CADMIUM-INDUCED CHANGES IN MAIZE LEAVES AND THE PROTECTIVE ROLE OF SALICYLIC ACID

A.Ivanova[1*], A. Krantev[1], Zh. Stoynova[1], L. Popova[1]

[1]Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl 21, 1113 Sofia, Bulgaria

Summary. Seeds of maize (Zea mays L., hybrid Norma) were sterilized and divided into two groups: untreated (the seeds were soaked in water) and pre-treated (the seeds was soaked in 500 μM salicylic acid (SA) solution for 6 h). Three–day–old dark grown seedlings, were placed in polyethylene pots filled with modified Hoagland solution. $CdCl_2$ was added at concentrations of 10, 15 and 25 μM, leading to low, moderate and severe stress, respectively. Plants were grown in a growth chamber and after 14 days of growth, i.e. 3 days after soaking, the plants were harvested for analyses. The plant samples were extracted with chloroform – methanol (1:1, v/v), the extracts were transesterified and the obtained fatty acids (FA) were analyzed by gas chromatography. Cd^{2+}- treatment of the untreated plants led to insignificant changes in the relative amounts of monoenoic and dienoic fatty acids, and of the long-chain FA as well. The applied low Cd-stress led to an increased amount of trienoic acids and decreased saturated acids. The severe stress had the opposite effect on the untreated maize plants. Pre-treatment of maize plants with SA seemed to have an insignificant effect on the amounts of monoenoic and long-chain FA. The low and moderate stress led to decreased saturated FA and increased trienoic FA. The severe stress caused FA- profiles similar to those of the control plants. The strong decrease of trienoic FA and increase of saturated FA was an indication that the biosynthetic pathway 18:0 →18:3

*Corresponding author, e-mail: albena55@yahoo.com

was disturbed. It is evident that the severe Cd-stress led to serious changes in the lipid membranes.
The changes in FA-profiles of pre-treated plants were less expressed in comparison to those of the untreated plants. It can be concluded that SA plays a protective role on the lipid membranes of the Cd-treated maize plants.

Key words**:** cadmium, fatty acids, plant protection, salicylic acid, stress, *Zea mays*.
Abbreviations**:** SA – salicylic acid; FA – fatty acids; FAME – fatty acids methyl esters.

INRODUCTION

Cadmium is a trace element witch has not biological functions, but is highly toxic to plants and animals (Alloway, 1990). It enters the environment mainly from industrial processes and phosphate fertilizers and can reach high levels in agricultural soils. The oxidizing conditions of weathering in soil release Cd as the soluble and mobile Cd^{2+} ion, which is easily assimilated by plants. Taken up in excess Cd disturbed almost all physiological processes in plants (see reviews: Barcelo and Poschenrieder, 1990; Van Assche and Clijsters, 1990; Krupa and Baszynski, 1995; Siedlecka, 1995; Vassilev and Yordanov, 1997) and affected the lipid membranes (Quariti et al., 1997).

Salicylic acid (SA) as a potent signaling molecule in plants is involved in eliciting specific responses to biotic and abiotic stresses (Janda et al., 1999; Tasgin et al., 2003;). Furthermore, SA is also known to be involved in plant protection to cadmium toxicity (Pal et al., 2002; Metwally et al., 2003).

This study was undertaken to determine the physiological, biochemical and lipid changes in maize plants treated by SA during Cd-induced stress, to investigate whether this plant regulator is involved in the induction of defense response and to test the hypothesis that the observed protection of SA on photosynthesis against Cd stress is mediated by its effect on antioxidant defense system.

MATERIALS AND METHODS

Plant growth and treatment with Cd

Seeds of maize (Zea mays L., hybrid Norma) obtained from Agricultural Research Institute, Martonvasar were sterilized and divided into two groups. One half of the seeds were soaked in water (samples 1-4 Table 1). The other half of the seeds was soaked in 500 μM SA solution for 6 h. (samples 5-8 Table 1), and then the both groups were allowed to germinate on moist filter paper in dark. Three-day-old, dark grown seedlings, were placed in polyethylene pots filled with 0.6 L modified Hoagland solution (0.3125 mM KNO_3, 0.45 mM $Ca(NO_3)_2$, 0.0625 mM KH_2PO_4, 0.125 mM $MgSO_4$ x $7H_2O$, 11.92 μM HBO_3 , 4.57 μM $MnCl_2$ x 4 H_2O, 0.191 μM $ZnSO_4$ x 7 H_2O, 0.08 μM $CuSO_4$ x 5 H_2O, 0.024 μM $(NH_4)_6Mo_7O_{24}$ x 4 H_2O, 15.02 μM $FeSO_4$ x 7 H_2O, 23.04 C Na_2 EDTA x 5 H_2O). The nutrient solution was aerated twice a day, and changed three times in a week. $CdCl_2$ was added at a concentration of 10, 15 and 25 μM (samples 2-4 and 6-8 respectively Table 1), leading to low, moderate and severe stress, respectively. Plants were growth in a growth chamber at a day/night cycle 16h/8h, at 22/18 °C, relative humidity between 50 % and 60 %, and 120μmol $m^{-2}s^{-1}$ PAR. After 12 days of growth, i.e. 3 days after soaking, the plants were harvested for analyses.

Isolation of the total lipid extracts

All leaf samples (8.5-12.5g) were cut into small pieces and extracted twice with 65 ml chloroform – methanol (1:1 v/v). The extracts were combined and diluted with water until two layers were obtained. The lower layer was evaporated under vacuum and the obtained total lipophilic extracts were kept at -30 °C. Table 1 shows dry weights of the samples and yields of lipophilic extracts.

Analysis of the total lipophilic extracts

Part of lipophilic extracts (50 mg from each sample) was transported

in small vials with teflon screw caps. Five ml of 15 % acetyl chloride in absolute methanol was added and the vials were heated 4h at 55 °C. After cooling the samples were diluted with water and the obtained fatty acids methyl esters (FAME) were extracted with hexane (2 x 5 ml). The FAME in combined hexane extracts were purified by preparative thin–layer chromatography on 20 x 20 cm silica gel G (Merck) plates (layer thickness 0.5 mm) with mobile phase hexane – acetone (95:5, v/v). The spots of the FAME were visualized under UV – light, scrapped off with the silica-gel layer and eluted with hexane. The amount of each sample was determined gravimetrically (see Table 1).

The FAME were analyzed by gas chromatography on Hewlett Packard 5890 (Hewlett Packard, Palo Alto, California, USA) equipped with FID and capillary column SP WAX 52CB (30 m x 0.25 mm). The temperature was programmed from 165 to 230 °C at a rate of 4 °C min^{-1} and a 10 min hold at 230 °C. The temperature of injector was 260 °C, and of the detector was 280 °C; the carrier gas was nitrogen (1.45 x 10^{-3}Pa).

RESULTS AND DISCUSSION

Effect of SA and Cd^{2+}on the amount of biomass, total lipophilic extracts and FAME

It is evident that SA-treatment increased the amounts of the total lipophilic extracts and the lipid composition (expressed as FAME), but there were not statistically significant differences in the amounts of the leaf biomass (compare samples 1 and 5, Table 1)

The treatment with Cd^{2+} led to an increase of the total lipophilic extracts. The moderate Cd-stress (10μM) led to increased FAME, but the higher Cd^{2+} solutions caused decreased FAME amounts (samples 3 and 4, Table 1). The decrease of lipids (respectively FAME) was observed in Cd–stressed wheat (Malik et al., 1992), barley (Vassilev et al., 2004), tomato (Krupa and Baszynski, 1985; Krupa and Baszynski, 1989; Quariti et al., 1997; Ben Ammar et al., 2005) and mustard (Gaur and Gupta, 1994; Nouairi et al., 2006) plants.

Pre-treatment of maize plants with SA resulted in an increase of lipid

Table 1. Effect of SA and Cd^{2+} on the amount of biomass, total lipophilic extracts and FAME in maize leaves.

SAMPLES			Dry wt	Total lipophilic extract	FAME
№	SA [μM]	$CdCl_2$ [μM]	[g]*	[mg.g^{-1}DW]*	[mg.g^{-1}DW]**
1	-	-	0.7 ± 0.1	150 ± 6	6.3 ± 0.5
2	-	10	0.6 ± 0.1	183 ± 8	10.2 ± 0.8
3	-	15	0.6 ± 0.1	175 ± 7	1.8 ± 0.1
4	-	25	0.5 ± 0.1	170 ± 7	3.2 ± 0.2
5	500	-	0.8 ± 0.1	185 ± 8	10.9 ± 0.9
6	500	10	0.7 ± 0.1	214 ± 11	11.0 ± 0.9
7	500	15	0.5 ± 0.1	100 ± 4	10.4 ± 0.8
8	500	25	0.6 ± 0.1	175 ± 7	4.7 ± 0.4

* Results ± SD from three parallel experiments.
** Results obtained from three parallel preparative thin-layer chromatographic and GC - procedures.

content by low and mild Cd-stress. Only the severe Cd-stress led to a decrease in FAME content, similar to those observed in not pre-treated with SA plants (compare samples 4 and 8, Table 1). Probably, by high Cd^{2+} - concentrations in the nutrient solution the SA cannot prevent the effect of Cd on lipid membranes of maize plants.

Effect of SA and Cd^{2+}on the fatty acid composition in maize leaves

Treatment with Cd^{2+} (samples 2 - 4, Table 2) led to a decrease in the content of the short– chain (C_{14} – C_{15}) and an increase in the long–chain fatty acids (C_{20} – C_{24}). Possibly the Cd – treatment caused activation of enzymes, responsible for elongation of C_{18} – acids.

The higher Cd–stress (sample 4, Table 2) increased the content of

Table 2. Effect of SA and Cd^{2+} on the fatty acid composition in maize leaves.

SAMPLES			Fatty acids (wt % of total) *											
№	SA (μM)	Cd^{2+} (μM)	14:0	14:1	15:0	15:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0
1	-	-	3.3	3.2	1.2	1.9	25.1	3.7	3.1	3.8	14.8	36.3	1.0	1.4	1.3
2	-	10	0.4	2.2	0.9	1.8	23.4	3.5	2.6	3.7	16.0	42.4	0.8	1.0	1.4
3	-	15	1.2	2.7	1.0	1.5	27.2	4.0	3.2	3.7	15.8	35.6	1.0	1.5	1.7
4	-	25	1.0	3.1	1.4	2.2	33.2	3.5	4.2	5.0	14.3	26.3	1.4	2.1	2.4
5	500	-	1.5	2.4	0.8	1.8	22.9	3.5	2.6	4.5	16.4	40.4	0.7	1.3	1.1
6	500	10	1.7	2.2	0.8	1.4	21.5	4.1	2.2	3.4	18.5	41.1	0.7	1.1	1.2
7	500	15	0.6	1.4	0.2	1.2	20.5	3.1	2.3	4.5	18.4	46.3	0.4	0.6	0.4
8	500	25	1.8	3.5	1.1	1.6	21.9	3.5	2.4	2.9	13.7	43.9	0.8	1.4	1.6

*) Values obtained from three parallel measurements; the SD (related to peak proportions on the chromatogram) are as follow: ± 0.3 for 16:0 and 18:3; ± 0.2 for 18:2 and ± 0.1 for the others.

saturated acids (16:0 and 18:0) and decreased the content of linolenic (18:3) acid. This is a typical reaction of plant lipid membranes to environmental stress, leading to decreased lipid membranes permeability. The same effect was observed in other plants, too (Jemal et al., 2000; Nouairi et al., 2006).

The FA changes in maize plants treated with low doses of Cd were apparently smaller. A similar effect was observed in tomato plants (Quarity et al., 1997). The more severe Cd-stress led to decreased linolenic acid content, as observed in other Cd-treated plants (Krupa and Baszynski, 1989; Jemal et al., 2000; Vassilev et al., 2004; Nouairi et al., 2006).

The changes in the content of the oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids showed a reserve trend. The same was observed in mustard seeds treated with Cd^{2+} (Gaur and Gupta, 1994). The content of saturated acids increased, but the amount of hexadecaenoic acids remained constant.

Pre-treatment of maize plants with salicylic acid seemed to have an insignificant effect on their fatty acid composition (sample 5, Table 2). There was a slight increase of linolenic acid and a decrease of all saturated FA.

The mild Cd-stress (10μM) applied on the pre-treated with SA plants led to the same changes in their FA-composition, as in the non-treated (compare samples 2 and 6, Table 2). The amount of the saturated acids decreased while the content of linoleic and linolenic acid increased.

The severe Cd-stress (25μM) applied on the pre-treated with SA plants led to FA-profiles, similar to those of the control plants (compare samples 1 and 8, Table 2). In all cases, the changes in fatty acid composition after severe Cd^{2+}-stress in the pre-treated with SA plants were opposite, as in the non-treated plants (compare samples 4 and 8, Table 2). Antagonistic influences of Cd and SA have been observed also in other plants (Drazic and Mihailovic, 2005; Drazic et al., 2006). Based on the results presented in Table 2 it could be concluded that SA plays a protective role on the lipid membranes of Cd-treated maize plants.

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