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Altered xanthophyll cycle and fluorescence quenching indicate light-dependent early events in paraquat-treated resistant *Erigeron canadensis*

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ABSTRACT Violaxanthin de-epoxidation and chlorophyll fluorescence quenching in the presence of paraquat (Pq) were studied in intact attached leaves of Pq-susceptible (PqS) and Pq-resistant (PqR) biotypes of *Erigeron canadensis* under different light conditions. Initially, similar changes were induced in the two biotypes, but the effects relaxed only in the PqR plants, indicating a Pq elimination process. The penetration of Pq into the chloroplasts of PqR plants proved to be somewhat restricted and highly light-dependent, as revealed by both the light response curves of violaxanthin de-epoxidation and fluorescence quenching and the short-term high-light pre-illumination experiments. An irregular down-regulation of the non-photochemical fluorescence quenching (NPQ) processes was observed, reflected by lower steady-state zeaxanthin and NPQ levels as compared with the corresponding non-treated high-light controls. It is concluded that light is essential not only for the initiation of the mechanism of resistance to Pq, but also for the penetration of Pq into the chloroplasts in the PqR *E. canadensis*. Also, the Pq elimination process may cause a modification to the regulation of the non-radiative energy dissipation in PqR plants in the presence of Pq.
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Paraquat (Pq, methylviologen), a strong autooxidable electron acceptor in PS I, has been widely used in weed control. Pq treatment can cause damaging effects via generating toxic oxygen species even at low light intensities. Pq enhances the linear photosynthetic electron transport rate and ΔpH formation across the thylakoid membranes, providing favourable conditions for xanthophyll cycle de-epoxidation (Büch et al. 1994; Pfündel and Bilger 1994; Thiele and Krause 1994).

As a consequence of the repeated use of Pq, resistance to Pq has emerged in several weed species (Pölös et al. 1988; Fuerst and Vaughn 1990; Preston et al. 1992), but the exact mechanism of resistance has not been revealed. Restricted movement of Pq, enhanced activity of the Halliwell-Asada pathway or the sequestration of Pq have been suggested as possible mechanisms. There is a characteristic transitory inhibition of the photosynthetic activity measured as CO_2 fixation, O_2 evolution and variable fluorescence (Lehoczki et al. 1992). All of these parameters reach their minima by the first or second hour of Pq treatment suggesting that Pq can reach the site of action in the chloroplasts in the PqR plant. Pq treatment under different light regimes (Váradi et al. 1990; Lehoczki et al. 1992) indicated a basic role for light in the resistance mechanism.

Excessive light as well as Pq generates highly reactive oxygen species that can cause oxidative damage to the photosynthetic apparatus. Oxygenic photosynthetic organisms have evolved multiple photoprotective mechanism to cope with the potentially damaging effects of light and oxidative stress. The xanthophyll cycle is known to be one of the main photoprotective mechanisms in higher plant leaves (Demmig-Adams and Adams 1990; Pfündel and Bilger 1994). Recently, it was reported that the xanthophyll epoxidation cycle may protect the photosynthetic apparatus by several mechanisms (Havaux and Niyogi 1999).

This widely accepted key role of the xanthophyll cycle in photoprotection and oxidative stresses and the observed role of light in the Pq action and Pq resistance mechanism led the authors to propose that the xanthophyll cycle might be a useful tool in studies of the elementary processes of the Pq resistance mechanism. Preliminarily, an unusual response of the xanthophyll cycle and NPQ processes was found in the PqR biotype of *E. canadensis* during the first hours of Pq treatment as compared with the PqS biotype and the untreated PqR control (Váradi et al. 1998) and details of further experiments were published in Váradi et al. (2000).

This report presents the most interesting results of analyses of the xanthophyll cycle and fluorescence quenching on Pq-treated PqS and PqR *E. canadensis*. Plants were treated under dim-light conditions, and the transient effects of illumination in the presence of Pq were then followed after a 10 min dark period. This experimental approach using dark-adapted Pq-treated plants allowed us to investigate light-induced transient processes that would be difficult to observe under natural conditions, in order to reveal some light-dependent elements of the Pq resistance mechanism.

Materials and Methods

Seeds of PqR and PqS biotypes of *E. canadensis* collected at various locations in Hungary (Pölös et al. 1988) were germinated and grown in soil containers in the greenhouse for 2-3 months and then transferred to a natural environment (natural light conditions with a daily maximum of about 1600 μmol m^{-2} s^{-1} PPFD). Rosette-stage plants aged 14-16 weeks with fully-developed leaves were used for experiments.

All treatments and measurements were carried out under laboratory conditions. Formulated paraquat (Gramoxone,

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25% active ingredient) diluted to 0.1 mM Pq was sprayed to the leaves under dim-light (15-20 μmol m^{-2} s^{-1} PPFD) conditions (treated leaves were then kept in the dark for 10 min before light treatments or measurements). Treated leaves were exposed to different light regimes as indicated in the Results and Discussion.

Fluorescence measurements were carried out after an additional 10-min dark adaptation in a modulated fluorimeter (Hansatech) with 1s 3000 μmol m^{-2} s^{-1} saturating pulses. NPQ was calculated according to Bilger and Björkman (1990). Xanthophyll cycle pigments were determined by HPLC (Váradi et al. 1994).

Results and Discussion

NPQ demonstrated a marked increase (up to 2.2) in the first hour of Pq treatment, and there was then an expressed decline in both biotypes. After 4 hours, however, NPQ in the PqR biotype approached the initial level (NPQ=0.9, corresponding to low-light conditions in the absence of Pq, in contrast with the appropriate high-light control, where NPQ=2.5), while it was negligible by the end of the 4th hour of Pq treatment in the PqS plant (reflecting a disrupted and non-functioning photosynthetic apparatus).

The light-driven de-epoxidation of violaxanthin (V), resulting in an accumulation of zeaxanthin (Z) via antheraxanthin (A) in the thylakoids, was characterized using the epoxidation index, E_i (where E_i=(V + 0.5xA)/(V + A + Z)). There was a decrease in E_i (mainly due to zeaxanthin accumulation) within the first 5 min of illumination of Pq-treated leaves of both biotypes, similarly to that observed in non-treated dark-adapted control leaves when illuminated (data not shown). In the PqS biotype, however, the magnitude of this Pq + light-induced drop in E_i appeared to be more drastic and nearly independent of the light intensity, while a clear light intensity dependence (between 100 and 1400 μmol m^{-2} s^{-1} PPFD) was observed in the PqR biotype. Moreover, after this light intensity-dependent first drop in E_i in the leaves of the PqR biotype, a recovery process started immediately and the E_i values approached their asymptotic levels after 1 or 2 hs of Pq treatment. These steady-state levels also showed a characteristic light intensity dependence in the PqR biotype, but the most interesting feature was the recovery of E_i to levels (0.7 in the case of 1400 μmol m^{-2} s^{-1} PPFD) much higher than the steady-state control in the PqR plants (0.38 for the untreated control exposed to 1400 μmol m^{-2} s^{-1} PPFD).

In a separate experiment, the low-light (LL) Pq treatment of PqR leaves was repeated by using a 5-min high-light (HL; 1100 μmol m^{-2} s^{-1} PPFD) pre-illumination before the usual 10-min dark adaptation. In the HL pre-illumination period, Pq induced a marked reduction of E_i close to that of the 5-min HL control (without Pq) and E_i relaxed at a similar rate under the 10-min dark period. There was, however, a second and larger transient decline in E_i when the low-light was switched on in the presence of Pq and the asymptotic level of that transient was near E_i=0.7 (similar to the continuous HL+Pq treatment). These results suggested that in the case of the PqR biotype the light-mediated early uptake of Pq may be the factor determining the Pq effect, but not the light intensity dependence of the electron transport rate in the presence of Pq.

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