Comparison of the decay of slow delayed luminescence in triazine-susceptible and -resistant biotypes of *Erigeron canadensis* L.

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The decay of flash-induced delayed luminescence was compared in the seconds to minutes time region in triazine-susceptible and -resistant chloroplasts. The slow luminescence component assigned to $S_2Q_B^-$ and $S_3Q_B^-$ recombination decayed more rapidly in resistant as compared to susceptible chloroplasts ($t_{1/2} \sim 8.5$ and 44 s, respectively) indicating that the midpoint potential of Q_B is lowered in the resistant chloroplasts. The luminescence intensity depended on the number of exciting flashes. The oscillation pattern was opposite in the two biotypes and could be computer simulated with the assumption that the Q_B/Q_B^- ratio was about 1 in sensitive and 1.5 in resistant chloroplasts. Thus atrazine resistance also induces a change in the oxidation-reduction state of the Q_B pool.

Delayed luminescence *Triazine resistance* *Herbicide* *Photosystem II* *Secondary quinone acceptor* *Photosynthesis*

1. INTRODUCTION

It is well established that in triazine-resistant plants, structural changes in the thylakoid membrane are also accompanied by changes in the properties of certain electron transport components [1–3]. On the basis of fluorescence induction studies it has been suggested that the midpoint redox potential of the secondary quinone acceptor, Q_B, is lowered in triazine-resistant as compared to triazine-susceptible thylakoids [4]. This redox change of Q_B shifts the equilibrium $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$ to the left resulting in an increased initial concentration of the primary quinone acceptor Q_A^- upon illumination. The higher initial concentration of Q_A^- is reflected in a higher initial level of variable fluorescence in resistant biotypes relative to susceptible ones [4,5].

The decrease in the redox potential of the Q_B/Q_B^- redox couple also manifests itself in thermoluminescence investigations [6]. The B thermoluminescence band, which is assigned to Q_B, appears at 30°C in the glow curve of susceptible chloroplasts, while it shifts to about 15°C in that of resistant ones. It has been demonstrated that thermoluminescence and delayed luminescence of chloroplasts are generated by the same charge recombination mechanism [7,8]. In agreement with this it has been shown recently that a slow component of delayed luminescence with a half-

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Abbreviations: atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; dinoseb, 2,4-dinitro-6-(2-butyl)phenol; PS, photosystem; Q_A, primary quinone electron acceptor of PS II; Q_B, secondary quinone electron acceptor of PS II

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time of about 44 s corresponds to the B thermoluminescence band [8,9]. On the basis of thermoluminescence results it can be expected that the slow component of delayed luminescence, like the B thermoluminescence band, will differ significantly in triazine-susceptible and -resistant chloroplasts. The aim of this study was to compare the slow phase delayed luminescence decay in chloroplasts isolated from triazine-susceptible and -resistant biotypes in order to introduce delayed luminescence analysis as a new technique for the fast determination of triazine resistance in plants.

2. MATERIALS AND METHODS

Seeds of triazine-resistant and -susceptible *Erigeron canadensis* L., *Amaranthus retroflexus* L. and *Solanum nigrum* L. were kind gifts from Dr P. Solymosi (Research Institute for Plant Protection, Budapest, Hungary), Dr G. Vytautas (Research Institute for Heavy Chemical Industries, Veszprém, Hungary) and Dr J. Gasquez (INRA Laboratoire de Malherbiologie, BP 1540, 21034 Dijon Cedex, France), respectively. The triazine-resistant *Nicotiana plumbaginifolia* was selected by Á. Cséplö and P. Medgyesy (Institute of Plant Physiology, Biological Research Center, Szeged, Hungary) from protoplast cultures [10].

Chloroplasts were isolated from 2–3-month-old, greenhouse-grown seedlings, as described in [11]. The suspension of chloroplasts contained 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, and 50 mM Hepes (pH 7.5) buffer with 30 μg Chl/ml. Herbicides were added to the chloroplast suspension in the dark and the samples were incubated for 10 min before delayed luminescence measurements.

Delayed luminescence was excited in a 1 cm cell with xenon flashes (General Radio, Stroboslave, 3 μs, 0.5 J). The emitted delayed luminescence was observed by opening of an Uniblitz shutter. The emitted light was detected by an EMI 9558B photomultiplier. The photomultiplier signal was amplified by a home-made differential amplifier and the signal was stored in a multichannel analyser (ICA 70) or in a storage oscilloscope (PM 3310, Philips) and plotted on an X-Y recorder (EMG-200). The oscillation of delayed luminescence was simulated by model calculation as described [9].

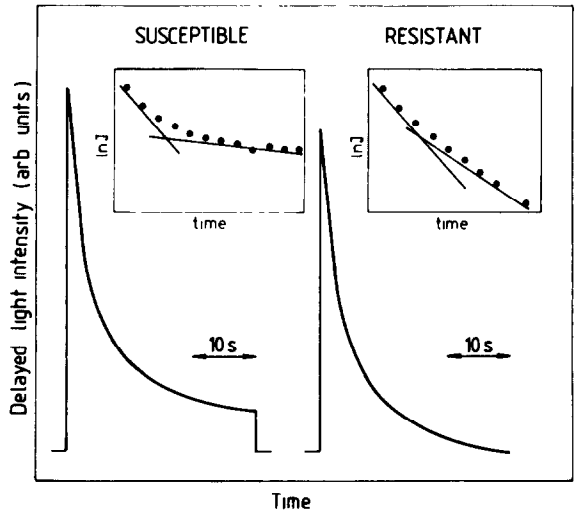

Fig.1. Decay of the delayed luminescence intensity after an exciting flash in triazine-susceptible and -resistant *E. canadensis* chloroplasts. Insets: curve resolutions into exponentials.

3. RESULTS AND DISCUSSION

Fig.1 shows the flash-induced delayed luminescence of chloroplasts isolated from triazine-susceptible and -resistant *E. canadensis*. In the seconds to minutes time region, the decay curves show a marked difference which becomes more obvious if one compares the insets showing the resolutions of the curves into exponentials. The delayed luminescence of the susceptible biotype consists of two components with half-times of ~4.1 and ~44 s. The half-times of the two components are in good correlation with those of the delayed luminescence components detected in spinach chloroplasts [9]. On the other hand in resistant chloroplasts besides the same fast component ($t_{1/2}$ ~ 4.3 s) the slow component exhibits a considerably shorter half-time ($t_{1/2}$ ~ 8.5 s) than that of the slow component in the susceptible biotype.

Our delayed luminescence observations closely resemble the results obtained in thermoluminescence investigations. The peak position of the B thermoluminescence band which corresponds to the slow component of delayed luminescence [9], was shifted from 30°C to about 15°C in the glow curve of resistant chloroplasts as

compared to the sensitive ones [6]. It has been suggested that the slow delayed luminescence component and the B thermoluminescence band originate from $S_2Q_B^-$ and $S_3Q_B^-$ recombination [8,9,12]. The rate of the charge recombination reaction depends on the redox span between the interacting positively charged donor and negatively charged acceptor molecules [6,12]. The oxidation-reduction potential of the S_2 state is not influenced by triazine resistance [6]. Therefore we can assume that both the acceleration of the slow delayed luminescence component and the shift of the B thermoluminescence band can be accounted for by a decrease in the midpoint potential of Q_B in resistant chloroplasts relative to susceptible ones. Delayed luminescence experiments carried out with triazine-resistant and -susceptible biotypes of *S. nigrum*, *A. retroflexus* and *N. plumbaginifolia* resulted in similar observations as obtained for *E. canadensis* chloroplasts suggesting that delayed luminescence is a suitable method for the identification of triazine resistance in plants.

Several observations indicate that electron transport inhibitors of PS II can be classified into two main groups: DCMU and phenolic-type herbicides [13–17]. At low concentrations both DCMU and phenolic herbicides interrupt electron transport between Q_A and Q_B. However, the phenolic-type inhibitors have an additional inhibitory site at the donor side of PS II [18–21]. It has been found that in the presence of DCMU-type inhibitors the main thermoluminescence band of chloroplasts appears at about 10°C while addition of a phenolic herbicide shifts the band to about −10°C [6]. On the basis of this thermoluminescence result we expected that the effect of DCMU- and phenolic-type inhibitors upon the delayed luminescence would also result in significantly different decay patterns.

When 500 nM atrazine, a concentration which is enough to inhibit electron transfer between Q_A and Q_B, was added to the susceptible chloroplasts the slow phase of delayed luminescence completely disappeared with a concomitant intensification of the fast component (cf figs 1 and 2). This observation is in agreement with the fact that the slow delayed luminescence component originates from $S_2Q_B^-$ and $S_3Q_B^-$ recombination while the fast one arises from $S_2Q_A^-$ and $S_3Q_A^-$ recombination [9]. In resistant *E. canadensis* chloroplasts due to the high

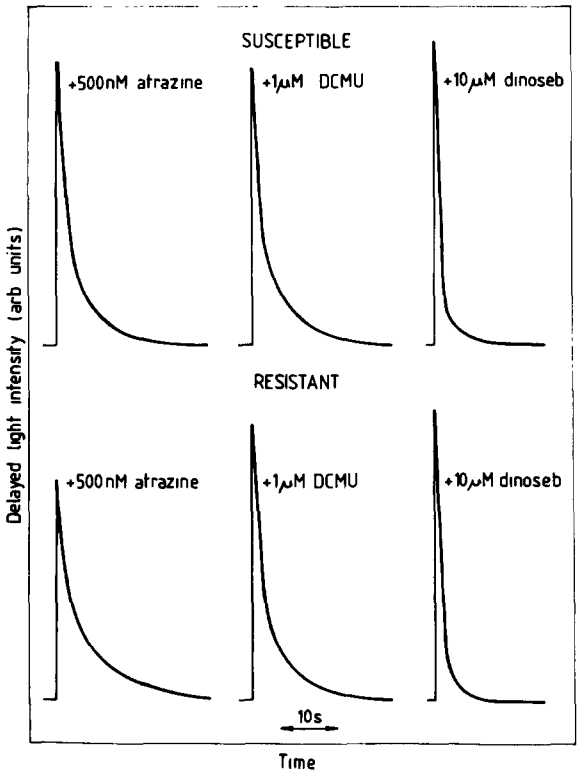

Fig.2. The effect of DCMU- and phenolic-type inhibitors on the delayed luminescence of triazine-susceptible and -resistant *E. canadensis* chloroplasts. Inhibitors were added at the indicated concentrations and delayed luminescence was excited by a single flash.

resistance ratio [6] 500 nM atrazine had practically no effect on the recombination process (cf figs 1 and 2). Addition of DCMU completely eradicated the slow phase of delayed luminescence in both susceptible and resistant chloroplasts but did not influence the half-time of the fast component (fig.2). On the other hand the decay of delayed luminescence was considerably faster in chloroplasts treated with the phenolic-type herbicide, dinoseb than in atrazine- or DCMU-treated chloroplasts ($t_{1/2}$ ~0.65 and ~0.60 s in susceptible and resistant chloroplasts, respectively). This observation indicates that dinoseb inhibits electron transport not only at the acceptor side of PS II but it has an action site at the donor side of the reaction center as well. It can be assumed that the fast delayed luminescence component appearing in the presence of dinoseb is generated by the recombina-

tion of Q_A^- with positive charges stored on an unidentified donor of PS II which is localized at a shorter redox distance from the reaction center than the water-splitting enzyme system. On the basis of the observed different effects of DCMU and dinoseb on the decay course of the delayed luminescence of chloroplasts we suggest that the delayed luminescence technique is a suitable method, enabling one to distinguish between DCMU- and phenolic-type herbicides.

The oscillation of the intensity of the slow delayed luminescence component as a function of the exciting flash number is mainly determined by the Q_B/Q_B^- ratio [9]. It is also known that triazine resistance brings about a large change in the semiquinone equilibrium of Q_A and Q_B and consequently in the Q_B/Q_B^- ratio [4,22]. Thus we could expect alterations in the flash-induced oscillation pattern of delayed luminescence of resistant chloroplasts as compared to sensitive ones. Indeed, while in sensitive chloroplasts the intensity of the slow delayed luminescence component was higher after two flashes than after one flash excitation, in resistant chloroplasts the opposite oscillation pattern could be observed: one flash excitation caused a higher delayed luminescence intensity than two flashes (fig.3). A similar change was observed in the oscillation of delayed luminescence during dark adaptation of spinach chloroplasts. With the oxidation of the secondary acceptor pool the oscillatory maximum was gradually shifted from the second flash to the first flash of a flash series [9]. On the basis of this similarity we can assume that the opposite pattern of oscillation in the resistant biotype relative to the sensitive one can be attributed to an increased Q_B/Q_B^- ratio in the resistant chloroplasts. Using various Q_B/Q_B^- ratios we tried to simulate the measured oscillatory pattern by model calculations [9]. Satisfactory fits were obtained by the assumption that the Q_B/Q_B^- ratio is about 1.0 for sensitive and 1.5 for resistant chloroplasts. Thus in addition to the lowering of the redox potential of the Q_B/Q_B^- redox couple in triazine-resistant plants as compared to the susceptible ones, the relative amount of Q_B^- is also lower in resistant chloroplasts.

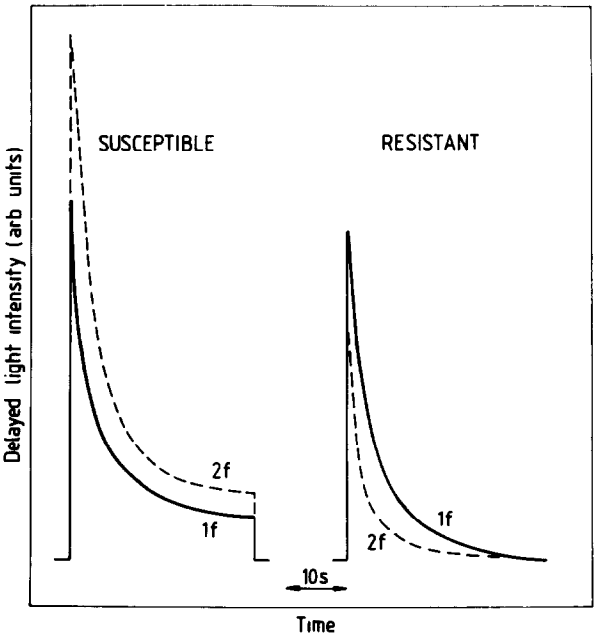

Fig.3. Decay of the delayed luminescence intensity in triazine-susceptible (left) and -resistant (right) *E. canadensis* chloroplasts after one (——) and two exciting flashes (– – –).

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