BULG. J. PLANT PHYSIOL., 2002, 28(1–2), 59–65

CHANGES IN LIPID COMPOSITION OF *Phaseolus vulgaris* LEAVES AFTER SIMULATING ACID RAIN TREATMENT

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Received April 25, 2002

Summary. In the present study we have examined the influence of single spraying with simulated acid rain (pH 1.8) on the content of main lipid classes and their fatty acid composition in the thylakoid membranes of bean plants. Acid stress caused considerable changes in the investigated parameters. A decline in the content of linolenic acid, a highly unsatureted fatty acid which is an indicator of lipid peroxidation was observed. This decrease should result in decreased fluidity of the membrane lipid bilayer. The MGDG/DGDG ratio considerably increased at the 3rd hour after acid spraying, which is an indicator of membrane injury and of the lower packing properties.

Key words: *Phaseolus vulgaris* L., acid rain, lipids, fatty acids

Abbreviations: DGDG – digalactosyl diacylglycerols; FA – fatty acid; FAME – fatty acid methyl esters; LOOH – lipid hydroperoxides; MGDG – monogalactosyl diacylglycerols; MDA – malonyldialdehyde; PL – phospholipids; TAG – triacylglycerols.

Introduction

Some of the most important pollutants present in the atmosphere with harmful effects on vegetation are O_3, SO_2 and NO_x. The latter two are capable of forming acidic compounds that are deposited as either dry or wet deposition. The acid rains cause damages on the nature even in regions distant from the direct source of emission. The acid rain components (mainly SO_2 and NO_x) generating reactive free oxygen radicals that may cause inhibition of photosynthesis, enzyme breakdown, membrane damage, DNA al-

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terations, all resulting in a reduced plant growth (Hippeli and Elstner, 1996). It is generally accepted that one of the first results of stress is the alteration in the structure and function of cell membranes, i.e. the occurrence of a gel-phase lipid-domain in the liquid-crystalline bilayer, which produces an increase in thylakoid membrane permeability and microviscosity (Ferrari-Iliou et al., 1984; Liljenberg, 1992). This leads to a disturbance of the association between membrane lipids of thylakoids and proteins as well as the enzyme activities and transport capacity of the bilayer (Caldwell and Whitman, 1987).

In our previous papers we reported on the effects of simulated acid rain with different pH (in the range from 4.6 to 1.8) on the structure and functional activity of the photosynthetic apparatus of *Phaseolus vulgaris* L. It was established that acid treatment (pH 4.6 and 2.6) did not cause noticeable changes in photosynthetic CO_2 fixation and photochemical activity of bean plants. However, pH 2.2 and 2.4 treatments caused mild stress, whereas those of pH 2.0 and 1.8 caused acute stress. Simulated acid rain (pH 1.8) strongly decreased the photosynthetic CO_2 assimilation, the oxygen evolution, as well as the PS2 activity (Velikova et al., 1997, 1998). This treatment led also to increased CO_2 compensation point and non-photochemical quenching, and changed the shape of CO_2 and light curves of photosynthesis (Velikova et al., 1999). Single acid spraying (pH 1.8) was accompanied by an increased H_2O_2 production, lipid peroxidation, as well as by changes in peroxidase and catalase activities (Velikova et al., 2000).

In this paper we present the effect of the simulated acid rain (pH 1.8) given by single spraying of bean plants on the content of main lipid classes and their fatty acid composition in primary leaves.

Materials and Methods

Experiments were carried out with 10-day-old bean plants (*Phaseolus vulgaris* L. cv. Cheren Starozagorsky) after the emergence of the first composite bean leaf. Plants were grown in a climatic chamber at a photon flux density of about 120 $\mu mol.m^{-2}.s^{-1}$, temperature of 23–25 ºC and a 12 h photoperiod (Velikova et al., 1997). The simulated acid rain was prepared according to Seufert et al. (1990) and contained the following components: NH_4NO_3 (1.3 g/l), $MgSO_4.7H_2O$ (3.1g/l), Na_2SO_4 (2.5g/l), $KHCO_3$ (1.3g/l), $CaCl_2.2H_2O$ (3.1g/l). After dilution of initial solution 1:100, pH value was adjusted to 1.8 with 1N H_3PO_4 and 1N H_2SO_4. The cocktail used to spray the control plants has the same composition as the acid rain but the pH value is 5.6. Tween 80 (0.5%, v/v) was used as surfactant. Each plant was sprayed with 2 ml simulated acid rain or cocktail pH 5.6. Both control and treated plants were sprayed only once. The vessels were covered with lids, which did not permit the acid rain to contaminate the nutrient solution.

For isolation and analysis of the main lipid classes the leaves were homogenized with $CHCl_3$–MeOH (1:1, v/v) and refluxed for few minutes to inactivate the enzyme systems. The lipid extraction of the samples was performed according to Bligh and Dayer (1959). The main lipid classes: monogalactosyl diacylglycerols (MGDG), digalactosyl diacylglycerols (DGDG) and phospholipids (PL) were isolated by preparative thin-layer chromatography as described by Ivanova *et al.* (1993) and transesterified. The analysis of the obtained fatty acid methyl esters (FAME) was performed by gas chromatography after addition of internal standart (heptadecanoic acid). The amount of each lipid class was determined on the basis of the FAME mass using converting factors as follows: 1.0 for TAG; 1.4 for MGDG and PL and 1.8 for DGDG (Elenkov et al., 1993). The measurements were carried out 3 and 24 h after acid rain treatment. All results were represented as means ± SE from 3 independent series of experiments. Experiments were repeated three times, with two replicates per experiment, using leaves of about 5 plants per replicate. Significant differences were determined by the Student's *t*-test.

Results and Discussion

Previous work has indicated that treatment of bean plants with simulated acid rain (pH 1.8) induced an increase of H_2O_2 and MDA (end product of lipid peroxidation) content (Velikova et al., 2000). The possible relationship between the extent of lipid peroxidation and behaviour of membrane lipids was examined further in the present study by monitoring changes in fatty acid (FA) composition and lipid fluidity of membranes in acid rain-treated leaves of bean plants. In Table 1 the changes in the amounts of the main lipid classes in the primary bean leaves at the 3rd and 24th h after treatment are presented. The applied stress led to more substantial changes in the amounts of MGDG and DGDG. The amount of MGDG was twice lower comparing to the control after 3rd h of the treatment. Then it increased and was near to the control level (at the 24th h). On the contrary, the amount of DGDG was twice higher at the 3rd h and then decreased, but nevertheless it was higher than in control plants. Similar changes with these of MGDG were observed in the amounts of TAG. The concentration of TAG in acid treated plants significantly decreased 3 h after spraying. The level was only 45% of the control. Then its content gradually increased. The MGDG/DGDG ratio, which is an indicator of the packing properties of thylakoid membranes, was 4-fold lower in stressed plants at the 3rd h after acid spraying. It was due to the sharp decrease in the MGDG concentration. Later, the differences in MGDG/DGDG ratio in stressed plants were less pronounced. An increased MGDG/DGDG ratio was found by Süss and Yordanov (1986) in bean plants acclimated to high temperature.

The FA composition of thylakoid membranes in control leaves (pH 5.6) and leaves treated with simulated acid rain (pH 1.8) is illustrated in Table 2. The main FA in all

Table 1. Influence of simulated acid rain (pH 1.8) on the content of main lipid classes in bean leaves 3 and 24 h after acid treatment (mean ± SE, n = 3)

Variants	Lipid classes	Concentration [mg g^{-1}(d.m.)]	[% of *total]	MGDG/DGDG ratio
Control pH 5.6	MGDG	17.6 ± 1.8	50.7	1.7
	DGDG	10.3 ± 1.1	29.7	
	PL	5.7 ± 0.6	16.4	
	TAG	1.1 ± 1.1	3.2	
	*total	34.7 ± 3.5	100.0	
pH 1.8 – 3 h	MGDG	8.1 ± 0.8	21.7	0.4
	DGDG	21.6 ± 2.1	57.9	
	PL	7.1 ± 0.7	19.0	
	TAG	0.5 ± 0.1	1.3	
	*total	37.3 ± 3.8	100.0	
pH 1.8 – 24 h	MGDG	16.3 ± 1.7	42.0	1.2
	DGDG	13.9 ± 1.4	35.8	
	PL	6.8 ± 0.7	17.5	
	TAG	1.8 ± 0.2	4.6	
	*total	38.8 ± 4.0	100.0	

* Total sum of the lipid classes (MGDG, DGDG, PL and TAG) in bean leaves identified in the present study

classes was linolenic acid (18:3). In control, it represented more than 90% in MGDG and 67% in DGDG, while in PL and TAG it was about 40% of the total fatty acid content. Acid spraying caused significant changes in the FA composition, particularly 3 h after spraying. The amounts of the saturated fatty acids (16:0 and 18:0) remarkably increased in MGDG. Palmitic acid (16:0) was notable enhanced in TAG, but stearic acid (18:0) was not changed. There were changes in oleic acid (18:1) concentrations. It increased in all main lipid classes, but in different extents, the most substantial changes being observed in MGDG and PL. The concentration of linoleic acid (18:2) was increased in DGDG and especially in MGDG, but in TAG it decreased. The amounts of linolenic acid decreased in all main lipid classes, especially in PL at the 3rd h after treatment. This decline in linolenic acid, a highly unsatureted FA, is indicative of lipid peroxidation. The decrease in FA unsaturation and accompanying lipid peroxidation should result in decreased fluidity of the membrane lipid bilayer, caused by acid rain treatment.

Our results are in accordance with the data of other authors. Covello et al. (1989) reported that exposure of bean leaves to SO_2 or bisulfite induced peroxidation of thylakoid lipids, decreased fluidity of the thylakoid membranes and inhibition of PS2 ac-

Table 2. Influence of simulated acid rain (pH 1.8) on the fatty acid composition of the main lipid classes in bean leaves 3 and 24 h after acid treatment (mean ± SE, n = 3)

Variants	Lipid classes	Fatty acid (% w/w)					
		16:0	16:1	18:0	18:1	18:2	18:3
Control pH 5.6	MGDG	2.8±0.1	1.1±0.04	0.6±0.03	0.2±0.01	3.0±0.09	92.1±5.9
	DGDG	18.1±0.6	3.0±0.1	3.4±0.1	0.5±0.01	7.6±0.2	67.3±4.3
	PL	29.2±1.1	11.7±0.4	4.7±0.2	0.5±0.01	12.0±0.4	41.8±2.7
	TAG	26.9±1.0	12.8±0.5	4.1±0.2	1.8±0.02	9.0±0.3	45.3±2.9
pH 1.8 – 3 h	MGDG	9.0±0.3	0.2±0.01	3.0±0.1	1.5±0.02	5.5±0.02	80.8±5.2
	DGDG	17.6±0.6	2.6±0.1	2.3±0.1	0.9±0.01	12.6±0.04	63.8±4.1
	PL	30.6±1.1	9.2±0.3	5.3±0.3	2.7±0.04	17.8±0.5	34.3±2.2
	TAG	41.9±1.4	32.1±1.3	3.7±0.2	3.9±0.05	5.5±0.2	12.9±0.8
pH 1.8 – 24 h	MGDG	5.3±0.2	0.7±0.02	1.2±0.06	0.6±0.01	3.9±0.1	88.0±5.7
	DGDG	17.3±0.6	2.3±0.09	4.0±0.2	1.9±0.02	12.0±0.4	62.5±4.0
	PL	28.5±1.0	6.8±0.2	4.6±0.2	2.2±0.03	20.0±0.6	37.9±2.4
	TAG	22.7±0.8	8.2±0.3	5.4±0.3	3.4±0.05	12.1±0.4	48.2±3.1

tivity. Lipid peroxidation has been observed in isolated bean membranes after ozone treatment (Pauls and Thompson, 1980). It can be initiated by hydroxyl radicals, in the presence of transition metal ions or the prooxidant ascorbate (Gutteridge and Halliwell, 1990). After abstraction of a hydrogen atom from a methylene group of an unsaturated FA, the carbon radical is stabilized by rearrangement to a conjugated diene. This species reacts with oxygen and forms a peroxy radical that again is capable of hydrogen abstraction from another FA, leading to the formation of lipid hydroperoxides (LOOH). LOOHs decompose in the presence of transition metals or enzymes, such as glutathione peroxidase, glutathione transferase or lyases, into a variety of compounds, including short chain aldehydes, pentane and ethane (Schraunder et al., 1997). The conjugated double bonds within a LOOH reduce the membrane fluidity and form kinks within the membrane, which may influence integral or peripheral membrane proteins (Chandra et al., 1996). Lipid peroxidation occurs not only during cell damage, but may be an early effect of ozone treatment possibly involved in signaling (Kondo et al., 1992).

We suggested that the effects observed in consequence of acid rain treatment could be due to an increased intracellular accumulation of H^+ and harmful ions, such as sulfate and phosphate, contained in the cocktail (Velikova et al., 1999). This probably led to impaired membrane permeability, enhancement of stroma acidity, uncoupled electron transport and insufficient accumulation of ATP and NADPH, which affected carbon metabolism. The results reported here showed that the remarkable changes in

main lipid classes, caused by acid rain treatment, perhaps contributed to the lowering of the functional activity of the photosynthetic apparatus.

Acknowledgments: This research was supported by the National Science Fund (Project MU – BAV – 7/1995) of the Ministry of Education and Science.

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