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Cellular and Ultrastructural Changes in Mesophyll and Bundle Sheath Cells of Maize in Response to Water Stress

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

ABSTRACT

Ultrastructural changes were correlated with leaf water potential, relative water content, and abscisic acid levels in the leaf. Mesophyll cells were more prone to damage than bundle sheath cells at a leaf water potential of −18.5 bars. Tonoplast breakdown and cell disruption occurred in 25% of the mesophyll cells. On rewatering, these disrupted cells did not recover. In bundle sheath cells, starch, lost at about −13.5 bars leaf water potential, reappeared within 2.5 hours of rewatering.

Although there have been many studies of water stress and senescence, relatively few of them (8, 11, 12) have been concerned with the ultrastructural changes which occur. This paper describes the changes which were observed in the cellular organization and ultrastructure of leaves of maize plants subjected to water stress under controlled environmental conditions. These changes are correlated with ABA levels and measurement of leaf water potential and relative water content.

MATERIALS AND METHODS

Plant Material. *Zea mays* L. (var. Wisconsin 575) plants grown in peat-sand-vermiculite potting mix under controlled environmental conditions with adequate supplies of a modified Hoagland's nutrient solution, made up as follows: 2.0 mM NH_4NO_3; 1.35 mM $Ca(NO_3)_2\ 4H_2O$; 0.184 mM KH_2PO_4; 0.063 mM K_2HPO_4; 1.25 mM KNO_3; 0.25 mM $MgSO_4\ 7H_2O$; 0.5 mM Na_2SO_4; zinc was supplied as the sulfate at 0.012 μg/ml, manganese as the chloride at 0.145 μg/ml, copper as the sulfate at 0.005 μg/ml, boron as boric acid at 0.13 μg/ml, molybdenum as molybdic acid 0.002 μg/ml; and iron as sequestrene NaFe at 6 μg/ml. Day/night temperatures, vapor pressure deficits, and equivalent relative humidities were 25 C/20 C, 10/5 millibars, and 68/78%, respectively. Daylength was 12 hr, and the photosynthetically active radiation (400–700 nm range) was approximately 170 w m^{-2}. Water stress was imposed by withholding nutrient solution; a number of plants were maintained on a full watering regime.

Table 1. *Summary of Changes in Ultrastructure, Leaf Water Potential, Relative Water Content, and Abscisic Acid Levels in Maize Leaves during Increasing Water Stress*

Day	ψ_l	RWC	ABA	Changes Occurring
No.	*bars*	*%*	*ng cm⁻²*	
1, 2	−6.0	97	0.5	None. Normal C_4 structure.
3	−10.5	80	7.0	Level of starch in bundle sheath cells reduced. Stomata closed.
4	−13.5	73	7.5	No starch in bundle sheath cells. Cytoplasmic vesicles appeared in bundle sheath and mesophyll cells.
7	−18.5	55	9.0	Bundle sheath chloroplasts randomly distributed around cell. Tonoplast breakdown in 25% of mesophyll cells resulting in complete cell disruption.

Plants having at least 10 leaves were selected for the experiments and the eighth or ninth leaf, the oldest leaf being counted as leaf one, was used for all measurements. Samples were normally taken 2 hr after the start of the photoperiod, and all measurements and samples on any one occasion were made on, or taken from, the same leaf. Because of the destructive nature of the sampling a number of plants were used in the experiment, each plant being sampled on two or three occasions.

Abscisic Acid Analysis. Samples of five leaf discs, each 15 mm diameter, were used for ABA analysis. The discs were extracted in an ammoniacal methanol-chloroform-water mixture, and the extract was purified by solvent partition and TLC as described by Beardsell and Cohen (2). Subsequent measurement of ABA was made using gas chromatography in the electron capture mode.

Plant Water Status. Leaf water potential was measured with a pressure chamber (3, 10), and the relative water content of the leaves was determined by the method of Barrs and Weatherley (1), using a 1-hr floating period. These values are given to the nearest 0.5 bar and 1%, respectively.

Microscopy Samples. Leaf samples for electron microscopy were taken from both stressed plants and from nonstressed controls. Samples from control plants were taken on every occasion. Samples were fixed in 3% gluteraldehyde, 2% formaldehyde at pH 7.2, postfixed with osmium, and embedded in epoxy resin for sectioning. For light microscopy 2-μm sections were cut and stained with toluidine blue to examine gross cellular changes.

RESULTS AND DISCUSSION

For the first 2 days after water was withheld, ψ_l[1] and RWC were characteristic of well watered control plants, being −6

[1] Abbreviations: ψ_l: leaf water potential; RWC: relative water content.

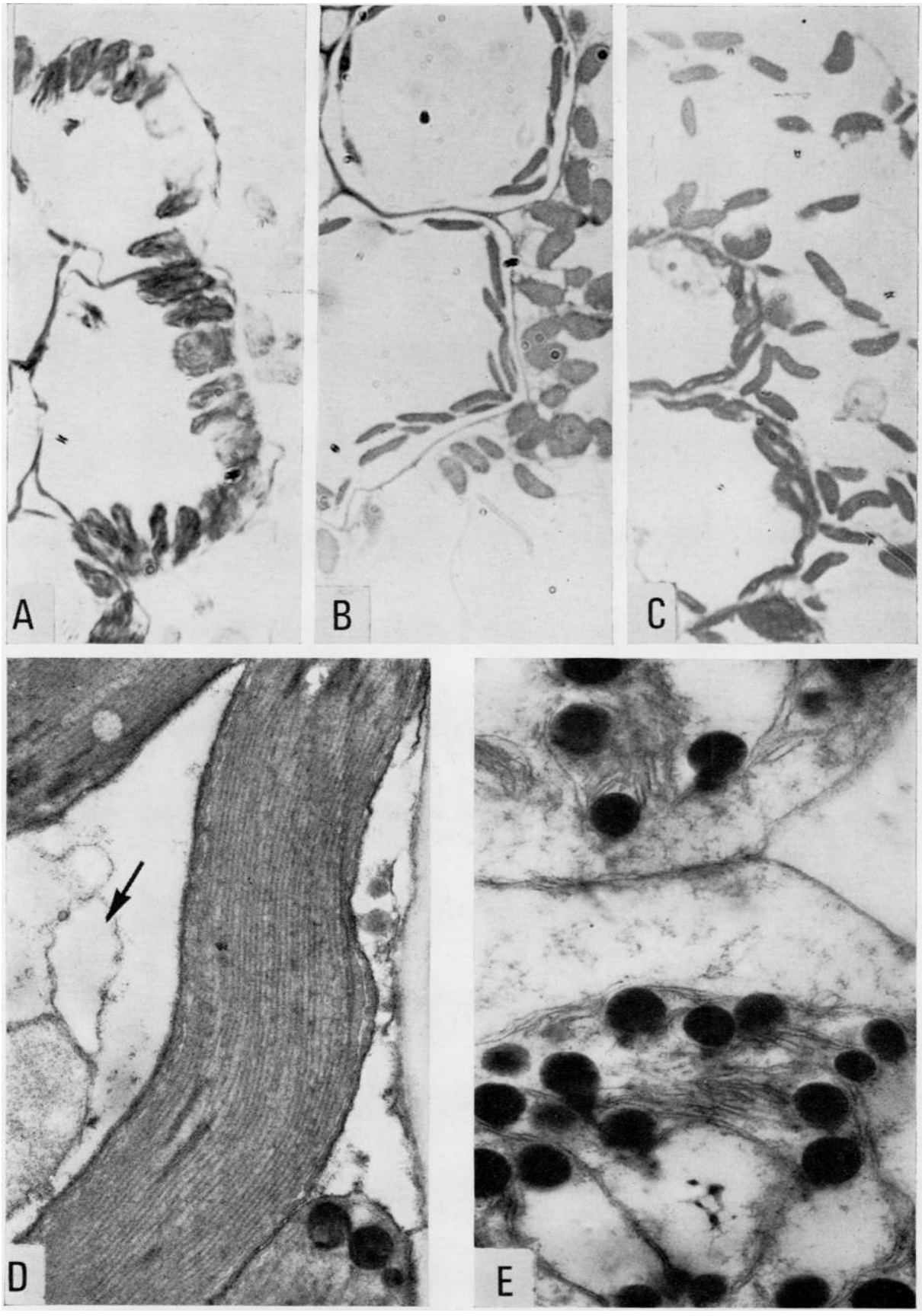

FIG. 1. A: Bundle sheath cells showing the normal arrangement of chloroplasts, on the side of the cell adjacent to the mesophyll. Starch grains can be seen in the chloroplasts. × 220. B: Random arrangement of bundle sheath chloroplasts around the cell when under water stress (ψ_1 −18.5 bars, RWC 55%). Starch has been lost from the chloroplasts. × 220. C: Twenty hours after rewatering (ψ_1 −3.5 bars, RWC 97%) starch has reformed in the chloroplasts and they appear to be returning to their original position. × 220. D: Bundle sheath on day 4 of stress. Starch has disappeared from the chloroplasts, a few osmiophilic globules have formed at their edges, but there is no disruption of the plastid. Cytoplasmic vesicles can be seen (arrow). × 32,000. E: Mesophyll chloroplasts voided into the vacuole of the cell after breakdown of the tonoplast. There are many osmiophilic globules and gross distribution of both stromal and granal lamellae. The outer chloroplast membrane has not yet broken. × 32,000.

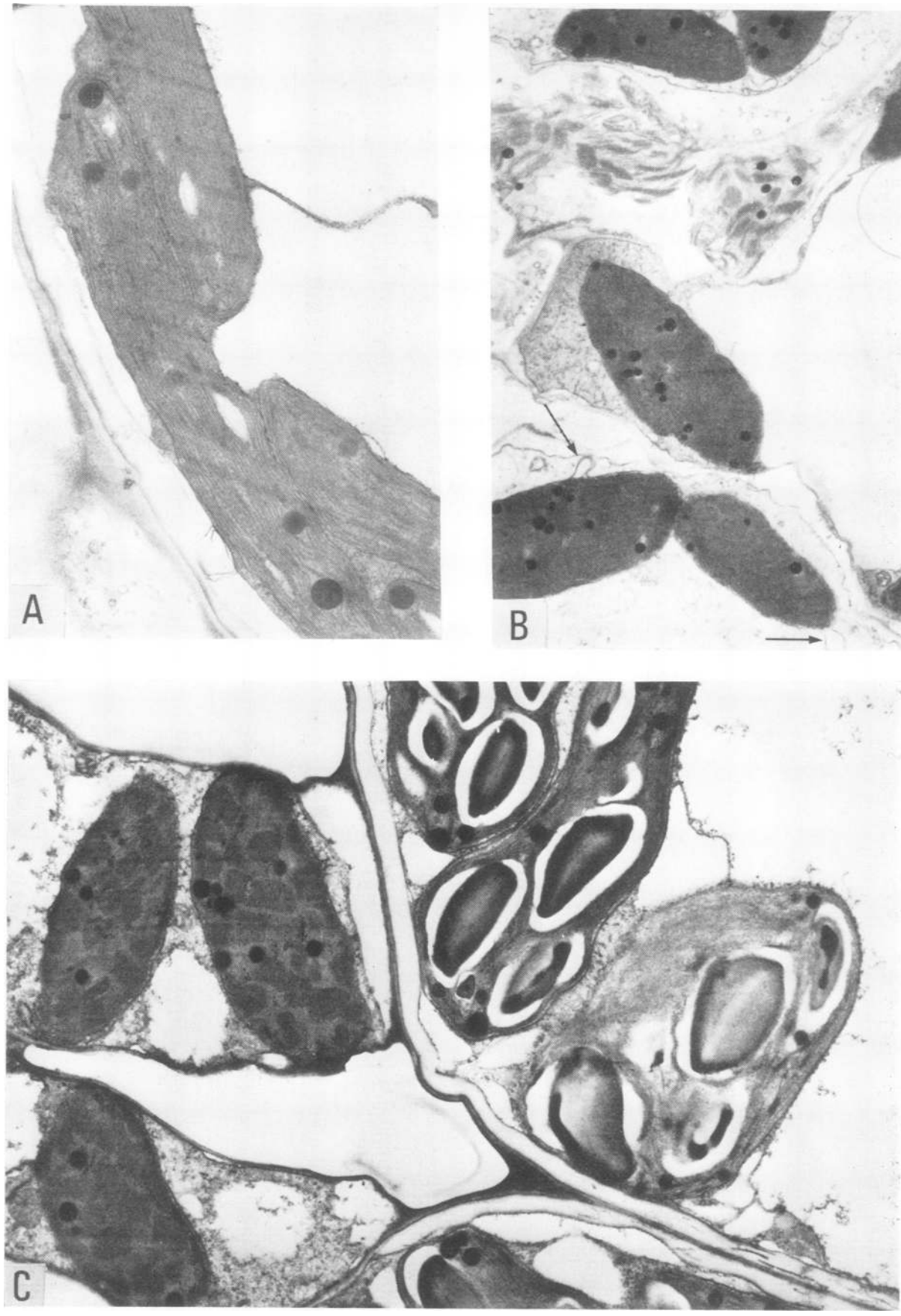

FIG. 2. A: Bundle sheath chloroplasts in a cell adjacent to undamaged mesophyll cells showing deposition of starch 2.5 hr post-rewatering. × 30,000. B: Individual mesophyll cell showing lysis of chloroplasts 2.5 hr after rewatering (ψ_1 −11.7 bars). Neighboring cells appear normal except for ballooning within the outer chloroplast membranes (arrows). × 10,000. C: Four days post-rewatering. Starch deposition has returned to normal and the mesophyll plastids have lost the swollen outer membranes, though some cytoplasmic vesicles still exist. × 12,600.

bars and 97%, respectively, and the level of ABA was 0.5 ng cm^{-2} leaf surface. There were no visible symptoms of stress. The organization and ultrastructure of the mesophyll and bundle sheath cells also appeared normal. The position of the bundle sheath chloroplasts was characteristic for maize and other malate-forming C_4-pathway grasses, being on the side of the cell adjacent to the mesophyll (5).

On the third day ψ_1 had fallen to −10.5 bars, RWC to 80%, and ABA had risen to 7.0 ng cm^{-2} leaf surface (Table I). ABA did not fall below this level until ψ_1 exceeded −10 bars in the rewatering phase. The level of starch in the bundle sheath cells had fallen, and the stomata (measured with a diffusion porometer (6), modified by H. G. McPherson and J. S. Talbot (personal communication)) were closed.

On the 4th day (ψ_1 = −13.5 bars, RWC = 73%) starch had disappeared from the bundle sheath chloroplasts. Mittelheuser and van Steveninck (8) noted in wheat, a C_3 plant, that applied ABA at 3.8 μM markedly reduced the level of starch in the chloroplasts. In the present material, an ABA level of 7.0 ng cm^{-2} leaf surface would be equivalent to at least 3.3 μM. It is not possible to determine if the disappearance of starch was related to the increased ABA levels or to the closure of the stomata.

By the 7th day (ψ_1 = −18.5 bars, RWC = 55%), the bundle sheath chloroplasts had changed their original position, and had become randomly distributed around the cell (Fig. 1, A, B, and C).

Ultrastructure. Bundle sheath cells showed no gross ultrastructural damage other than the formation of small vesicles in the cytoplasm at about −13.5 bars ψ_1. The chloroplasts, apart from the loss of starch and the formation of osmiophilic granules around the edge of the chloroplast, remained intact (Fig. 1D). Even at −18.5 bars ψ_1, cell integrity was maintained and no further damage could be seen.

The mesophyll cell plastids appeared normal at −13.5 bars ψ_1, apart from ballooning of the outer chloroplast membrane in some instances. Small vesicles had also appeared in the cytoplasm of these cells. When ψ_1 had fallen to −19 bars, the tonoplast appeared to have broken in about 25% of the mesophyll cells and complete disruption had occurred, the cells becoming filled with chloroplast debris. In the remaining 75% of the mesophyll cells, there was swelling of the outer chloroplast membrane, but grana and stroma lamellae remained well defined. This contrasted strongly with the structure of the chloroplasts immediately after breakdown of the tonoplast, where the swelling was much more pronounced and the internal structures were disorganized (Fig. 1E).

The apparent breakdown of the tonoplast at about −19 bars ψ_1 occurred in a random fashion. There seemed to be no rationale as to why individual cells were affected. They were not always the cells furthest from the vascular tissue, nor were they always adjacent to each other. Shaw and Manocha (11) noted similar changes in the tonoplasts of detached, senescing wheat leaves.

Recovery. Plants were rewatered 6.5 hr after the start of the photoperiod, and samples taken 2.5 hr later showed that starch deposition had commenced in the bundle sheath chloroplasts adjacent to undamaged mesophyll tissue (Fig. 2A). At this time, ψ_1 had risen from −18.5 bars to −11.5 bars and RWC from 55% to 70%, but no decrease in the level of ABA was detected.

Twenty hours after rewatering (ψ_1 = −3.5 bars, RWC = 97%) the bundle sheath chloroplasts had returned to their original position adjacent to the mesophyll cells. The level of ABA had fallen to approximately twice that of the control plants, but the stomata were still closed.

Mesophyll cells in which the tonoplast was disrupted showed no signs of recovery, and presumably died. In mesophyll cells in which the tonoplast had remained intact, however, no lasting structural damage occurred to the chloroplasts, and the ballooning of the outer chloroplast membrane observed during stress was still present 2.5 hr after rewatering (Fig. 2B), but disappeared during the following day. Granal stacking and stroma lamellae in these undamaged mesophyll chloroplasts remained unaltered.

After 4 days some small cytoplasmic vesicles still persisted in both mesophyll and bundle sheath cells, although ψ_1, RWC, and ABA levels had returned to normal. By this stage the bundle sheath choroplasts were full of starch (Fig. 2C).

Samples from the well watered control plants showed none of the structural changes described above and maintained the appearance characteristic of healthy material. Intercellular chloroplasts, derived from cells cut open when fresh leaf tissue is sampled, are commonly swollen and broken much in the manner of the chloroplasts observed here. Since no such swelling or breakage of chloroplasts was noted in control samples, which were sampled in the same way as stressed plants, it seems unlikely that this phenomenon has given rise to any misinterpretation of the results. The changes we discuss may, therefore, with reasonable caution, be attributed to water stress, although the aqueous nature of the fixative may also affect the finer points of ultrastructure before complete fixation is achieved.

Water potentials of the levels induced in these experiments have been found in the field in Idaho (4) and locally (Beardsell, unpublished results).

The causes of the breaking of the tonoplast and the swelling and disruption of the chloroplasts are not known, but it is of note that *Sorghum*, a genus long considered to be more drought-resistant than maize (7, 9), shows much greater resistance to damage of this kind at similar levels of water stress (unpublished results).

In preliminary work with *Sorghum*, under the same conditions as reported here, starch was detectable in the bundle sheath plastids until at least the 7th day after cessation of watering (ψ_1 = −19.5 bars, RWC = 74%), although in smaller amounts than in the well watered controls. ABA levels in the tissue were about 5 ng cm^{-2} leaf surface, *i.e.*, similar to those found in maize at a comparable level of stress. Damage to mesophyll chloroplasts also seemed less marked, with only slight swelling of the outer membrane. The tonoplast was still intact, although some cytoplasmic vesicles were present.

The maintenance of the integrity of the tonoplast may therefore be an important factor in the ability of the mesophyll cells, and hence of the whole plant, to withstand water stress.

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