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BRIEF COMMUNICATION

Morphometric analysis of chloroplasts of cotton leaf and fruiting organs

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Abstract

We examined morphological and ultrastructural differences in chloroplasts of cotton leaves and the fruiting organs, bract, and capsule wall to advance our understanding of their commonly observed differences in photosynthetic efficiency. Chloroplasts from leaves were large (7.1 μm long in cross section), lens shaped with a well developed membrane system differentiated into grana and stroma lamellae that occupied the large cross-sectional area (12.3 μm^2) of the organelle. A few small plastoglobuli and starch grains were scattered in the stroma region. The bract chloroplasts were correlative of leaf chloroplasts in size (6.8 μm in length) and shape with the exception that the bract chloroplasts exhibited greater thylakoid number per granum (15.8) than the leaf chloroplasts (10.5). In contrast to leaf and bract, the capsule wall chloroplasts were smaller in size (4.3 μm) and cross sectional area (6.8 μm^2) than either the leaf or bract. The most intriguing feature of the capsule wall chloroplasts was its domination by large starch granules (5.3 μm^2) in the stroma which filled the whole chloroplast coercing the membrane system to move towards the periphery of the organelle. Grana number and thylakoids per granum were lowest in the capsule wall chloroplasts.

Additional key words: bract, capsule wall, *Gossypium hirsutum*, grana, starch, thylakoids, transmission electron microscopy.

The chloroplast is a highly plastic organelle and changes its shape in response to external stimuli incited by various abiotic and biotic factors, the distortion of shape is ascribed to accretion of various substances such as starch grains and plastoglobuli in the stroma of the chloroplast (Björkman *et al.* 1972, Bondada *et al.* 1995, Pritchard *et al.* 1997). Metamorphosis of the chloroplast, encouraged by the accumulation of these substances is frequently accompanied by disintegration of the thylakoid membrane system (Kutík *et al.* 1993). For instance, an excessive starch build-up stimulated by nitrogen deficiency (Kutík *et al.* 1993) or CO_2 enrichment, altered chloroplast shape and destroyed the membrane assembly (Pritchard *et al.* 1997). Damage to the membrane system coupled with alterations in its morphology may disrupt the functional integrity of the chloroplast (Kutík *et al.* 1993), hence, chloroplast membrane integrity is crucial to enhance photosynthetic efficiency.

In cotton, other plant organs such as the bracts of the floral bud and the capsule wall of the boll (the fruit) exhibit some degree of autonomy by accomplishing photosynthetic activity. For instance, Wullschleger and Oosterhuis (1990) documented that bracts and the capsule wall participated in gas exchange activities analogous to leaves. Hence, the bracts and the capsule wall are also integral to the growth and development of the boll (Elmore 1973, Wullschleger and Oosterhuis 1991). However, the leaves are more physiologically active with greater values of photosynthesis, stomatal conductance, and transpiration than the bracts and the capsule wall (Constable and Rawson 1980, Wullschleger and Oosterhuis 1991). The incongruities in photosynthetic

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rates between these organs could be elucidated by probing the ultrastructure of their chloroplasts. In our previous studies, we delineated anatomical and surface ultrastructural differences between these three organs to account for their divergent photosynthetic activity (Bondada *et al.* 1994, Bondada and Oosterhuis 2000). The objective of this study was to perform comparative morphometric analysis of chloroplasts between cotton leaves, bracts, and the capsule wall. A study of this nature would aid in establishing a functional relationship with photosynthesis in the three organs and further enrich our understanding of their divergent photosynthetic behavior leading to improved cotton productivity.

Cotton (*Gossypium hirsutum* L. cv. Stoneville 506) plants were grown under irrigated field conditions at the Agricultural Research and Extension Center in Fayetteville, Arkansas, USA. Seeds were planted in rows 1-m apart on 17 May 1991 and thinned after emergence to a population of *ca* 7.2 plants m^{-2}. Fertilizer consisted of N, P and K (3.2, 1.4 and 2.6 g m^{-2}) incorporated before planting and an additional side dressing of 3.0 g(N) m^{-2} 55 d after planting. The plots were furrow-irrigated and maintained well-watered throughout the season. Leaf material for observation with transmission electron microscopy (TEM) was collected from upper-canopy fully-expanded 20-d old sun-lit leaves. Tissue samples from the capsule wall of the fruit (boll) and associated bracts were taken from the first fruiting position on the sympodial branch at main-stem node 10. The experimental design was completely randomized with four replications. Five samples of leaf, bract, and capsule wall from a plant in each replication were harvested for examination with transmission electron microscopy (TEM).

Several 1 - 2 mm^2 pieces were excised from mid-laminar area of leaves and bracts and middle portion of the capsule wall with a razor blade, fixed in 3 % glutaraldehyde overnight, washed with 0.1 M potassium phosphate buffer, pH 7.2, and postfixed in 2 % osmium tetroxide overnight. The leaf samples were then dehydrated in an acetone series and embedded in Spurr's resin (Spurr 1969). Gold and silver ultrathin (70 - 90 nm) transverse sections were cut with a glass knife (*Ted Pella Inc.*, Redding, CA, USA) and mounted on 200-mesh copper grids. The leaf sections were then double stained with 2 % uranyl acetate (Stempack and Ward 1964) and lead citrate (Reynolds 1963) and examined under a transmission electron microscope (*Philips 201*, Amsterdam, The Netherlands) at 60 kV.

Ten to fifteen chloroplasts per section representing different mesophyll cells from each replication of leaf, bract, and capsule wall were photographed at a primary magnification of ×15 000. Contact prints were used to determine the number of plastoglobuli, starch grains, grana and thylakoids per granum. Starch grain and chloroplast profile (visible cross-sectional) areas per chloroplast were quantified with an image analysis system (*Image-Pro Plus*, *Media Cybernetics*, Silver Spring, MD, USA). Grana with at least three thylakoids per granum were used for the measurements of membrane characteristics.

The chloroplasts of leaves were lens shaped (Fig. 1*A*) with a cross sectional length of 7.1 μm and a cross-sectional area of 12.3 μm^2 (Table 1). A previous study illustrated that the leaf chloroplasts were equipped with well developed thylakoids appressed into grana connected to other grana by stroma lamellae (Bondada *et al.* 1994). These pouch-shaped thylakoids, the basic morphological unit of photosynthesis are responsible for creating a large surface-to-volume ratio (Horton 1999). The chloroplasts of leaves had the greatest grana number (Table 1) which perhaps conferred them with the largest membrane surface area. A large surface area of the membrane is constructive since photosystems (PS) 1 and 2 are embedded in the membrane system, hence, the greater the surface area, the greater the entrapment of light energy (Lichtenthaler 1968). Small starch granules coupled with a few plastoglobuli appeared in the stroma (Table 1). Such ultrastructural configurations of chloroplasts represent the ideal membrane system to maximize photosynthesis (Anderson 1999).

The ultrastructural features of the bract chloroplasts were analogous to leaf chloroplasts (Fig. 1*B*), however, differences occurred with respect to thylakoids per granum. Although leaves had the greatest grana number, the thylakoids per granum was greatest in the bract chloroplasts (Table 1), a membrane architecture distinctive of shade chloroplasts (Björkman *et al.* 1972, Anderson 1999). Membrane domains with appressing of thylakoids into enormous grana are expected in the chloroplasts of bracts since they develop deep under the canopy with reduced light conditions. Since photosystem 2 (PS 2) is localized to grana and shade chloroplasts have lesser PS 2 complexes than sun chloroplasts, shade chloroplasts adapt better to their environment by means of large granal stacks to facilitate collection of as much excitation energy as feasible by PS 2 without excessive spillover of excitation energy from PS 2 to PS 1 (Anderson 1999). The number of grana in the bract chloroplasts was lower than the leaf chloroplasts (Table 1). Encrypted in the membrane system are the chlorophyll molecules, and hence a decline in grana abundance would contribute to reductions in chlorophyll concentrations in the bracts. This explains the low chlorophyll concentrations of the bract compared with the leaves reported earlier by Wullschleger and Oosterhuis (1990).

The chloroplast of the capsule wall (Fig. 1*C*) were smaller in size (4.3 μm long in cross section) and area (6.8 μm^2 in cross section) than chloroplasts of either leaf or bract (Table 1). The most intriguing feature of the capsule wall chloroplast was the accretion of starch granules that distorted the shape of the chloroplast to a sphere and pressed the membrane system against the chloroplast envelope (Fig. 1*C*). These starch granules

retained most of the cross-sectional area (~ 79 %) of the chloroplast (Table 1). The excessive build-up of starch granules in the stroma is a reflection of depressed translocation of photoassimilates, a paradigm of source-sink imbalance between the capsule wall and the lint.

A chloroplast undergoing metamorphosis by reserving excess starch may hinder photosynthesis by the mechanism of feedback inhibition (Pritchard *et al.* 1997). Furthermore, the starch granules pushed the membrane system towards the periphery pressing the thylakoids

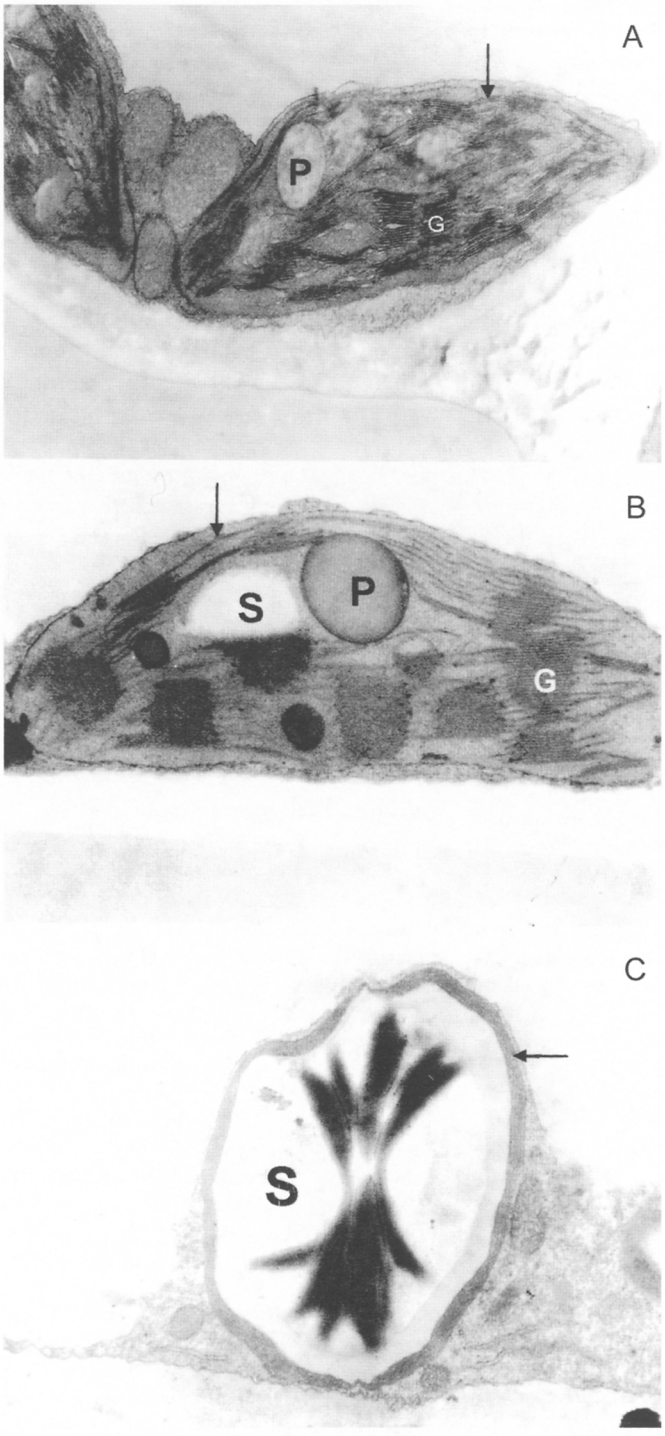

Fig. 1. Transmission electron micrographs of *A* - chloroplasts of leaf showing well developed grana (*G*), stroma lamellae (*arrow*), and plastoglobuli (P) (×12500); *B* - bract with grana (G), stroma lamellae (*arrow*), starch (S), and plastoglobuli (P) (×13000); and *C* - capsule wall showing a large starch (S) granule that occupied most of its area by pushing the membrane system (*arrow*) towards the periphery (×13000).

Table 1. Chloroplast characteristics of cotton leaf, bract, and capsule wall. Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Ch. length - length of the chloroplast cross section [μm], Ch. area - area of chloroplast cross section [μm^2], Gr. No. - grana number per chloroplast cross section, Thy/Gr - number of thylakoids per granum, Pg. No. - number of plastoglobuli per chloroplast profile (longest dimension), Pg. size - plastoglobuli size per chloroplast profile, St. No. - number of starch grains per chloroplast profile, St. area - area of mesophyll chloroplast starch grain profiles [μm^2] per chloroplast profile.

	Ch. length	Ch. area	Gr. No.	Thy/Gr	Pg. No.	Pg. size	St. No	St. area
Leaf	7.1a	12.3a	16.4a	10.5b	3.2a	18.5a	3.2a	4.7a
Bract	6.8a	11.5a	7.4b	15.8a	2.3a	15.3a	2.5a	3.6a
Capsule wall	4.3b	6.8b	3.8c	3.3 c	1.9a	14.9a	2.1a	5.3a

unusually close together (Fig. 1*C*). Such an event is known to physically prevent light from reaching the thylakoids and causing photosynthesis (Salisbury and Ross 1992). Hence, it appeared that starch accumulation triggered events inhibitory to photosynthesis. The number of grana and thylakoids per granum were the lowest in the capsule wall chloroplast (Table 1) where they were mostly pressed against the envelope (Fig. 1). This may be the reason why the chlorophyll levels and photosynthesis in the capsule wall were the lowest of all the three organs as reported by Wullschleger and Oosterhuis (1990).

In conclusion, the chloroplasts of leaves exhibited greatest integrity by displaying an abundance of well developed grana and stroma lamellae oriented parallel to the long axis of the chloroplast cross section. Although, morphologically and structurally, the bract chloroplasts were analogous to leaf chloroplasts, it exhibited lower grana number but greater number of thylakoids per granum than the leaf chloroplasts. The capsule wall chloroplasts were the smallest with the smallest grana number and number of thylakoids per granum. Overwhelming area of the chloroplast was dominated by starch granules that modified its regular shape to a sphere. Taking into account these ultrastructural disparities in chloroplasts, one may be able to further explain the observed differences in photosynthesis between the leaf, bract, and the capsule wall.

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