BRIEF COMMUNICATION

Effect of reddening of cotton (*Gossypium hirsutum* L.) leaves on the ultrastructure of mesophyll cells

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Abstract

The ultrastructure of cotton leaves, exhibiting reddening as symptom of physiological disorder, was examined by means of transmission electron microscopy. Osmiophilisation of the membrane compartment was established. Massive agglomerations on the tonoplast in the vacuole of cells under the adaxial epidermis were observed, and were referred to as electron-dense osmiophilic substance, most probably of anthocyanin nature. In chloroplast stroma a zone of low electron density enclosing numerous osmiophilic aggregations of unclear chemical character was differentiated. Fragmentation and severe destruction of thylakoids in chloroplasts of reddening cotton leaves was not detected.

Additional key words: chloroplast; osmophilic aggregation; peristromium; peroxisome; starch; transmission electron microscopy.

"Red leaf disease" is a physiological disorder in cotton observed in some regions of Sudan, India (Dastur *et al*. 1960, Dhopte 1990), and in the last ten years in the Aegean region of Turkey, causing economic losses due to reduced yield and damaged fibre quality. The phenomenon is provoked by abiotic stress related to disturbed soil ion equilibrium leading to K shortage and accumulation of Na ions in cotton leaves (Yagmur *et al*. 2003). As an important crop, cotton is largely studied in respect to abiotic stress constraints, such as nutrient deficiency, salinity, UV-B radiation, high temperature, *etc*. (Nepomuceno *et al.* 1998, Zhao *et al*. 2001, Kakani *et al*. 2003, Meloni *et al*. 2003). Scarce data, however, are available on cotton reddening and the underlying biochemical, physiological, and ultrastructural mechanisms. In a previous work we reported a dramatic accumulation of anthocyanins, drop of chlorophyll content, and raise of peroxidase activity and proline content in red cotton leaves. The malonyldialdehyde content, however, was not increased, which points to a conserved membrane integrity. The above data suggest a stress situation in which anthocyanins may play a protective role (Edreva *et al.* 2002) as shown in other plants subjected to stress (Chalker-Scott 1999). A survey on functional activity of photosynthetic apparatus revealed that in red cotton leaves photosynthesis was lowered due to both decreased photochemical activity and stomatal limitations (Velikova *et al*. 2002).

The purpose of the present paper was to study the ultrastructure of mesophyll cells of red cotton leaves. A special emphasis was laid on the chloroplasts as structural counterparts of the functional changes occurring in the photosynthetic apparatus upon cotton reddening.

Cotton (cv. Nazilli 84) plants were grown in Izmir district of Aegean region. Upper (25-d-old) leaves of plants at flowering-boll formation stage were used. Samples were taken in the middle of September when leaf reddening was fully expressed. Two stages of reddening were assayed: light symptoms (small, slightly-red coloured spots) and severe symptoms (large, deeply red coloured spots). Green upper leaves of plants without symptoms of reddening were used as controls.

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For transmission electron microscopy (TEM) 1 mm^2 pieces were collected from leaves and fixed in 3 % glutaraldehyde (m/v) in 0.1 M sodium phosphate buffer (pH 7.4) for 12 h at 4 °C. The samples were post-fixed in 2 % (m/v) OsO_4 in the same buffer for 4 h at room temperature. After dehydration the material was embedded in *Durcupan* (*Fluka*). Ultra-thin sections were cut from palisade parenchyma using a *Reichert* ultra-microtome. Examination was performed with *JEOL 1200EX* electron microscope.

Mesophyll cells of controls (symptom-less leaves) were characterized by a well-differentiated plastid apparatus, numerous, relatively small-sized mitochondria of sacular type, and less numerous peroxisomes. Single, midsize starch granules were rarely discernible (Fig. 1*A*). The energetic organelles were in structural contact through relatively large associative regions (Fig. 1*B*), with chloroplasts essentially contributing to their formation. Chloroplasts exhibited both typical and not-typical structural characteristics. Their form varied from elongated-elliptic to near-round. A large peristromium, oriented mainly to the cell wall, was formed in the peripherally located chloroplasts. In some cases the peristromium was a uniform wide zone underlying the chloroplast envelope. The inner membrane system was typically structured, composed of relatively high grana (30–35 thylakoids) and well-developed stromal thylakoids. However, it did not always parallel the longitudinal axis of chloroplast—a peculiarity that is not related to the formation of peristromium. In some chloroplasts a part of thylakoids (both granal and stromal) were almost perpendicular to the chloroplast envelope (Fig. 1*B*). In other chloroplasts the membrane system was separated in two uneven parts without visible thylakoid connection (Fig. 1*B, arrow*). Another non-typical feature was the location of single, peripheral stromal thylakoids (Fig. 1*A*, *arrow*) that were freely oriented in the peristromium and seemed to be only unilaterally connected with the grana. These characters of the chloroplast membrane system of control plants can hardly be interpreted as destructive changes in response to the environmental conditions. They may be rather referred to as ontogenic features typical for cotton leaves older than 20 d, in which chloroplast deformations are described (Bondada and Oosterhuis 2002).

The structural organization of mesophyll cells in leaves with light symptoms of reddening was consider-

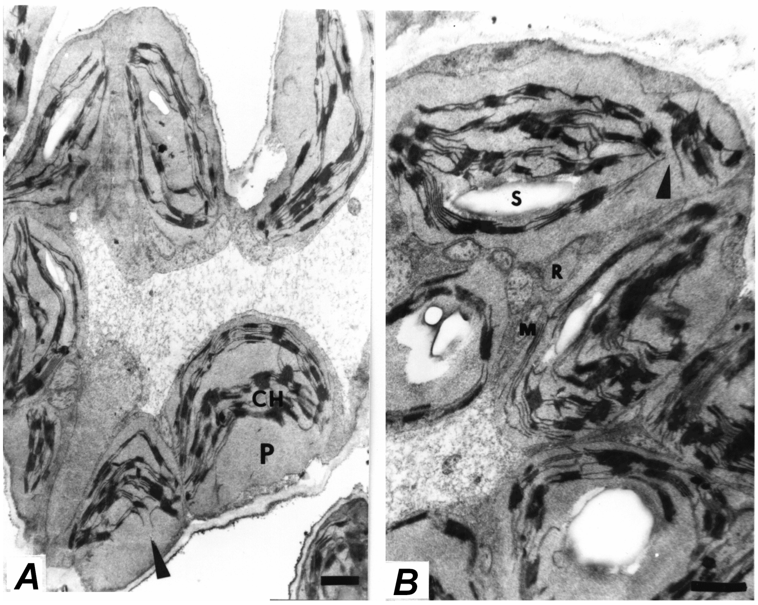

Fig. 1*A*,*B*. Ultrastructure of mesophyll cells and chloroplasts in leaves of control cotton plants. CH = chloroplast, M = mitochondrion, P = peristromium, R = peroxisome, S = starch grain. Bar = 1 µm. For arrows, see the text.

ably different as compared to controls (Fig. 1*C*). Osmiophilisation of the membrane compartment was established in all vesicular formations in the vacuole, boundary membranes, and envelopes of double membrane-enclosed organelles (Fig. 1*C*). Massive agglomerations on the tonoplast in the vacuole were observed (Fig. 1*C*, *arrow*). These agglomerations in cells under the adaxial epidermis are referred to as electron-dense, osmiophilic substance. Their chemical nature is not clearly understood. According to Pecket and Small (1980), Cormier *et al*. (1990), and Hodges and Nozzolillo (1996) anthocyanins are synthesized in cytoplasmic vesicles which are then transported to the vacuole, where they coalesce, forming a bounded anthocyanoplast. In our experiments we did not confirm the presence of anthocyanoplasts in the vacuole. Anthocyanin pigments may be supposed as constituents of the osmiophilic granules located in the above-described structures of red cotton leaves. Moreover, anthocyanin breakdown and deposition of tannins in the cell are related (Borriss and Libbert 1984). This suggests that products of anthocyanin condensation and/or breakdown may also be deposited as osmiophilic aggregations.

The most specific structural changes that are characteristic for reddening leaves were observed in the chloroplasts. In chloroplast stroma a zone of very low electron density enclosing numerous osmiophilic agglomerations was differentiated (Fig. 1*D*, *arrow*). The zone covered approximately half of the chloroplast volume, was near-round, and differently filled-up by osmophilic agglomerations which were similar to the above-described electron-dense formations in the cells. This questions the chloroplast specificity of agglomerations, adding debatable elements to their structural and functional characteristics. The remaining part of chloroplast volume was engaged by single, various-sized starch granules and peripheric inner membrane system with no clearly shaped peristromium. The starch granules lacked a structured saccharide zone and an enzyme-containing capsule. In some chloroplasts fragmentation of stromal thylakoids in grana-connecting regions was observed (Fig. 1*E*, *arrow*).

The chloroplast structural organization of mesophyll cells in leaves with severe symptoms of reddening was similar to that observed in leaves with light reddening symptoms. However, the ratio of chloroplast compartments was different (Fig. 1*F*): the osmiophilic aggregations covered large part of the chloroplast volume. They were irregularly shaped due to elongation of chloroplasts. The remaining part of chloroplasts was engaged by 1–2 large starch granules, pushing the strongly reduced inner membrane system towards the periphery (Fig. 1*G*).

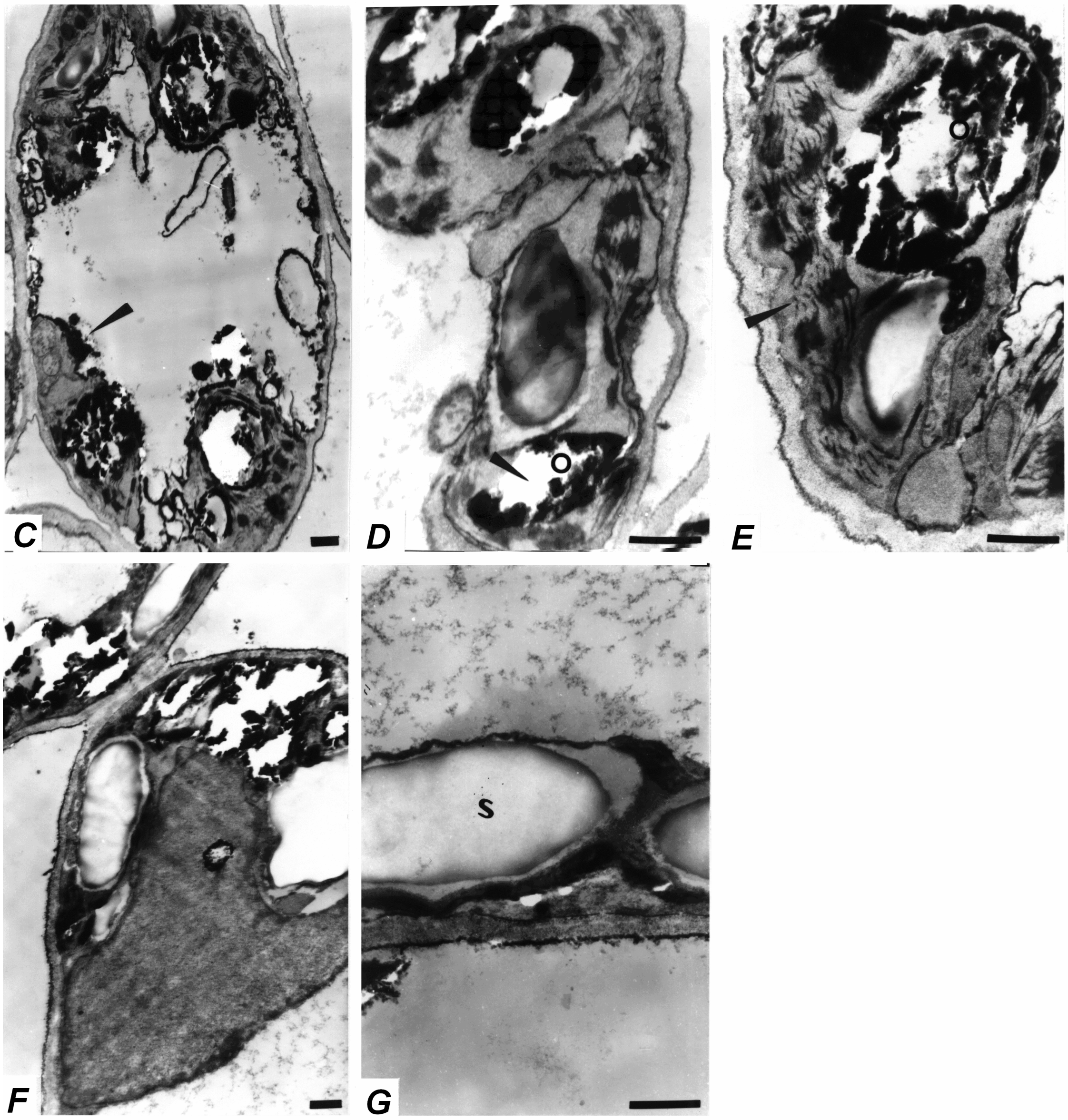

Fig. 1*C*-*G*. Ultrastructure of changes in structural organisation in mesophyll of cotton leaves with light (*C*, *D*, *E*) or severe (*F*, *G*) symptoms of reddening. O = osmophilic aggregation, S = starch grain. Bar = 1 µm. For arrows, see the text.

Independent of the observed deformations in chloroplast ultrastructure, fragmentation and severe destruction of thylakoids in red leaves were not observed. This agrees with our previous results pointing to membrane intactness in these leaves as judged from malonyldialdehyde test (Edreva *et al*. 2002). Stability of chloroplast membranes was also reported in other stress situations, such as acid rain in bean leaves (Stoyanova and Velikova 1997, Gabara *et al*. 2003). Thus the lowering of photosynthetic activity in red cotton leaves (Velikova *et al*. 2002) could be attributed to both functional and structural chloroplast changes. Stomatal and non-stomatal factors, such as the decreased photochemical efficiency (Velikova *et al*. 2002) could also be responsible for photosynthesis decline. Similar data are reported for cotton plants subjected to salt stress (Meloni *et al*. 2003) and K deficiency (Bednarz *et al*. 1998, Zhao *et al*. 2001). The presence of large starch granules in chloroplasts of red leaves (Fig. 1*G*) is indicative of an attenuated flow of assimilates from source tissues to reproductive sinks. This may be explained by the decreased photosynthetic rate, and could result in reduction of lint yield in reddening cotton.

The formation of osmiophilic electron-dense agglomerations in the chloroplasts of red cotton leaves could be related to the disturbance of chloroplast functions. Similar agglomerations were observed in senescent or abscised leaves (Ragetli *et al*. 1970, Colquhoun *et al*. 1975) and in response to air pollution and metal toxicity (Godzig and Knabe 1973, Sresty and Rao 1999). Their genesis and chemical character, however, remain still unclear.

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