

Comparison of Photosynthetic Activities in Triazine-Resistant and Susceptible Biotypes of *Chenopodium album*

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Triazine-resistant and susceptible *Chenopodium album* plants were grown at low and at high light irradiances. At the lower light irradiance the dry matter production of the resistant and the susceptible plants were almost similar. At the higher irradiance the resistant biotype had a significantly lower production. Fluorescence studies showed that the photochemical yield and the photosystem II electron transport rate were lower in the resistant biotype. It could be demonstrated in intact leaves that the lower productivity of the resistant biotype is caused by a higher sensitivity to photoinhibition. However, when studying effects of photoinhibition on electron flow and photophosphorylation in isolated thylakoids of the two biotypes, no significant differences between resistant and susceptible plant materials were observed. It is suggested that the difference between resistant and susceptible biotypes connected with processes protective against photoinhibition in intact leaves, are lost during the isolation of thylakoids.

Introduction

Triazine-resistance of several plant species is caused by an alteration of the D1 protein of photosystem II (PS II). At site 264, serine is replaced by glycine [1–3]. This alteration causes resistance to atrazine and other members of the triazine family [4, 5]. There appears a small resistance to diuron-type herbicides, while sensitivity to phenol-type herbicides is increased [6, 7].

The alteration of the D1 protein does not only result in triazine-resistance, but also in a decrease of the binding affinity for plastoquinone in the Q_B -binding niche in the D1 protein [8]. This causes a 3-fold decrease in the rate of electron flow from Q_A^- to Q_B [9]. It was suggested that the changed kinetics of the Q_A/Q_B reaction does not simply decrease primary photosynthetic efficiency *via* a direct effect on photosynthetic electron flow, but that the mutation in the D1 protein affects also other functional aspects of the PS II complex important for regulation of photosynthesis and biomass production, *e.g.*, the turnover of the D1 protein [9].

The triazine-resistance trait has been transferred to several crop plants, *e.g.*, rapeseed (*Brassica na-*

pus L.), chinese cabbage (*Brassica campestris* L.) and foxtail millet (*Setaria italica* L.). Unfortunately, a significant reduction in yield accompanies the resistance trait in most species studied. Field studies of resistant rapeseed showed decreased growth and crop yield. The above-mentioned slower rate of electron flow between Q_A and Q_B has been suggested as the cause of the reduction in photon yield, lowered maximum photosynthesis and ecological fitness [*e.g.*, 10, 11].

An influence of the impaired electron transport between Q_A and Q_B on the overall electron transport rate was questioned by Jansen *et al.* [7]. The lower rate of electron flow between Q_A and Q_B in the resistant biotype is still about 20 times faster than the oxidation of reduced plastoquinone. The latter reaction remains the light limiting step having a half-time of about 20 ms. These authors demonstrated that the electron transport between water and plastoquinone has a lower rate and a lower quantum yield in isolated chloroplasts of resistant plants. However, no significant differences were found for the rate and quantum yield of whole chain electron transport.

It was recently suggested that the differences between resistant (R) and susceptible (S) plants occur only when grown at high light irradiance and are much less when grown at low irradiance [12–14]. Hart and Stemler [12] compared R and S *Brassica napus* plants, grown under low photon flux density. They found that the slow electron flow

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from Q_A to Q_B was still present in the R biotype, but photon yield and light-saturated oxygen evolution were similar in the two *B. napus* biotypes. These authors proposed [14] that the differential reduction in photon yield and photosynthesis often observed in R biotypes when plants are grown at high photon flux density is the result of an increased sensitivity to photoinhibition.

We measured growth of R and S biotypes of *Chenopodium album* plants grown at high (HL) and low (LL) light irradiances. Intact leaves of R plants showed lower photochemical quenching, lower photochemical yield and lower PS II electron flow rate compared to leaves of S plants. HL leaves of R biotypes appeared to be more sensitive to photoinhibition than HL leaves of S biotypes; the differences between the LL plants were much less. However, thylakoids isolated from R and S plants of both HL and LL appeared to show insignificant differences in their sensitivity for photoinhibition. Possible reasons for the difference in sensitivity for photoinhibition in leaves compared with that in isolated thylakoids of R plants are discussed.

Materials and Methods

The growth from seed to seedling of triazine-resistant (R) and susceptible (S) biotypes of *Chenopodium album* L. was previously described [7]. Three weeks after germination the seedlings were placed under two different irradiances: a high light irradiance (HL) of $120 \text{ W} \cdot \text{m}^{-2}$ (PAR) and a low light irradiance (LL) of $30 \text{ W} \cdot \text{m}^{-2}$ (PAR) for 16 h/day during a period of three weeks. The dry matter of the above ground parts of R and S biotypes were determined of plants grown at both irradiances.

Different parameters of chlorophyll *a* fluorescence were measured [15]. Whole leaves of both biotypes of *C. album* grown at the two irradiances were used. Measurements of modulated chlorophyll fluorescence emission from the upper surface of the leaf were made using a pulse amplitude modulation fluorimeter (PAM-101) [16]. These measurements were carried out at about 25°C . Furthermore, a photoinhibition treatment (PIT) was carried out, consisting of a period of preillumination with photoinhibition light ($800 \text{ W} \cdot \text{m}^{-2}$, PAR) at 5°C for 15 min after which the F_v/F_m val-

ues were determined. These values were compared with the dark control (period of 15 min in the dark at 5°C).

After growing R and S *Chenopodium album* plants for three weeks, chloroplasts were isolated [17] and stored at -80°C . Electron transport was measured as oxygen exchange [18]. The Hill and the Mehler reaction were used for measuring the PS II and/or the PS I dependent electron transport. Cyclic and non-cyclic photophosphorylation was measured as light-induced pH changes observed at pH 8 in the presence of an ATP-forming system. The photoinhibitory treatment of these two types of measurements consisted out of different periods of preillumination with strong light ($470 \text{ W} \cdot \text{m}^{-2}$, PAR). At the end of the preillumination period the appropriate reagents were added to measure the different electron flow and photophosphorylation reactions.

Results and Discussion

When triazine-resistant (R) and susceptible (S) *Chenopodium album* plants were grown at low light irradiance (LL) the dry matter production of R and S plants was almost similar; at the higher light irradiance (HL) the R biotype showed a significantly lower productivity (Table I). Fluorescence studies with intact leaves did not show as much of the difference between HL and LL conditions (Fig. 1–4). Nevertheless, the values of the R leaves were always lower than those of the S leaves with the tendency that the differences were somewhat larger at the HL conditions.

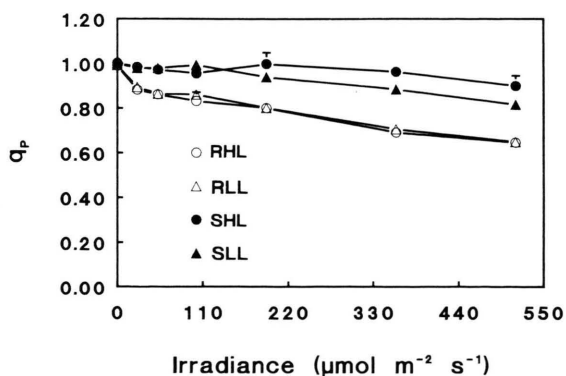


Fig. 1. Photochemical quenching (q_p) at different irradiances of intact leaves of R and S plants, grown under high light (HL) and low light (LL) irradiances.

Table I. Dry weight production of resistant (R) and susceptible (S) *Chenopodium album* plants grown at high light (HL) and at low light (LL) irradiances.

RHL	SHL	RLL	SLL
1.14 ± 0.10	1.65 ± 0.21	0.09 ± 0.02	0.11 ± 0.03

Dry matter in grams per plant. HL is $120 \text{ W} \cdot \text{m}^{-2}$; LL is $13 \text{ W} \cdot \text{m}^{-2}$ (PAR).

We have previously reported that, although the overall electron flow rate of R and S was similar, the rate of PS II dependent electron flow in chloroplasts isolated from R plants was lower than that in S chloroplasts [7]. This could be confirmed by the fluorescence measurements, illustrated in Fig. 1–4. Photochemical quenching (q_p) was lower for the R leaves at all irradiances (Fig. 1). This indicates that the fraction of open PS II reaction centers of R is always lower. A lower fraction of open reaction centers may induce a higher probability of absorbed photons to be dissipated as heat, *i.e.*, increase of non-photochemical quenching (q_N). Actually, a higher q_N was only found for R leaves grown at HL, measured at low actinic irradiances. However, at higher irradiances and at all irradiances for the LL leaves, q_N appeared to be lower for R than for S leaves (Fig. 2). Apparently, especially at higher irradiances, the lower rate of electron flow at PS II in R plants leads to a lesser amount of proton production at the water splitting site. This may cause a smaller proton gradient and may contribute to a lower q_N . The quantum yield of PS II electron flow (Φ_p) was lower for R leaves

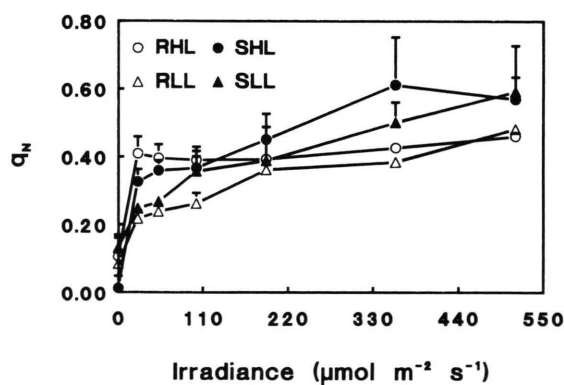


Fig. 2. Non-photochemical quenching (q_N) at different irradiances of intact leaves of triazine-resistant (R) and susceptible (S) *C. album* plants, grown at high light (HL) and low light (LL) irradiances.

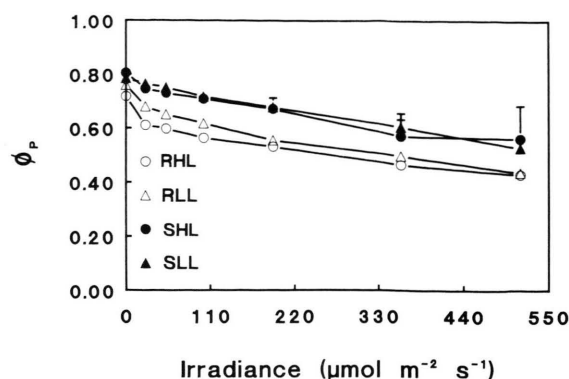


Fig. 3. Photochemical quantum yield of PS II electron flow (Φ_p) at different irradiances of intact leaves of R and S plants, grown under high light (HL) and low light (LL) irradiances.

at both HL and LL conditions (Fig. 3). From the fluorescence data the rate of PS II dependent electron flow (J) could be calculated: the R leaves exhibited lower rates than the S leaves at all irradiances (Fig. 4). Similar results from fluorescence studies using R and S *Brassica napus* plants were recently reported by Sundby *et al.* during the 9th Int. Congress of Photosynthesis at Nagoya [19].

While the results of the fluorescence measurements confirm earlier reports (*e.g.* [7]) that PS II electron flow in R proceeds at a lower rate than in S plants, there was little difference between the HL and LL conditions with respect to PS II performance. The lower dry matter production of R plants at HL (Table I) must, therefore, be due to other processes. Because the lower electron flow rate be-

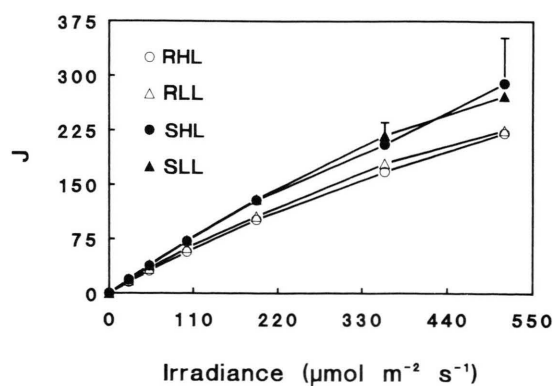


Fig. 4. Photosystem II electron transport rate (J) at different irradiances of intact leaves of R and S plants, grown under high light (HL) and low light (LL) irradiances.

Table II. Photoinhibition measured as F_v/F_m values of leaves of R and S plants, grown under HL and LL irradiances.

	RHL	SHL	RLL	SLL
Dark	0.80 ± 0.01	0.85 ± 0.01	0.81 ± 0.01	0.84 ± 0.01
PIT	0.58 ± 0.02	0.69 ± 0.04	0.51 ± 0.03	0.58 ± 0.04

F_v/F_m was measured after the leaves were kept at 5 °C during 15 min, while one half of the leaf was darkened (dark) and the other half illuminated at 800 W · m⁻² (PIT).

tween Q_A and Q_B induces a more reduced state of Q_A in R plants, it is very probable that R plants are more sensitive to photoinhibition, especially when grown at high irradiance. The effects of a photoinhibitory treatment of intact leaves is illustrated in Table II. Although plants adapted to high light suffer less from photoinhibition, it appears that the R biotype is indeed more sensitive to photoinhibition than the S plants; the difference is less when grown at low irradiance.

When chloroplasts were isolated from R and S plants and given a photoinhibitory treatment (PIT), no significant differences on chloroplastic activities were observed between R and S biotypes. The effect of PIT was measured on three types of

PS II and/or PS I dependent electron transport reactions (Fig. 5). In all three cases there was no significant difference between R and S biotypes. In Fig. 6 results are presented of effects of PIT on PS II and/or PS I dependent photophosphorylation reactions. Remarkable is the very fast decrease of the PS I dependent cyclic photophosphorylation with PMS during photoinhibition. But also in this case there appeared no significant differences between R and S.

The differential effect of photoinhibition *in vivo* and *in vitro* may be caused by the fact that *in vitro* energy conversion by photosynthesis, energy dissipation by protective mechanisms and recovery processes do not function or function less efficient-

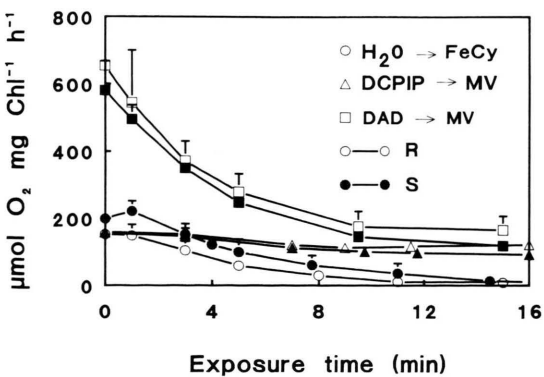


Fig. 5. Effect of photoinhibition treatment on the PS II and/or PS I dependent electron transport of chloroplasts isolated from R (open symbols) and S (closed symbols) plants. The basic assay medium contained 0.3 M sorbitol, 50 mM tricine pH 7.6 and 5 mM MgCl₂. The water to ferricyanide (FeCy) reaction medium contained in addition 5 mM NH₄Cl and 1 mM FeCy. The reaction medium of dichlorophenolindophenol (DCPIP) to methyl viologen (MV) contained in addition 1 μM DCMU, 2 mM dithioerythritol, 2 mM ascorbate, 40 μM DCPIP and 20 μM MV. The assay medium of diaminodurene (DAD) to MV contained in addition to the basic medium 1 μM DCMU, 2 mM dithioerythritol, 0.5 mM DAD and 20 μM MV. Photoinhibition light was 470 W · m⁻² (PAR).

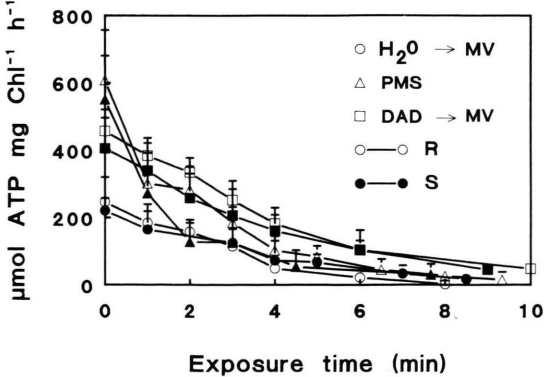


Fig. 6. Effect of photoinhibition treatment on the PS II and/or PS I dependent photophosphorylation reactions of chloroplasts isolated from R (open symbols) and S (closed symbols) plants. The basic assay medium contained 0.3 M sorbitol, 100 mM KCl, 5 mM MgCl₂, 1 mM K₂HPO₄ and 1 mM ADP; initial pH was 8.0. The water to MV reaction contained in addition 100 μM MV. The cyclic photophosphorylation was measured with 20 μM phenazine methosulfate (PMS). For the DAD → MV reaction 1 μM DCMU and 0.5 mM DAD was added to the basic medium. Photoinhibition light was 470 W · m⁻² (PAR).

ly. The higher sensitivity of R biotypes *in vivo* must be due to a lower activity of one or more of these processes, which are all protective against photo-inhibition. It is important to mention that Sundby *et al.* [19] reported that the D1 protein turnover rate was different in R biotypes of *Brassica napus* compared with that in the S plants. In general, it

appears that differences between R and S biotypes observed in intact leaves may not be present in chloroplasts isolated from them.

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