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Effects of Water Stress on the Ultrastructure of Leaf Cells of *Sorghum bicolor*

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KENNETH L. GILES, DANIEL COHEN, AND MICHAEL F. BEARDSELL
Plant Physiology Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand

ABSTRACT

The subcellular changes which occurred in sorghum leaves during increasing water stress and subsequent rewatering are described. Stomata were closed, abscisic acid levels were elevated, and the amounts of starch in the bundle sheath chloroplasts were much reduced by −14 bars leaf water potential. Swelling of the outer chloroplast membrane, and reorganization of the tonoplast to form small vesicles from the large central vacuole, occurred by a leaf water potential of −37 bars. Complete structural disruption of the tonoplast, as previously described for maize was not found. On rewatering, large amounts of starch reappeared within three hours. These findings strengthen the hypothesis that maintenance of tonoplast integrity is an important factor in the ability of plants to withstand drought.

Several comparative studies of water relations and drought resistance have suggested that sorghum is less adversely affected by water stress than maize (1, 4, 6-9). Root factors (7) and the ability of sorghum to keep its stomata open at a lower ψ_l[1] than maize (9) have been put forward as explanations.

We have suggested that sorghum may also be less susceptible than maize to cellular damage brought about by water stress (3). This communication describes the subcellular changes which occurred in sorghum leaves as a result of water stress and provides comparative data for maize.

MATERIALS AND METHODS

Sorghum bicolor (hybrid NK145) plants were grown under controlled environmental conditions in the Climate Laboratory as described previously (3). Day/night temperatures and vapor pressure deficits were 25 C/20 C and 10/5 millibars, respectively. Daylength was 12 hr and the photosynthetically active radiation (400-700 nm) was approximately 170 w m^{-2}. Water stress was imposed by withholding nutrient solution while a number of control plants were maintained on a full watering regime.

The method of ABA analysis, by solvent partitioning and TLC, followed by gas chromatography in the electron capture mode has been described by Beardsell and Cohen (1), and the fixation processes for electron microscopy samples by Giles *et al.* (3). Leaf water potentials were measured with a pressure chamber: the corresponding values of leaf relative water content may be obtained from the leaf moisture characteristic curve (1). Stomatal diffusion resistances were determined with a porometer of the type described by Kanemasu *et al.* (5). All samples and measurements were made on the 11th, 12th, or 13th leaves of plants having at least 15 fully mature leaves (leaf 1 being the oldest leaf).

[1] Abbreviation: ψ_l: leaf water potential.

RESULTS AND DISCUSSION

No changes in the normal morphology and ultrastructure (Fig. 1) were observed until ψ_l had fallen to about −14 bars. At this ψ_l there had been a marked reduction in the amount of starch in the bundle sheath chloroplasts (Fig. 2), the stomata were closed and the ABA level had risen approximately 13-fold to 3 ng cm^{-2}. At no level of stress did we find the rearrangement of the chloroplasts noted in maize at a ψ_l of −18 bars (3).

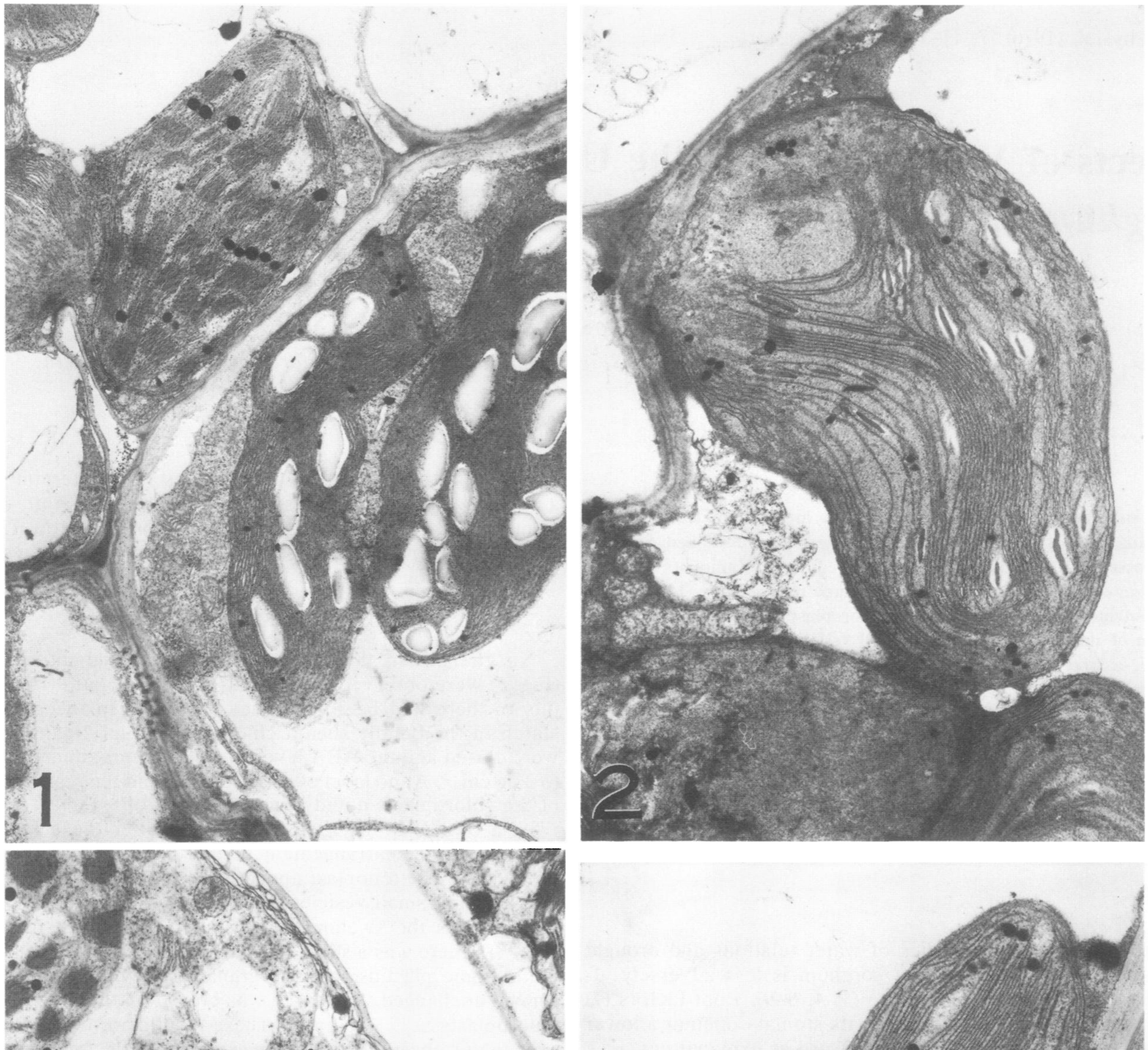
At a ψ_l of −37 bars on the 7th day after the cessation of watering some rearrangement had occurred within the mesophyll cells. The tonoplast appeared to have fragmented, giving rise to many small vesicles, but the only apparent chloroplast damage was the swelling of the outer chloroplast membrane (Fig. 3). There was a slight disarrangement of the stroma lamellae in some plastids, but the granal organization appeared to remain unchanged. No starch was present in the mesophyll cell chloroplasts at −37 bars ψ_l. These results are in marked contrast to our observations with maize, where the tonoplast broke down causing cell disruption at a ψ_l of −18 bars and the chloroplasts, coming into contact with the vacuolar fluid, swelled and burst. The cells were thus totally disrupted.

As in maize (3) the bundle sheath cells of sorghum seemed more resistant than mesophyll cells to damage under water stress conditions. Although starch was completely lost from the bundle sheath cells of sorghum before ψ_l reached −37 bars, the chloroplasts showed no swelling or disarrangement of the lamellae. In the absence of starch, small grana could be seen (Fig. 4).

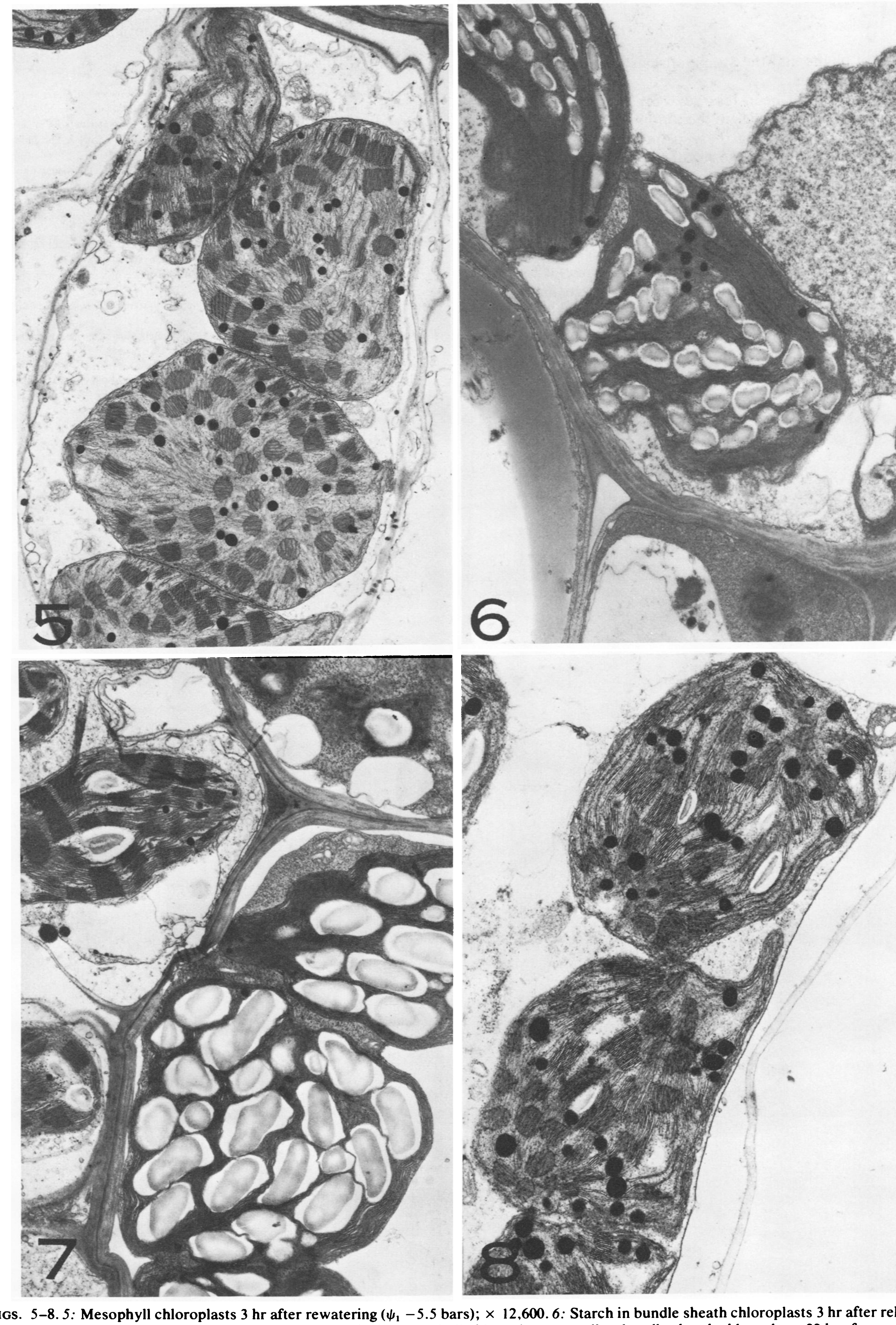
On the 7th day stress was relieved by rewatering 4 hr after the start of the photoperiod. In the most severely stressed plant (ψ_l = −37 bars before rewatering) ψ_l rose to −5.5 bars within 3 hr and the swelling of the outer membrane of the mesophyll chloroplasts had decreased (Fig. 5). Bundle sheath cells had developed large amounts of starch (Fig. 6), possibly exceeding those found in the controls. Within these 3 hr the stomata had started to reopen and ABA levels had fallen to one quarter of those obtaining prior to rewatering. The samples for Figures 3 to 6 inclusive were all taken from the same leaf.

Twenty-two hours after rewatering, vesicles were still present in the mesophyll cells, and starch had further increased in the bundle sheath plastids and had reappeared in some mesophyll plastids (Fig. 7).

Forty-six hours after rewatering (ψ_l = −2.8 bars), the tonoplast vesicles in the mesophyll cells had virtually disappeared (Fig. 8), and the ultrastructure of mesophyll and bundle sheath cells appeared to be normal. ABA levels had fallen to those of



FIGS. 1–4. *1:* Unstressed bundle sheath chloroplasts containing starch, and chloroplasts of neighboring mesophyll cell; × 12,500. *2:* Bundle sheath chloroplasts at ψ_1 −14 bars showing a marked reduction in starch content; × 15,000. *3:* Mesophyll cell from leaf at ψ_1 −37 bars. Several small vesicles (V) have formed from the tonoplast, and the chloroplast outer membranes show some swelling (arrows); × 12,500. *4:* Bundle sheath chloroplasts at ψ_1 −37 bars.



FIGS. 5–8. *5:* Mesophyll chloroplasts 3 hr after rewatering (ψ_1 −5.5 bars); × 12,600. *6:* Starch in bundle sheath chloroplasts 3 hr after relief from stress (ψ_1 −5.5 bars), × 12,600. *7:* Increased starch deposits in mesophyll chloroplasts as well as bundle sheath chloroplasts 22 hr after rewatering (ψ_1 −3.3 bars); × 12,600. *8:* Normal appearance regained in mesophyll chloroplasts 46 hr after rewatering (ψ_1 −2.8 bars); × 12,600.

the control plants and the stomatal resistance was the same as that of the controls.

Plants which had been less severely stressed (*e.g.*, to −25 bars ψ_l) showed no changes in the tonoplast or outer chloroplast membrane. On rewatering these plants normal levels of ABA and stomatal resistance were regained more quickly than in the plants stressed to a ψ_l of −37 bars.

It should be borne in mind that the values of ψ_l at which the various changes occur may depend both on the stage of growth and on the conditions under which the plant was grown (2). These findings confirm our earlier observation that leaf cells of sorghum were less damaged than those of maize at low water potentials (3), and strengthen the hypothesis that maintenance of membrane structure is an important factor in the ability of plants to withstand severe water stress.

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