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Water Stress Effects on the Content and Organization of Chlorophyll in Mesophyll and Bundle Sheath Chloroplasts of Maize[1]

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ABSTRACT

The consequences of drought stress on the organization of chlorophyll into photosynthetic units and on the chlorophyll-protein composition of mesophyll and bundle sheath chloroplasts of *Zea mays* L. were studied. It was found that the majority of chlorophyll lost in response to water stress occurs in the mesophyll cells with a lesser amount being lost from the bundle sheath cells. All of the chlorophyll loss could be accounted for by reduction in the lamellar content of the light-harvesting chlorophyll *a*/*b*-protein, a rather specific target for water stress. The decreased content of this chlorophyll-protein accounts for the elevated chlorophyll *a*/*b* ratios and the reduced photosynthetic unit sizes of the two cell types in stressed plants. The implications of the selective catabolism of this major membrane component are discussed.

The response of crop plants to water deficits has been investigated with a wide range of techniques (*cf.* 12, 13). In general, as water deficits increase in the soil, leaf water potentials decline, leading to closure of stomata, visible wilting, and dramatic impairment of many metabolic functions. Prolonged deficits can result in retardation of growth, reduction in yield, and even death of the plants. It is therefore of great importance not only to characterize and quantitate whole plant water stress phenomena, but to investigate and evaluate critically the molecular consequences of water stress. In this way, it may be possible to identify the specific biochemical targets of water stress and to understand the mechanism and nature of control of plant water stress responses.

The present study examines the consequences of drought stress on the organization of Chl into photosynthetic units and the Chl-protein composition of mesophyll and bundle sheath chloroplasts of maize (*Zea mays* L.). An attempt is made to account specifically for Chl losses due to water deficits and to determine how these losses might relate to the functional organization and efficiency of the PSU.[4]

MATERIALS AND METHODS

Plant Material. Seeds of *Zea mays* L. cv. Funk's 4808 were grown in a gravel/vermiculite mixture in 25.5-cm pots in the controlled environment chambers of the Duke University Phytotron. Plants were grown at 30/25 C day/night temperature regimes with a 12-hr photoperiod. The plants were watered three times daily with half-strength Hoagland solution. When the plants were 55 days old, they were divided into a control group which was watered on the described schedule, and a stress group from which water was withheld for 8 days and thereafter the plants were returned to the normal watering schedule. In all cases, we attempted to select leaves of the same physiological age by sampling only the fourth and fifth leaves from the top of the plants.

Chlorophyll Determinations. Relative leaf Chl content was determined *in vivo* on a Zeiss DMR-21 integrated sphere spectrophotometer as described previously (4). Chl content for chloroplast lamellar analyses was determined in 80% acetone extracts (6) while Chl content for PSU size measurements (see below) was determined by measuring the absorbance of the red maximum of Triton extracts of chloroplast and using an extinction coefficient of 60 mM^{-1} cm^{-1} (16).

Water Potential Measurement. Leaf water potentials were determined on leaf discs (0.6 cm diameter) using Peltier-cooled thermocouple psychrometers. Sampling was done within 1 hr after the start of the daily illumination period and at least five samples were taken for each determination.

Characterization of Chloroplast Lamellae. Mesophyll and bundle sheath cells were separated using a differential grind technique similar to that described by Woo *et al.* (19) excepting that a Polytron tissue disintegrator was used in place of an Omni-Mixer. Purity of the cell types was monitored during homogenization by light microscopy. Chloroplast lamellae were prepared from the two cells types (5) and were solubilized in either SDS or Triton X-100 (3). The SDS-treated lamellae were fractionated by SDS-polyacrylamide gel electrophoresis (18) into two Chl-protein complexes; complex I, the SDS-altered form of the P700-Chl *a*-protein; and complex II, the light-harvesting Chl *a*/*b*-protein (17).

The concentration of P700 in the Triton extracts of chloroplasts and in the P700-Chl *a*-protein isolated by hydroxylapatite

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[4] Abbreviations: PSU: photosynthetic unit.

chromatography of these extracts (15) was calculated from the light-induced absorbance change at 697 nm using a differential extinction coefficient of 64 mM^{-1} cm^{-1} (11). PSU size was calculated from the molar ratio of total Chl to P700 in the chloroplast Triton extract. The relative proportions of Cyt *f* and b_6 present in the isolated P700-Chl *a*-protein complex and in the total chloroplast Triton extracts were determined from chemical difference spectra (15).

RESULTS AND DISCUSSION

Water Potential. The leaf water potentials during the drying cycle and recovery are summarized in Figure 1. The data shown are the results of one of eight replicate experiments. Control plants, which were watered three times daily throughout the experimental period, maintained high leaf Ψ_w (−1 to −3 bars) for the 14-day period (Fig. 1). Drought-stressed plants showed a sharp decrease in leaf Ψ_w to about −17 bars after 3 days of stress, a value similar to that found in a recent study (10). The leaf Ψ_w of these plants remained fairly constant between −16 and −19 bars from day 3 to day 8 of drought stress. Upon rewatering, the recovery of Ψ_w of the leaves was almost as rapid as the rate of drying in that leaf Ψ_w reached the control Ψ_w of −2 to −3 bars within 3 days. The Ψ_w changes occurring during drought stress and subsequent recovery described here were typical of those found in other studies (13).

Chlorophyll Content. Relative leaf Chl content was determined on the same leaves sampled for water potential measurements. We attempted to select leaves of the same physiological age by sampling the leaf appearing in the same position relative to the top of the plant. Maize is a determinate plant, and during the period of the experiment, the full complement of leaves was attained. As a consequence, during the latter stages of the experiment, the leaves being sampled were physiologically somewhat more mature than those sampled at the start and were gradually accumulating more Chl. This accumulation is a typical response for growing leaves (14) and appears in the control plants as a 30% increase in Chl content during the 14-day experimental period (Fig. 1).

Upon withholding water, the leaf Chl content dropped (solid line and circles, Fig. 1) slowly at first (between days 1 and 6) and then more rapidly when the leaf Ψ_w reached about −16 bars (Fig. 1). The Chl content remained at this low level (27% lower than the initial level) during the subsequent 2 days of stress. Rewatering resulted in rapid Chl accumulation after a short lag period (Fig. 1). The rate of regreening was very rapid and the leaves reached their initial Chl content within 3 days. However, they never did attain the increased levels of Chl seen in the controls at the end of the experiment.

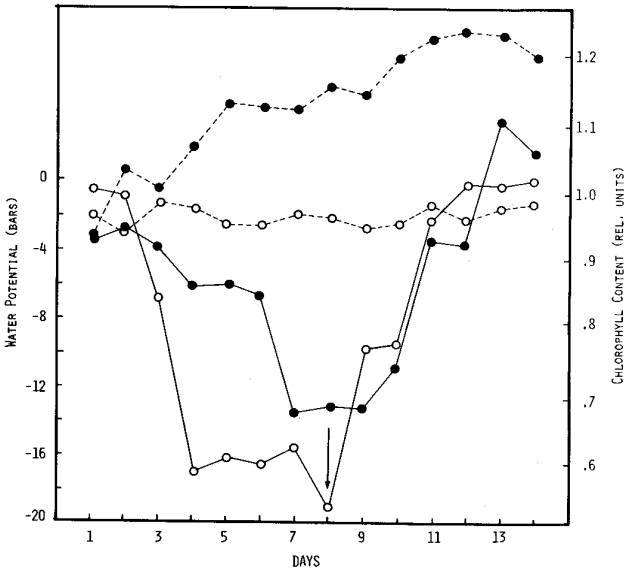

FIG. 1. Leaf Chl content (●) and water potential (○) in drought stressed (——) and nonstressed (- - -) maize plants. Water was withheld on day 1 in the stressed group and the plants rewatered (arrow) on day 8.

Characteristics of the Chloroplast Types. The Chl-protein composition and organization into PSUs in mesophyll and bundle sheath chloroplasts from stressed and control leaves are summarized in Table I. Values for the control were measured on leaves harvested on day 1 at the start of the drying cycle, while the stress material was analyzed on day 8 prior to rewatering. The stress leaves lost about 27% of their Chl and had much higher leaf Chl *a*/*b* ratios than controls. The isolated mesophyll and bundle sheath cells from the control had Chl *a*/*b* ratios in the range typically found for these cell types (7). As a result of water stress, the Chl *a*/*b* ratio of the mesophyll cells increased from 2.8 to 4.5 with a concomitant reduction, from 53 to 35%, in the lamellar content of the light-harvesting Chl *a*/*b*-protein, the site of Chl *b* in the membrane (1, 2). In addition, there was a reduction in the ratio of total Chl to P700 (PSU size) in these cells. The difference (18%) in the percentage of total Chl represented by that Chl in the light-harvesting Chl *a*/*b*-protein between the stressed and nonstressed conditions fully accounts for the difference in PSU size (18%) under the two conditions. Similarly, in response to water stress, the bundle sheath cells showed a reduction in the fraction of total Chl represented by the light-harvesting Chl *a*/*b*-protein (8%) which was equal to the reduction in the PSU size (8%). Futhermore, the total percentage reduction in PSU size (18% for the mesophyll plus 8% for the bundle sheath = 26%) and in the percentage reduction of total Chl contained in the light-harvesting protein (26%) is essentially the same as the percentage reduction in total leaf Chl content (27%). All of the Chl lost during drought stress can be attributed to loss of pigment in the light-harvesting Chl *a*/*b*-protein, consequences of which are elevated Chl *a*/*b* ratios and reduced PSU sizes in the two cell types. The observation that the ratio of the electron transport components of photosystem I (P700, Cyt *f* and Cyt b_6) remained constant (Table I) in both mesophyll and bundle sheath preparations of stressed and nonstressed tissues indicates that there has not been selective loss of photosystem I electron transport assemblages due to water stress. A previous study on severely desiccated *Fucus* (9) showed stability of P700 activity under low tissue water content.

The losses in the lamellar content of the light-harvesting Chl *a*/*b*-protein in response to water stress can be attributed either to greatly enhanced catabolism of the complex or to severe retardation of synthesis (assuming rapid turnover under normal growing conditions). Alberte *et al.* (3) showed that the synthesis of this component during greening was inhibited at least 50% under mild leaf water stress (−8 bars) conditions, and that the reduced rate of synthesis could account for the lag in Chl accumulation under these circumstances. It is likely that the lag in Chl accumulations seen after rewatering in the present study (until the leaf Ψ_w increased above −10 bars) (Fig. 1) is attributable to inhibi-

Table I. Characteristics of Drought Stressed and Non-stressed Maize Leaves and Chloroplast Lamellar Characteristics of Isolated Mesophyll and Bundle Sheath Cells of the Stressed and Non-stressed Tissues

	Control		Stress	
mg Chl/gm fresh wt	1.8		1.3	
Chl a/b Ratio	3.0		4.4	
Water Potential (Ψ_w)	−2 bars		−18 bars	
	Mesophyll	Bundle Sheath	Mesophyll	Bundle Sheath
Chl a/b Ratio	2.8	3.5	4.5	4.4
Total Chl/P700 (PSU)	365	315	300	290
Percentage of Total Chl				
Complex I	12	11	11	12
Complex II	53	45	35	37
Free Pigment	36	44	55	51
P700/cyt f/cyt b_6	1:1:2	1:1:2	1:1:2	1:1:2

tion of synthesis of this Chl-protein. Whether degradation of this membrane component is enhanced under water stress conditions remains to be examined.

The rate of regreening during recovery from water stress is remarkable (Fig. 1). Thus, both the metabolites as well as the energy required to synthesize Chl and carotenoid and membrane proteins and lipids must be readily available, despite the known reduction in synthetic ability, both metabolic and energetic, of chloroplasts and mitochondria in water-stressed tissue (13). It is therefore of value to understand the recovery process, since the recovery potential of a crop plant under field conditions may prove to be at least as important as its ability to survive water deficits.

CONCLUSIONS

It is clear from the present study that the majority of Chl lost from maize leaves subjected to drought stress is lost from the mesophyll cells. The reasons for this preferential loss could be attributed to the fact that the mesophyll cells are farther removed from the vascular supply of water than the bundle sheath cells, and hence develop greater cellular water deficits which lead to a greater loss of Chl. Alternatively, the mesophyll chloroplasts may be subject to greater Chl losses because they contain more of the light-harvesting Chl *a*/*b*-protein (complex II, Table I) which appears to be labile under even mild water stress conditions (3). It is equally possible that both situations contribute to the Chl loss. Attempts to explain some of the molecular consequences of water stress on chloroplasts in the present study and previously (3) have shown that the synthesis and perhaps the degradation of a specific membrane protein is highly sensitive to the water status of the tissue. Since this membrane component accounts for about 50% of the total Chl and 50% of the total protein of well washed chloroplast membranes (17), it represents a major component of the chloroplast which appears to be a rather specific target of water stress. Studies by De Silva and co-workers (8) have shown that loss of chloroplast membrane integrity under large water deficits ($\Psi_w = -15$ to 20 bars) is correlated to greatly enhanced activity of acid phosphatases localized on or near chloroplast membranes. Other studies (*cf.* 12) support these findings in that one of the most common and major consequences of tissue water deficits is destruction of intracellular membrane systems. Because the light-harvesting Chl *a*/*b*-protein is a major intrinsic membrane component, it is likely that losses in this component may lead not only to perturbation of the structural organization of the chloroplast membrane, but also to a reduction in the efficiency of the membrane-dependent electron transport of photosynthesis. The rapid loss and resynthesis of this component in response to tissue water status strengthens the view (2) that this Chl-protein is a very plastic component of the PSU. That this Chl-protein is rapidly catabolized under stress conditions suggests strongly that it may be a readily mobilized source of amino nitrogen for maintenance protein synthesis as well as a source of carbon skeletons for energy production during stress.

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LITERATURE CITED

1. Alberte, R. S. and J. P. Thornber. 1974. The correlation between chlorophyll *a*/*b* ratio and proportions of chlorophyll-protein complexes in green plants. Plant Physiol. 53: S-63.
2. Alberte, R. S. and J. P. Thornber. 1975. The plasticity of the major pigment-protein complex of photosynthetic membranes. Plant Physiol. 56: S-10.
3. Alberte, R. S., E. L. Fiscus, and A. W. Naylor. 1975. Effects of water stress on the development of the photosynthetic apparatus in greening leaves. Plant Physiol. 55: 317–321.
4. Alberte, R. S., J. D. Hesketh, G. Hofstra, J. P. Thornber, A. W. Naylor, R. L. Bernard, C. Brim, J. Endrizzi, and R. J. Kohel. 1974. Composition and activity of the photosynthetic apparatus in temperature-sensitive mutants of higher plants. Proc. Nat. Acad. Sci. U. S. A. 71: 2414–2418.
5. Alberte, R. S., J. P. Thornber, and A. W. Naylor. 1972. Time of appearance of photosystems I and II in chloroplasts of greening jack bean leaves. J. Exp. Bot. 23: 1060–1069.
6. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts: polyphenoloxidase in isolated chloroplasts. Plant Physiol. 24: 1–15.
7. Black, C. C., Jr. 1973. Photosynthetic carbon dioxide in relation to net CO_2 uptake. Ann. Rev. Plant Physiol. 24: 253–286.
8. De Silva, J. V., A. W. Naylor, and P. J. Kramer. 1974. Some ultrastructural and enzymatic effects of water stress in cotton (*Gossypium hirsutum* L.) leaves. Proc. Nat. Acad. Sci. U. S. A. 71: 3243–3247.
9. Fork, D. C. and T. Hiyama. 1973. The photochemical reactions of photosynthesis in an alga exposed to extreme conditions. Carneg. Inst. Yrbk. 72: 384–388.
10. Giles, K. L., M. F. Beardsell, and D. Cohen. 1974. Cellular and ultrastructural changes in mesophyll and bundle sheath cells of maize in response to water stress. Plant Physiol. 54: 208–212.
11. Hiyama, T. and B. Ke. 1972. Difference spectra and extinction coefficients of P700. Biochim. Biophys. Acta 267: 160–171.
12. Hsiao, T. C. 1973. Plant responses to water stress. Annu. Rev. Plant Physiol. 24: 519–570.
13. Kozlowski, T. T. Water Deficits and Plant Growth. Vol. I. Development, Control and Measurement (1968). Vol. II. Plant Water Consumption and Response (1968). Vol. III. Plant Response and Control of Water Balance (1972). Academic Press, New York.
14. Maksymowych, R. 1973. Analysis of Leaf Development. Cambridge University Press, London.
15. Shiozawa, J. A., R. S. Alberte, and J. P. Thornber. 1974. The P700-chlorophyll *a*-protein. Isolation and some characteristics of the complex in higher plants. Arch. Biochem. Biophys. 165: 388–397.
16. Thornber, J. P. 1969. Comparison of a chlorophyll *a*-protein isolated from a blue-green alga with chlorophyll-protein complexes isolated from green bacteria and higher plants. Biochim. Biophys. Acta 172: 230–241.
17. Thornber, J. P. 1975. Chlorophyll-proteins: light-harvesting and reaction center components of plants. Annu. Rev. Plant Physiol. 26: 127–158.
18. Thornber, J. P. and H. R. Highkin. 1974. Composition of the photosynthetic apparatus of normal barley leaves and a mutant lacking chlorophyll *b*. Eur. J. Biochem. 41: 109–116.
19. Woo, K. C., J. M. Anderson, N. K. Boardman, W. J. S. Downton, C. B. Osmond, and S. W. Thorne. 1970. Deficient photosystem II in agranal bundle sheath chloroplasts of C_4 plants. Proc. Nat. Acad. Sci. U. S. A. 67: 18–25.