *et al.* 2003 reported that SA activated the synthesis of Cars and xanthophylls and also enhanced the rate of deepoxidation with a concomitant change in Chl pigments and Chl *a*/*b* ratio in wheat. Furthermore, the foliar application of SA also proved to be equally fruitful in increasing the pigment contents in *Brassica napus* (Ghai *et al.* 2002).

In this experiment, the analysis of gas-exchange parameters showed significant changes. For example, E of high-dose clethodim-treated leaves were reduced to half value of the control. Moreover, g_s was reduced to 20% of control with 1,000 ppm clethodim. Contrary, clethodim accumulated much substomatal CO_2 (C_i) and reduced WUE of the plant. High C_i may be due to defects in photosynthesis or increasing of respiration rate. Furthermore, SA spraying before clethodim had improved P_N, g_s, and WUE where the values were significantly higher than those of the control. Similarly, SA enhanced WUE, E, and C_i in soybean and corn leaves (Kumar *et al.* 2000, Fariduddin *et al.* 2003, Hayat *et al.* 2005, Khan *et al.* 2003).

The behaviour of carbohydrates is contradictory among herbicides and plants (Magné *et al.* 2006). In relation to control, carbohydrates content was found to be increased after clethodim application. Accumulation or decline of carbohydrates was previously reported with other herbicides (Magné *et al.* 2006, Saladin *et al.* 2003). Breaking or totally blocking of the pathway of carbohydrate consumption may lead to carbohydrate accumulation even if the photosynthetic rate is lower than normal.

Protein contents of maize leaves are highly influenced by clethodim application. Soluble and total proteins recorded significant increases compared with the corresponding controls. A variety of environmental factors such as herbicides have been reported to influence the synthesis of plant proteins (William 1989). Accumulation of proteins caused by abiotic stresses such as herbicides can be explained on the basis of enhanced protein synthesis due to activation of N_2 assimilation or induction of the activity of enzyme. This accumulation may be one mechanism by which plants can resist the action of herbicides and xenobiotics. Glutathione S-transferases (GSTs) have a major role in herbicide detoxification and in protecting plants against oxidative damage (Kilili *et al.* 2004). GSTs form a large family of nonphotosynthetic enzymes known to function in the detoxification of xenobiotics. The *in vitro* activity of this enzyme is affected by SA application (Watahiki *et al.* 1995).

Biotic and abiotic stresses such as herbicides induced accumulation of proline (Radwan *et al.* 2007, Fayez 2000). In this experiment, proline content increased in clethodim-treated leaves and the increase was concomitant with clethodim sprayed dose. Moreover, SA induced elevated amounts of proline in leaves treated or untreated with clethodim. Hence, SA application may provide some protection to maize plants against the harmful effects caused by clethodim through accumulation of proline. It was reported that proline acts as a cryoprotectant (Hellergren and Li 1981), or as a protective agent for cytoplasmic enzymes and cellular structures (Schobert 1977). Previously, Shakirova *et al.* 2003, observed enhanced accumulation of proline in wheat seedlings treated with SA. They reported that accumulation of proline is one of the protective ways against abiotic stresses induced by external SA application.

In conclusion, clethodim herbicide affects negatively maize leaves by alteration of physiological, biochemical and metabolic processes leading to the appearance of certain morphological changes. Prespraying with SA can protect plants partially or totally from clethodim injuries. The protective role of SA can be evidenced through the improvement of photosynthesis, potentiation of the gas exchange and/or induction of some metabolites as proteins and proline or activation of some enzymes which in turn have a role in herbicide detoxification.

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