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BRIEF COMMUNICATION

Effects of diquat on pigment-protein complexes of thylakoid membranes in soybean and maize plants

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Abstract

Soybean (*Glycine max* Merrill) and maize (*Zea mays* L.) plants were exposed for 5 to 48 h to the herbicide diquat under "white light" (WL) or far-red radiation (FR) (photon fluence rate of 30 µmol m^{-2} s^{-1}). The WL enhanced diquat effect on chlorophyll content in soybean plants, while FR had the same effects on maize plants. After 5 h, diquat increased the content of polypeptides bound to light-harvesting proteins in both plants.

Additional key words: carotenoids, chlorophyll, light-harvesting complexes, photosystem 1, photosystem 2, polypeptides.

During photosynthetic electron transfer, diquat is reduced to cation radicals. Radicals formed in this way react with molecular oxygen, producing superoxides (O_2^-) which subsequently produce other toxic components such as H_2O_2 and OH-radical (Elstner *et al.* 1980). When plants are irradiated and herbicides of this group are applied, degradation of pigments and thylakoids takes place. According to Ashton and Crafts (1973), bipyridyls are more effective in the field in the late afternoon or at dusk when far-red (FR) prevails in the sun light spectrum than in the morning or at noon. Therefore, we compared the accumulation of pigments and polypeptides of chloroplast thylakoids in soybean and maize plants treated and untreated with diquat when exposed to different radiation qualities.

After emergence in darkness, seedlings of maize (*Zea mays* L. cv. ZPSC704) and soybean (*Glycine max* Merrill cv. ZPS015) were grown for 10 d as hydroponic cultures in growth chambers under 12-h photoperiod, irradiance of 300 µmol m^{-2} s^{-1}, temperature of 24/20 °C, and relative humidity of 70 %. The seedlings of both plant

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species were exposed to 3 mM diquat in darkness or under "white light" (WL; *Sylvania* cool fluorescent tubes *P96T12-CEW-VHO*, 380 - 750 nm, emission maxima at 456 and 600 nm, photon fluence rate of 30 µmol m^{-2} s^{-1}) or FR (xenon lamp *F5311/700K*, 675 - 750 nm, emission maximum at 725 nm, photon fluence rate of 30 µmol m^{-2} s^{-1}). Photon fluence rates were measured by the *Li-Cor* (Lincoln, USA) *Li-1888* Radiometer. Pigment contents were determined and calculated according to Lichtenthaler (1987). The method of Delepelaire and Chua (1979) was used for SDS-PAGE of thylakoid polypeptides. The gels for proteins were scanned densitometrically by an *Ultra Scan Laser Densitometer LKB 2202*. Light harvesting complex (LHC) proteins were reported as a sum of 25 - 30 kDa proteins, photosystem (PS) 1 as 110 and 66 kDa proteins, and PS2 as 50 - 45 kDa proteins and as a percentage of total proteins. Analysis of variance (*ANOVA*) for all variables was carried out by the *M Stat c* program.

In both plant species, the chlorophyll (Chl) *a*/*b* ratio in darkness and under WL or FR showed tendency decreased with length of exposure to diquat, which indicates a degradation of Chl *a* (Table 1). In soybean plants, WL enhanced diquat action more than FR, while FR had the same effect in maize plants. After 48 h, the Chl *a*/*b* ratio in soybean plants decreased 37.5 % under WL and 12 % under FR, compared with untreated plants. This ratio decreased 27 % under WL and 57 % under FR in maize plants.

Chl *a*/carotenoid (Car) ratios grew with the duration of diquat treatment indicating a degradation of Car. In soybean plants exposed to both WL and FR, diquat effect on Car increased significantly, while WL had the same effect on maize plants.

After the initial herbicidal shock, the plants tried to utilise the radiation in the best possible way by changing the composition of thylakoid membranes. Five hours after diquat was applied, WL and FR increased the content of polypeptides of the principal thylakoid component LHC2 in soybean and maize. The contents of reaction centre (RC) proteins of PS1 and PS2 decreased. 24 h after diquat application, the polypeptide content of the LHC complexes of thylakoid membranes and the contents of polypeptides of PS 1 and PS2 RC decreased, indicating that the plants were unable to elude such unfavourable conditions (Table 2).

Soybean and maize plants treated with diquat and exposed to WL and FR radiation showed 5 h after the treatment an adaptation tendency that was analogous to the effects found in shade adaptation of chloroplasts. Such effects were also observed in the chloroplasts of plants treated with bentazon (Meier and Lichtenthaler 1981) and *San 9785* (Leech *et al.* 1985). Exposed to diquat and WL or FR, soybean and maize plants responded similarly as regards the LHC2/RC2 ratio, *i.e.*, by enlarging their PS2 units. Larger PS2 units were consistent with the higher LHC/PS2 and PS2/PS1 ratios.

Car are accessory light-harvesting pigments, and they directly quench the excited state of Chl, which leads to singlet oxygen formation, and also removes singlet oxygen molecules (Demmig-Adams and Adams 1996, Yamamoto and Bassi 1996). The WL was significantly more efficacious in reducing Car content in maize than the FR was. In soybean plants, both WL and FR increased the destructive effect of diquat on the Car.

Table 1. Effect of diquat in the dark or under "white light" (WL) or far-red radiation (FR) on ratios of chlorophylls (Chl) and carotenoids (Car) in primary leaves of soybean and maize.

	Soybean Chl *a/b*			Chl *a*/Car			Maize Chl *a/b*					Chl *a*/Car				
	5 h	24 h	48 h	5 h	24 h	48 h	5 h	12 h	18 h	36 h	48 h	5 h	12 h	18 h	36 h	48 h
WL	2.1	2.3	2.4	5.3	5.6	4.6	2.6	2.5	2.3	2.5	1.1	4.9	4.8	4.9	5.5	15.3
WL + diquat	2.5	2.0	1.5	4.4	6.6	8.9	2.6	2.6	2.3	1.5	0.8	4.7	5.4	6.1	9.8	35.8
FR	2.2	2.1	1.7	3.4	3.4	3.9	2.6	2.4	2.4	2.1	2.1	4.9	4.6	4.9	4.6	4.9
FR + diquat	2.1	2.1	1.5	3.5	4.4	6.9	2.6	2.4	1.5	1.9	0.9	5.2	4.6	15.8	6.8	11.6
Dark	2.1	2.3	2.6	5.3	4.1	4.2	2.8	2.7	2.5	1.6	1.2	5.0	4.7	5.2	5.7	6.0
Dark + diquat	2.3	2.4	2.1	4.1	4.1	5.1	2.7	2.6	2.4	1.4	1.0	4.9	3.6	5.7	13.8	10.7
$LSD_{0.05}$	0.07			0.09			0.24					2.13				

Table 2. Effect of diquat in the dark or under WL or FR polypeptides of photosynthetic reaction centres (RC) and light-harvesting complexes (LHC) of photosystem (PS) 1 and PS2 of soybean and maize chloroplast thylakoids.

	Soybean PS1		PS2		LHC		PS2/PS1		LHC/PS2		Maize PS1		PS2		LHC		PS2/PS1		LHC/PS2	
	5 h	24 h	5 h	24 h	5 h	24 h	5 h	24 h	5 h	24 h	5 h	24 h	5 h	24 h	5 h	24 h	5 h	24 h	5 h	24 h
WL	7.1	3.0	23.2	35.4	38.6	34.3	3.3	11.8	1.7	1.0	6.9	5.2	22.2	33.8	36.7	29.8	3.2	6.5	1.7	0.8
WL + diquat	4.0	2.6	19.1	25.4	40.7	43.7	4.8	9.8	2.1	1.7	1.9	4.0	24.7	33.6	42.6	25.6	13.0	8.4	1.7	0.8
FR	5.3	2.6	24.6	23.8	23.9	43.7	4.6	9.2	1.0	1.8	2.9	5.8	28.4	25.7	37.1	27.9	9.8	4.4	1.3	1.1
FR + diquat	3.5	2.0	12.9	15.0	50.7	34.3	3.7	7.5	3.9	1.4	1.2	3.6	21.2	23.4	45.9	23.4	17.7	6.5	2.2	1.0
Dark	3.5	3.1	19.9	22.0	39.0	46.3	5.5	8.7	2.0	1.7	1.5	4.0	25.0	26.3	35.8	30.9	16.7	10.1	1.4	1.2
Dark + diquat	4.9	1.8	34.9	30.0	34.9	37.0	4.7	16.7	1.5	1.2	1.0	2.6	45.0	32.0	36.3	25.1	45.0	12.3	0.8	0.8
$LSD_{0.05}$	0.8		10.5		9.3		1.5		1.2		1.1		5.1		8.5		7.6		0.4	

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