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BRIEF COMMUNICATION

Quantitative Comparison of Chloroplast Ultrastructure in Mesophyll Cells of Triazine Resistant and Triazine Sensitive *Amaranthus retroflexus* L. Plants

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Abstract. Ultrastructure of chloroplasts in leaf mesophyll cells of triazine resistant and triazine sensitive *Amaranthus retroflexus* L. plants was evaluated stereologically. The most striking difference between both types of the chloroplasts was a small volume of starch inclusions in triazine resistant plants.

Triazines, variously substituted heterocyclic compounds with three nitrogen atoms in the benzene ring (see, *e.g.*, Baumann and Günther 1978) are commonly used herbicides, inhibiting photosynthetic electron transport between photosystems (PS) 2 and 1. Some weed plants have gained resistance to triazines by mutation slightly changing polypeptide chain of the D1 protein which binds the secondary electron acceptor of PS 2, a quinone compound B, as well as herbicide molecules (Gardner 1981). In resistant (R) plants, triazine molecules are probably more quickly released from the bond (van Rensen 1990). As a consequence of the change in D_1 protein structure, electron transport from PS 2 to PS 1 is lowered in triazine resistant plants which is probably the cause of ultrastructural alterations of chloroplasts in these plants in comparison with triazine susceptible (S) plants (Vaughn and Duke 1984). Lemoine *et al.* (1985) studied these alterations in *Amaranthus retroflexus* and other four common weeds.

The aim of our work was to evaluate, using a stereological method, differences between the ultrastructure of mesophyll tissue chloroplasts of the R and S *A. retroflexus* plants. The plants studied were grown from seeds collected from plants resistant or sensitive to the triazine herbicide atrazine (2-ethylamino-4-chloro-6-isopropylamino-1,3,5-triazine).

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Amaranthus retroflexus L. plants were grown in soil, in a growth cabinet (16 h irradiation of about 110 μmol m^{-2} s^{-1}, 8 h dark; 20/18 °C; 50/70 % relative humidity) in spring 1988 (series 1) and in spring 1989 (series 2). Seeds of the R and S plants were collected on the same locality (in northern and central Bohemia, respectively) always one year earlier. Transverse ultrathin sections from central part of the blade of fully developed third leaf of all plants were examined by transmission electron microscope after glutaraldehyde/osmium acid fixation, dehydration via ethanol/propylene oxide series, embedding into Spurr's low viscosity resin, contrasting of the sections with a saturated solution of uranyl acetate in 70 % ethanol followed by lead citrate solution after Reynolds. In each experimental series, three R and three S plants were evaluated; each plant was represented by 8 to 12 chloroplast sections. On electron microphotographs, relative partial volumes of chloroplast ultrastructural components (granal and intergranal thylakoids, peripheral reticulum, starch inclusions, plastoglobules, and stroma) were stated as per cent of chloroplast section area using rasters with regularly distributed points (more dense for minor chloroplast components, *i.e.* peripheral reticulum, starch inclusions, and plastoglobules; less dense for thylakoids; the per cent of stroma was counted). Absolute length and area of chloroplast sections were also stated on these microphotographs.

A. retroflexus L. belongs to the plants with C_4 photosynthesis. Fisher and Evert (1982) distinguished seven chloroplast types in its leaf tissues. For production of photosynthates in leaves, the chloroplasts in mesophyll (palisade and spongy parenchyma) cells and in vascular bundle sheaths cells are the most important. The last ones were mostly filled with starch (Fig. 1a) in both S and R plants. (However, it seemed to be less starch in these chloroplasts of R plants.) We evaluated quantitatively the ultrastructure of mesophyll cell chloroplasts only, not distinguishing between the palisade and spongy parenchyma cells. The ultrastructural differences between mesophyll cell chloroplasts of R and S plants concerned first of all the quantity of starch inclusions (Fig. 1b and 1c); more starch was in the chloroplasts of S plants, the chloroplasts of R plants often contained no starch at all.

This result was confirmed by the stereological evaluation (Fig. 2). Using the Student's pair *t*-test, this difference was statistically highly significant in contrast to the other compared characteristics of chloroplasts of the R and S plants in the series 1. In the series 2, the chloroplasts of R plants were larger than those of the S plants and they contained a smaller partial volume of starch, a larger partial volume of stacked (granal) thylakoids, and a smaller partial volume of plastoglobules; all these differences were statistically highly significant.

We confirmed in this way a smaller starch deposition in the chloroplasts of R plants than S plants (Lemoine *et al.* 1985 in *Amaranthus retroflexus*, other authors in other weed species), caused probably by a lowered photosynthetic

electron transport in the R plants. A larger degree of thylakoid stacking, reported by Vaughn and Duke (1984), Lemoine *et al.* (1985) and other authors for the R plants, that resembles chloroplast ultrastructure in leaves growing under low irradiance, was not confirmed by us unambiguously. Unfortunately, Lemoine et al. (1985) do not present results of stereological analysis of the R and S chloroplasts for *A. retroflexus* plants and other weeds studied. The differences in our results between both experimental series evaluated may have several reasons; the simplest one may be differences in quality of the seeds harvested in two successive vegetation seasons. The second reason may be a slightly different physiological age of the plants and leaves studied in both series, because, generally, during leaf development the chloroplast ultrastructure quantitatively changes, see Kutík (1985) for review.

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Fig. 1 and 2 at the end of the issue.