Effects of simulated acid rain on chloroplast ultrastructure of primary leaves of *Phaseolus vulgaris*

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Abstract

The ultrastructure of chloroplasts in the primary leaf of 10-d-old bean plants (*Phaseolus vulgaris* L., cv. Cheren Starozagorski) was studied 3, 5, 24, 48, 72 and 168 h after a single treatment with simulated acid rain (pH 2.4, 2.2, 2.0 and 1.8). Different changes in chloroplast structure till full destruction of organelles were traced. A determining factor for these changes was the histological localization of chloroplasts. In the chloroplasts of palisade parenchyma different degrees of expansion of thylakoids (3, 5, and 24 h after the single treatment), and conformational changes of the inner membrane system (48, 72 and 168 h) were observed. The chloroplasts of spongy parenchyma showed a significantly higher degree of structure resistance. The expansion of thylakoids was weak and did not depend on the duration of treatment. The ultrastructural changes of chloroplasts confirmed relative resistance of this species till pH 2.0.

Additional key words: bean, palisade and spongy parenchyma, thylakoids.

Introduction

The structure of chloroplasts depends on the leaf age and environmental conditions. The chloroplasts in leaves of *Phaseolus vulgaris* L. during the different stages of ontogeny were studied, *e.g.* by Whatley (1974, 1977, 1979, 1980). The adaptation of chloroplasts to high temperature (*e.g.*, Jordanov *et al.* 1986a), their structural changes induced by antibiotic treatment (Jordanov *et al.* 1986b) or by the heavy metals (Vázquez *et al.* 1987, Barceló *et al.* 1988, Maksymiec *et al.* 1995) have been also studied. The chloroplast structure of primary bean leaves subjected to acid treatment have been studied only by Swiecki *et al.* (1982). The present work focused

Received 24 June 1997, *accepted* 20 November 1997.
Acknowledgments: This research was supported by the National Found for Scientific Investigations (Grant MU-BAV-7) Ministry of Education, Science and Technology, Bulgaria.
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on the changes in the chloroplast ultrastructure induced by simulated acid rain of different pH. Together with changes in leaf anatomy (Stoyanova 1998) these changes will enable to approximate the threshold toxicity of simulated acid rain in the studied species in nature.

Materials and methods

The chloroplast ultrastructure of primary leaves of 10-d-old bean plants (*Phaseolus vulgaris* L., cv. Cheren Starozagorski) was studied after plant treatment once with simulated acid rain of different pH (2.4, 2.2, 2.0 and 1.8) (for detail see Stoyanova 1998). Plants of the same age but treated with pH 5.6 were used as controls.

For electron microscopy studies, material was taken from the middle parts of lamina in the visibly undamaged zones of the primary leaf 3, 5, 24, 48, 72 and 168 h after a single treatment. The material was fixed in 3 % glutaraldehyde in phosphate buffer (pH 7.4) for 12 h at 4 °C and postfixed in OsO_4 for 4 h at room temperature. After dehydration the material was embedded in *Durcupan* (*Fluka*, Switzerland) and cut with *Tesla* (*Tesla*, Prague, Czech Republic) ultramicrotome. Observations were carried out with *JEOL 1200 EX* (Japan) electron microscope.

Results and discussion

The plastid apparatus of mesophyll in the primary leaf of 10-d-old bean plants consisted of well differentiated chloroplasts. Their ultrastructure corresponded to that of the typical mature chloroplast in the primary leaf of *Phaseolus vulgaris* L. with continuous aligned lamellae and stacked grana described (Whatley 1974). The chloroplasts of palisade parenchyma were characterised by well developed inner membrane system (grana thylakoids and stroma thylakoids) (Fig. 1). The grana were of different height (from 4 to 15 - 18 thylakoids) and were linked by a few stroma thylakoids. In the stroma small single starch grains and plastoglobuli with diameter up to 80 nm were observed. The chloroplasts of spongy parenchyma also possessed well developed inner membrane system though built of lower number of large grana and a small number of stroma thylakoids. Starch accumulation was better pronounced. During the 7-d experimental period the chloroplasts of mesophyll in the control plants almost preserved their structural characteristics.

The structural changes in the chloroplasts of plants treated once with simulated acid rain depended on pH level (pH 2.4, 2.2, 2.0 and 1.8) and time of measurement (3, 5, 24, 48, 72 and 168 h after the treatment). The histological localization of chloroplasts was of decisive importance: the structural and functional differences in the structural and functional characteristics of palisade and spongy parenchyma determined different response to the acid rain. In palisade parenchyma of plants measured 3, 5 and 24 h after treatment the changes in chloroplasts were uniform (slightly pronounced swelling of thylakoids of stroma and peripheral grana, but without structural disorganisation of the inner membrane system - Fig. 2) and were

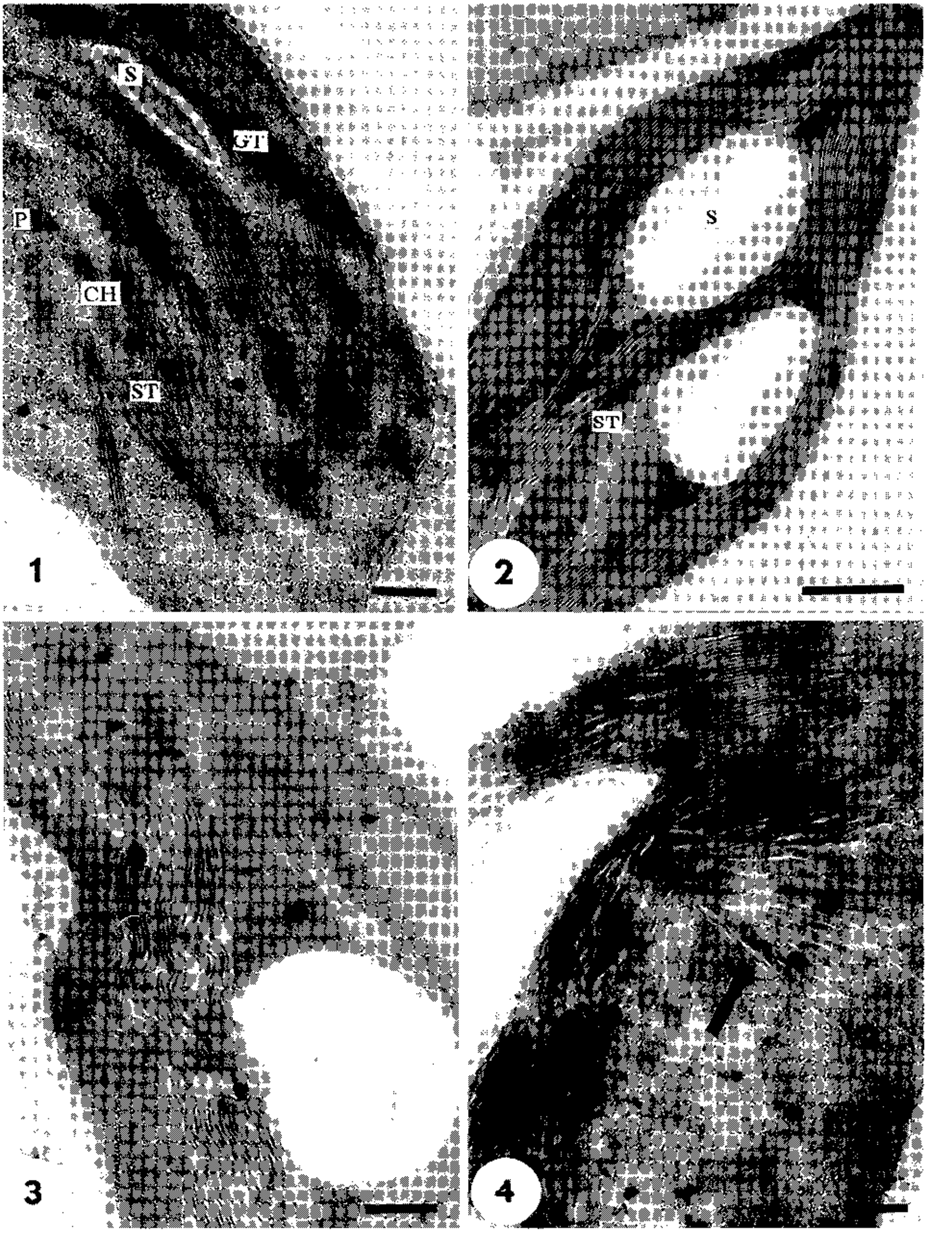


Fig. 1. Chloroplast ultrastructure in palisade parenchyma cells of the primary leaf of 10-d-old control bean plant (CH = chloroplast; GT = grana thylakoid; ST = stroma thylakoid; S = starch grain; P = plastoglobuli; *bar* = 200 nm).

Figs. 2 - 4. Structural changes in chloroplasts of the palisade cells 3, 24 or 48 h after treatment with simulated acid rain of pH 1.8.

Fig. 2. Expansion of stroma thylakoids (ST) and increase in starch (S) 3 h after treatment (*bar* = 500 nm).

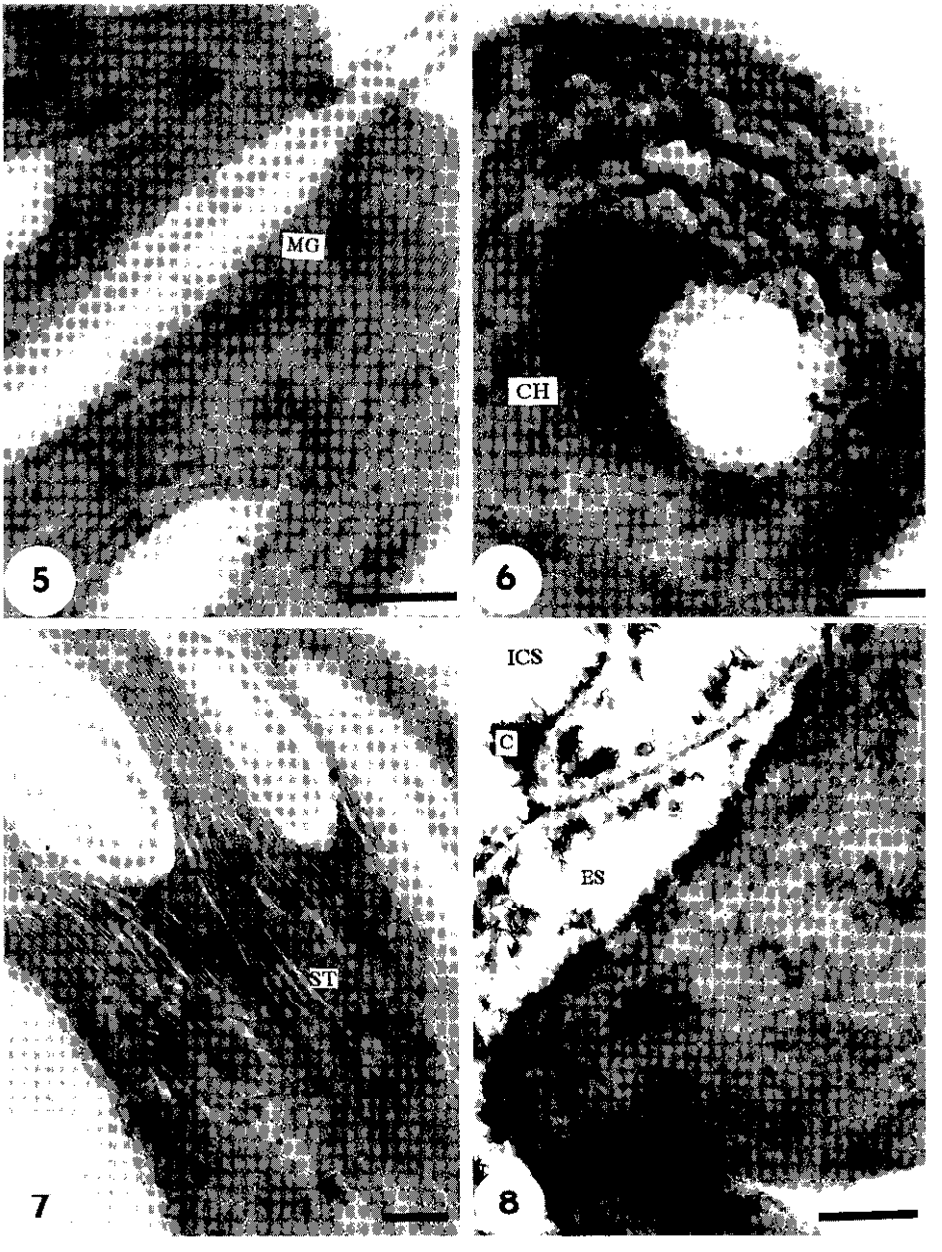
Fig. 3. Wavelike thylakoids 24 h after treatment (*bar* = 200 nm).

Fig. 4. Conformational changes of thylakoids (*arrow*) 48 h after treatment.

notable only in the variants treated with pH 1.8. In addition, 24 h after treatment with pH 1.8, chloroplasts possessing thylakoids with changed spatial orientation to wavelike were observed (Fig 3). Soikkeli and Tuovinen (1979) pointed out that the expanded thylakoid lumen and wavelike structure of membranes were the two basic changes of the inner membrane system of chloroplasts in plants subjected to air pollution. Similar changes were registered in chloroplasts of different plant species subjected to SO_2, NO_2 and O_3 (Miyake *et al.* 1984, Ascaso and Rapsch 1986, Psaras and Christodoulakis 1987, González *et al.* 1993). The acid rain under experimental and natural conditions led to an increase in the volume of thylakoid stroma and expansion of thylakoids in the needles of coniferous trees (Reinikainen and Huttunen 1989, Bäck and Huttunen 1992, Bäck *et al.* 1993, 1994, Turunen *et al.* 1995). This type of change of the inner membrane system was observed also in the chloroplasts of palisade parenchyma 48 and 72 h after treatment of bean plants with acid rain of pH 2.4, 2.2 and 2.0. A reaction of the inner membrane system, close to the observed in the experimental plants after short term acid treatment was observed in this species also after treatment with cadmium (Barceló *et al.* 1988). The sharp increase in the amount of starch grains in the chloroplasts of the palisade tissue only 3 h after treatment with pH 1.8 was of special interest. The starch grains were comparatively large with well shaped zone of saccharides and capsule containing enzymes (Fig. 2). The volume occupied by chloroplasts was no less than 80 % and the notion of a broken flow of assimilates was beyond discussion. A relation could be sought between the level of starch accumulation in chloroplasts in the analysed variants and the significantly lower photosynthetic rate as compared to control plants (Velikova *et al.* 1998).

Changes in chloroplasts 48 h after treatment with acid rain of pH 1.8 could be characterised as transformational changes of the inner membrane system leading to reorganisation of part of the thylakoids to almost perpendicular position to the longitudinal axis of the chloroplast (Fig. 4, *arrow*). A similar reorientation of the inner membrane system was observed in rice plants after 4-d ozone treatment (Toyama *et al.* 1989). It was accompanied with a weak expansion of part of the thylakoids. It is known that ozone reacts predominantly with the membrane lipids and proteins and causes changes in the membrane permeability. Most probably the simulated acid rain treatment induced in bean plants (under certain conditions) changes in the inner membrane system structurally analogous to changes induced by ozone treatment.

The structural changes in chloroplasts of the palisade parenchyma 168 h after the treatment depended mainly on pH: at pH 2.0 conformational changes of the inner membrane system without symptoms of thylakoid destruction were observed again. Only in this experimental variant, the membrane system was built of very low (2 - 5 thylakoids) grana and very high grana (40 - 50 thylakoids) identified as macrograna (Fig. 5). The formation of macrograna led to a new chloroplast architecture and was probably a result of a spatial transformation of thylakoids. It could be suggested that in functional aspect these changes were of compensatory character. The large association regions between chloroplasts and mitochodria could also be looked upon as a functional compensatory mechanism. Most probably under stress conditions the



Figs. 5 and 6. Structural changes in chloroplasts of the palisade cells 168 h after treatment.

Fig. 5. Conformational changes of the inner membrane system leading to formations of macrograna (MG) after treatment with acid rain of pH 2.0 (*bar* = 500 nm).

Fig. 6. Full destruction of the chloroplast (CH) after treatment with acid rain of pH 1.8 (*bar* = 500 nm

Fig. 7. Expansion of stroma thylakoids (ST) in chloroplasts of spongy cells 72 h after treatment with acid rain of pH 1.8 (*bar* = 200 nm).

Fig. 8. Formation of crystals (C) in the intercellular spaces (ICS) and the extraprotoplast space (ES) of cells undergone plasmolysis after treatment with acid rain of pH 1.8 (*bar* = 500 nm).

intracellular energetic system of photosynthesizing cells became unbalanced, and the role of the mitochondrial component increased. In the plants treated with acid rain of pH 1.8 different degrees of destruction of the inner membrane system were observed.

In the chloroplasts from the visibly undamaged areas of the leaves, part of the stroma and peripheral grana thylakoids were disorganized and merging of thylakoids was registered in some cases. The chloroplasts were totally destroyed in part of the cells (Fig. 6), usually in vicinity to necroses.

Under the same conditions the chloroplasts of the spongy parenchyma showed a significantly higher degree of structural resistance compared to the palisade parenchyma. The structural changes observed were uniform and did not depend on the time of measurement after the treatment. The significant changes were registered only in plants treated with simulated acid rain of pH 1.8: 48 and 72 h after treatment an expansion of the stroma and peripheral grana thylakoids was established (Fig. 7). Congestion and osmiophilisation of the stroma was observed for all variants. The changes in the apoplast of the spongy parenchyma and the protoplast of the cells were of considerable interest in plants treated with pH 1.8 and studied 168 h later. After such a long treatment almost all spongy cells had undergone plasmolysis. Formation of a great number of crystals both in the extraprotoplast space of cells undergone plasmolysis and their intracellular spaces (Fig. 8) was seen. The crystallization evidently started from the apoplast. In the variants treated with acid rain of higher pH, plasmolysis was not observed and the crystals were localized in the cell wall. The lack of crystals in the chloroplasts or in other cell components indicated that these crystalls might be residuals of components of the simulated acid rain.

The comparative analysis of the structural changes in the chloroplasts from palisade and spongy tissues points to the greater structural flexibility of the chloroplasts from the mesophyll. A sharp drop in photosynthetic rate was observed only 3, 5 and 24 h after the single treatment (Velikova *et al.* 1998), and 48 and 72 h after the treatment the photosynthetic rate increased probably due to the transformational changes of the inner membrane system leading to new chloroplast architecture. In functional aspect these changes could be classified as compensatory. Strong ultrastructural changes of chloroplasts going to full destruction were registered only after treatment with acid rain of pH 1.8. Thus the threshold level of pH for resistance of mesophyll at subcellular level was close to 2.0. The structural changes were not specific for the action of the simulated acid rain.

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