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BRIEF COMMUNICATION

Recovery of photosystem 2 and membrane lipid composition in triazine-treated soybean seedlings by vitamins

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Abstract

Triazine (0.6 mM) quenched the chlorophyll fluorescence emission at 686 nm, selectively reduced the biosynthesis of linolenic acid ($C_{18:3}$), lowered the ratio of unsaturated/saturated fatty acids in thylakoid membranes and percentage of total glycerides in the extracted oils, and extremely enhanced ethylene production by seedlings. These effects were partly reversed by the addition of 100 g m^{-3} of vitamins (thiamine > nicotinic acid > pyridoxine).

Additional key words: chlorophyll fluorescence, emission spectra, ethylene, thiamine, nicotinic acid, pyridoxine, linolenic acid, glycerides.

The uncontrolled increase in the consumption of herbicides, pesticides and chemical compounds is one of the important reasons for the rapid pollution of the environment. Commercialy important herbicides inhibit photosynthetic electron transport at various sites (Covello *et al.* 1989, Tollenaar and Mihaylovic 1991, Dekker 1993, Bertoluzza *et al.* 1995, Rai *et al.* 1995). Triazine herbicides act on the reducing side of photosystem 2 (PS 2) which is followed by lipid peroxidation (Asami *et al.* 1995, El-Shintinawy and Selim 1995). Photoinactivation of PS 2 is an early symptom of incipient photodamage by herbicides (Streb and Feierabend 1993). Changes in fatty acid saturation or in the ratio of proteins to lipids increase the thylakoid membrane fluidity which indicates the extent of adaptive modification of the lipid matrix (Barber 1985). Antioxidative systems including vitamins protect plants from oxidative stress induced by herbicides by enhancing antioxidant enzyme activity (Alscher 1989, Finckh and Kunert 1985, Kraus *et al.* 1995). The aim of the present study was to test the hypothesis that herbicidal damage of thylakoid membrane isolated from leaves of soybean plants affected by triazine involves an

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inhibition of PS2 as well as peroxidation of membrane lipids, and that vitamins (thiamine, nicotinic acid and pyridoxine) alleviate the herbicidal effect.

Soybean (*Glycine max* L.) seeds (from Sahka Agriculture Center) were soaked for 24 h in distilled water at room temperature. Always 30 seeds were placed on cotton in a 10 cm Petri dish. The Petri dishes were incubated under an irradiance of 40 W m^{-2} at 15-h photoperiod. Triazine (2-chloro-5-ethylamino-6-isopropyl-amine-5-triazine) and vitamins (thiamine, nicotinic acid and pyridoxine) were applied with normal watering to the 3-d-old seedlings. After 14 d the plants were used in experiments.

Thylakoid membranes were prepared as described by Osman and El-Shintinawy (1988). Leaves (10 g) were homogenated for 5 s in 60 cm^3 of isolation buffer (50 mM Tricine, pH 7.8, 50 mM NaCl, 3 mM $MgCl_2 . 6 H_2O$ and 0.5 mM EDTA) using a mixer. The homogenate was filtered through eight layers of cheesecloth and centrifuged for 2 min at 2000 *g*. The pellet was resuspended in 20 cm^3 of suspension medium containing 400 mM sorbitol, 50 mM KH_2PO_4, 2 mM $MgCl_2 . 6 H_2O$ and 0.1 % bovine serum albumin, pH 7.5. The suspension was centrifuged at 3000 *g* for 90 s. The pellet was suspended in 2 cm^3 of suspension buffer. Thylakoids containing 5 g(Chl) m^{-3} were used for fluorescence measurements. Chlorophyll (Chl) *a* concentration was determined spectrophotometrically in 80 % acetone extract according to Arnon (1949).

PS 2 activity was determined by measuring Chl *a* fluorescence emission spectra at room temperature using the *Perkin-Elmer LS50B* fluorometer. Measurements were done after maintaining the samples in complete darkness for 15 min. Fluorescence emission was detected in wavelength range of 600 - 700 nm with a slit width of 3.3 nm. The signals were stored and analyzed by a computer (*IBM*).

Lipids were extracted from soybean thylakoid membranes according to a modified procedure of Bligh and Dyer (1959). Fatty acid methyl esters were prepared with boron trifluoride methanol and analyzed by gas chromatography as described by El-Shintinawy and Selim (1995). Approximately 150 mg of isolated thylakoid membranes were used for fatty acid analysis.

To measure ethylene production, 10 germinating plants were placed in a 5.5 cm test tube sealed with a rubber serum cap and stored under a white light (40 W m^{-2}) at room temperature for 15 min. Gas samples (1.0 cm^3) were withdrawn with a syringe and injected in a *Shimadzu GC-15A* gas chromatography equipped with a packed alumina (60/80 mesh) column (1.6 m × 2.6 mm) and a flame ionization detector. Carrier gas (N_2) flow was 0.7 cm^3 s^{-1}. Injector, column and detector temperatures were 120, 75 and 150 °C, respectively.

Triazine inhibited root elongation (main root length 20.0 ± 1.5 *versus* 40.0 ± 2.0 mm in control plants). The roots were reddish, the cotyledons were dark and lost their softness. However, addition of 100 cm^3 m^{-3} of thiamine, nicotinic acid or pyridoxine (in the presence of triazine) corrected the abnormal growth. Root length reached 36.0 ± 2 mm with thiamine, 29.0 ± 1.5 mm with nicotinic acid and 28.0 ± 0.5 mm with pyridoxine.

The fluorescence band at 686 nm originates in the antenna Chl *a* molecules of PS 2 (Murata and Satoh 1986). A dramatic quenching of the fluorescence band at 686 nm accompanied with a peak shift of 3 nm (to 683) was recorded in thylakoid

membranes isolated form leaves treated with 0.6 mM triazine. This quenching reflects the inefficient energy transfer from the light harvesting complex to the reaction center of PS 2 (Cao and Govindjee 1990), probably as a result of structural alterations in the PS 2 complex (Briantais *et al.* 1989). The quenching was partly reversed by vitamins (thiamine, nicotinic acid or pyridoxine).

Table 1. Fatty acid composition of thylakoid membranes isolated from leaves of soybean seedlings, as affected by triazine and vitamins. Means ± SD of three experiments. nd - not detected.

Fatty acids	Control	0.1 mM triazine	0.6 mM triazine	0.6 mM triazine + 100 g m^{-3} of thiamine	nicotinic acid	pyridoxine
Myristic 14:0	3.1±0.2	4.9±0.1	6.1±0.3	2.4±0.6	7.7±0.2	1.5±0.2
Myristolic 14:1	2.4±0.1	0.9±0.5	nd*	nd	1.5±0.1	0.9±0.1
Palmitic 16:0	8.0±0.2	12.7±1.0	14.3±0.5	11.1±0.5	15.8±0.1	15.6±0.4
Palmitoleic 16:1	nd	nd	5.6±0.2	nd	nd	2.1±0.4
Stearic 18:0	2.3±0.5	nd	9.6±0.3	2.3±0.6	nd	3.9±0.7
Oleic 18:1	3.6±0.1	7.6±0.5	6.7±0.3	9.6±0.5	9.3±0.2	12.1±0.2
Elaidic 18:1'	5.9±0.5	nd	nd	nd	nd	nd
Linolic 18:2	17.7±1.0	17.5±0.1	22.8±0.1	17.9±0.4	18.8±0.4	18.2±0.2
Linoleic 18:3	57.1±0.2	56.4±0.2	35.0±0.1	56.8±0.4	49.9±0.5	45.6±0.2
Saturated [%]	13.4	17.6	30.0	15.4	20.4	21.1
Unsaturated [%]	86.6	82.4	70.0	84.3	79.6	78.1
Unsatur./satur.	6.5	4.7	2.3	5.6	3.9	3.7
Total glycerides [%]	6.0	3.2	1.7	4.7	3.2	3.4

To check the structural alteration of thylakoid membranes signalized by the fluorescence values, fatty acid composition of triazine and vitamin-treated membranes was identified using gas chromatography. Triazine (0.6 mM) lowered the amount of linolenic acid, C18:3 (the most abundant unsturated fatty acid of chloroplast) by about 39 % compared to control (Table 1). This reduction of linolenic acid affects the unsaturation needed to maintain optimal membrane structure in chloroplasts. Addition of the tested vitamins reversed this inhibition (thiamine > nicotinic acid > pyridoxine). The ratio of unsaturated/saturated [(16:1, 18:1, 18:1', 18:2, 18:3)/(14:0, 16:0, 18:0)] fatty acids is an indication of lipid peroxidation induced by herbicides (El-Shintinawy and Selim 1995). This ratio reached its minimum in the 0.6 mM triazine treated membranes, reflecting the disturbance of membrane saturation. However, addition of vitamins, in the presence of the same triazine concentration, enhanced this ratio. Furthermore, a dramatic decrease in the content of total glycerides by (73 %) was detected in the 0.6 mM triazine-treated sample compared to control. Again, thiamine addition raised the percentage of total glycerides.

Ethylene production is a non-destructive monitor of lipid peroxidation (Bouzayen *et al.* 1991). Triazine (0.6 mM) increased ethylene production from 120 ± 0.4 to 1200 ± 0.6 mol g^{-1}(f.m.) s^{-1}. Thiamine, nicotinic acid and pyridoxine lowered ethylene production to 360 ± 1.6, 660 ± 1.4 and 780 ± 1.4 mol g^{-1}(f.m.) s^{-1}, respectively.

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