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Changes in the Photosynthetic Apparatus of Maize in Response to Simulated Natural Temperature Fluctuations[1]

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JAMES A. TEERI, DAVID T. PATTERSON,[2] RANDALL S. ALBERTE,[3] AND RON M. CASTLEBERRY[4]
Barnes Laboratory, The University of Chicago, 5630 South Ingleside Avenue, Chicago, Illinois 60637

ABSTRACT

The response of the photosynthetic apparatus to low temperature periods differed among three hybrids of maize (*Zea mays* L.) grown in a phytotron. Light-saturated photosynthetic rates, leaf chlorophyll content, and mesophyll cell photosynthetic unit density all declined with increasing duration of low temperature. No single metabolic or physiological parameter appeared to control the response of the three hybrids to low temperature stress. Among all temperature treatments, net photosynthetic rate on a leaf area basis was more closely correlated with leaf chlorophyll content than with any other measured parameter. Final shoot dry weight was most highly correlated with stomatal conductance to CO_2.

Many species of higher plants exhibit depressed photosynthetic rates following exposure to low temperatures (10). Such decreases in photosynthesis are particularly pronounced in many C_4 species, especially in those of tropical origin. In maize, for example, Chl synthesis (19) and chloroplast development (22) are inhibited at low temperatures. In both field and laboratory studies the various deleterious effects of low temperature on C_4 species most commonly have been described at nonfreezing temperatures in the range of 2 to 12 C. Reduced leaf Chl content or inhibition of Chl synthesis has been observed at temperatures of about 16 to 20 C (4, 19, 22); the precise reasons for the associated decreased photosynthetic activity are not often clear, although reduced stomatal conductance can be a contributing factor. These physiological sensitivities of C_4 species to low temperature are supported by ecological evidence. In the Gramineae the geographic abundance of C_4 species is closely correlated with minimum daily temperatures of the growing season (23). Since it appears that the ability of plants to gain carbon during periods of suboptimal environmental conditions is closely related to the plasticity of the photosynthetic apparatus (20, 24), it is of value to define the nature of the acclimation at both the whole plant and molecular levels. In this respect it is known that there are dramatic changes in the size of PSUs[5] and the number of these energy-transducing sites per unit leaf area in response to both environmental (2, 7, 13) and genetic (4-6) modulation.

Previous experimental studies of the adaptability of the photosynthetic apparatus have usually compared leaf tissue or plants grown under relatively constant light (11, 13) or temperature (4, 10) conditions. Consequently, there is a paucity of information on the response of among-days variation in environmental factors which occur under natural conditions. Therefore, one of the goals of the present study was to examine the responses of CO_2 exchange properties of three genotypes of maize to natural among-day temperature variations. An attempt was made to define, at the molecular level, the consequences of different patterns of natural temperature fluctuation on the oranization of Chl into functional PSUs.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Plants of three commercial hybrids of maize (*Zea mays* L. cv. DK22, XL43, and XL95, supplied by DeKalb AgResearch, Inc.) were grown from seed in the controlled environment facilities of the Duke University Phytotron of the Southeastern Plant Environment Laboratories. The three hybrids are adapted to grow in different climates; hybrid DK22 is grown primarily in Canada, XL43 is a widely adapted hybrid grown in much of the corn belt, and XL95 is grown in the southeastern United States. The plants were grown in a substrate of 1 part gravel to 1 part vermiculite (v/v) under natural daylight with photoperiods extended to 16 hr with incandescent lamps. The pots were watered twice daily from germination through early growth, and three times daily by means of an automated watering system during the final 3 weeks of growth. Following day 15, nutrients were supplied during the daily watering periods with 0.5 strength Hoagland solution.

Eight replicates of each hybrid were grown in each of four temperature treatments (Table I) during the experiment. The daily temperature regimes consisted of an 8-hr high temperature period (800-1600 hr) followed by a 16-hr low temperature period. The high temperature period was timed so that the middle of the daily high temperature period coincided with the middle of the daily light period. At 41 and 42 days after planting, all plants were assayed for photosynthetic rates and other leaf physiological parameters (see below) and harvested. The four temperature treatments (Table I) included a control treatment (TI) which simulated an early growing season with no cold periods, and three treatments (TII-TIV) having different patterns and duration of low temperature superimposed on the control regime. In all low temperature treatments both the day and night temperatures were lowered to simulate natural diurnal amplitudes in these variables.

Photosynthesis Measurements. Leaf net photosynthetic rates were determined under high light intensities (1800-2000 μeinsteins m^{-2} sec^{-1} PAR) using a Beckman IR gas analyzer (model 865) and sample cuvette as described in Patterson *et al.* (21). All measurements were made at a leaf temperature of 31 C and a

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[2] Department of Botany, Duke University, Durham, North Carolina 27706. Present address: USDA-ARS, Southern Weed Science Laboratory, Stoneville, Mississippi 38776.

[3] Department of Biology, University of California, Los Angeles, California 90024. Present address: Department of Biology, Barnes Laboratory, The University of Chicago, Chicago, Illinois 60637.

[4] DeKalb AgResearch, Inc., DeKalb, Illinois 60115.

[5] Abbreviations: PSU: photosynthetic unit; T: treatment; PAR: photosynthetically active radiation.

TABLE I. Phytotron temperature regime for the four growth regimes

Day no.	Treatment number I	II	III	IV
1	23/11°C	23/11°C	23/11°C	23/11°C
18	"	17/5	17/5	17/5
22	"	"	23/11	"
26	29/17	29/17	29/17	"
37	"	"	17/5	"
41	"	"	29/17	29/17

CO_2 concentration of about 320 μl/l. The cuvette enclosed both the upper and lower surfaces of a 0.126 dm^2 segment of the leaf blade. Measurements of leaf diffusive resistance, CO_2 exchange rates, leaf Chl content, and composition of the photosynthetic apparatus were made on the same section of the most fully expanded leaf on the plant. At the time of assay (day 41 or 42) this was the 7th or 8th leaf of the plant. Following the above measurements, the plants were harvested and total shoot dry weight and leaf area were determined. Diffusive resistance for water vapor was measured for both adaxial and abaxial surfaces with a calibrated diffusive resistance porometer (Lambda Instruments, model LI-60). Total leaf diffusive resistance (r_{leaf}) was calculated from the porometer data as follows:

$$r_{leaf} = \frac{(r_{adaxial})(r_{abaxial})}{(r_{adaxial}) + (r_{abaxial})} \quad (1)$$

Following measurements of photosynthetic rates and diffusive resistance, a constant area section of the lamina was excised and homogenized in 80% acetone, and leaf Chl contents determined according to the methods of Arnon (9). The remaining portion of the leaf segment was then homogenized to release only mesophyll cells following procedures of Alberte *et al.* (2). Mesophyll chloroplast lamellae were isolated and solubilized in 1% Triton X-100 (5). Light-induced oxidation and dark reduction of P700 and Chl concentration were measured in the Triton extracts (5) and the PSU size determined from the ratio of total Chl to P700. The nmol of P700/unit leaf area were calculated to give the density of PSUs.

Linear regression equations and correlation coefficients (r) were calculated using photosynthetic rate/unit leaf area or final shoot weight as the dependent variable and stomatal conductance to CO_2, PSU density, Chl content/unit leaf area, or photosynthetic rate as the independent variable. Coefficients of determination (r^2) were calculated as indicators of the amount of variation in the dependent variable accounted for by the variation in the independent variable.

RESULTS

All three hybrids gained progressively less shoot dry weight from treatment I to treatment IV (Table II). In all treatments both shoot dry weight and total plant leaf area were greatest for the northern hybrid (DK22), intermediate for the midlatitude hybrid (XL43), and least for the southern hybrid (XL95). Plants of DK22 had progressively less leaf area from treatment I to treatment IV, while both XL43 and XL95 had an increase in leaf area in TII as compared to TI, with a progressive decrease in TIII and TIV.

In the three hybrids the photosynthetic rates of TII expressed on a leaf area basis (Table III) were equal to or greater than TI, the control. In all cases photosynthetic rates of TIII and TIV were much lower than controls (TI) or TII, with TIV rates always being the lowest. The differences in photosynthetic rates among hybrids for any given temperature treatment were small. In each of the hybrids there was a progressive decrease in leaf Chl content/dm^2 from TI to TIV with the most dramatic reduction occurring in TIII and TIV. There were no visually distinct chlorotic bands (15) produced in the leaves of any of the plants in response to any of the treatments.

The stress response differences among the three genotypes are most evident in the relationships of changes in photosynthetic rate (leaf area basis), leaf Chl content, PSU size and PSU density (Table III). For DK22, the decrease in photosynthetic rate in treatments II, III, and IV is accompanied by a decrease in leaf Chl content, which is closely paralleled by a decrease in the number of PSUs/unit leaf area, with very little change in PSU size. In XL43, between treatments I and II there is a relatively small change in leaf Chl content which is accompanied by a large increase in PSU size and a large decrease in PSU density. There is a large decrease in PSU size between treatments III and IV but very little change in photosynthetic rate (leaf area basis). In this case the photosynthetic rate is highly correlated with PSU density in these two treatments. Such a situation is to be anticipated when photosynthesis is measured under near saturating light intensities (7). The PSU size of XL43, the midlatitude line, was the most sensitive of the three hybrids to low temperature. As compared to the control treatment (TI), 8 days of low temperature (Table III) resulted in a 42% increase in PSU size of XL43, and 11% increase in XL95, and less than 1% increase in DK22.

In treatments III and IV, hybrid XL95 maintained similar PSU densities, with only a slight increase in PSU size in treatment IV. The leaf Chl content of XL95 was also similar in these two treatments. However, there was a relatively large (28%) decrease in photosynthetic rate in treatment IV as compared to treatment III. The decrease in photosynthetic rate is paralleled by a relatively large increase in total leaf diffusive resistance (Table II), which is probably the limiting or controlling variable for photosynthesis between these two treatments.

Considering all hybrids and all treatments, net photosynthetic rate/unit leaf area was most closely correlated with Chl content/unit leaf area; about 76% of the variation in photosynthetic rate could be accounted for by the variation in Chl content (Table IV). Variations in stomatal conductance and PSU density, respectively, could account for 69.5% and 65.0% of the variation in photosynthetic rate. Final shoot dry weight was correlated best with stomatal conductance to CO_2; there was significant correlation also with photosynthetic rate, Chl content/unit leaf area, and PSU density.

DISCUSSION

There appears to be no single metabolic or physiological parameter that controls the responses of the three tested maize

TABLE II. Response of leaf diffusive resistance, shoot dry weight and plant leaf area of three maize hybrids to four patterns of temperature fluctuation

Hybrid	Treatment number	Total leaf resistance sec/cm	Stomatal conductance to CO_2	Shoot dry wt g/plant	Plant leaf area cm^2/plant
DK22	I	1.6	.389	15.4	1752
	II	1.6	.389	11.3	1477
	III	1.8	.346	10.2	1097
	IV	4.9	.127	2.7	375
XL43	I	2.2	.283	10.1	1288
	II	1.8	.346	9.5	1361
	III	2.4	.260	8.3	980
	IV	5.2	.120	2.4	362
XL95	I	1.9	.328	9.1	1193
	II	1.5	.415	8.0	1241
	III	1.8	.346	6.8	851
	IV	3.4	.183	1.7	257

TABLE III. Effects of four patterns of temperature fluctuation on leaf photosynthetic rate, chlorophyll content, photosynthetic unit size, and photosynthetic unit density of three maize hybrids.

Hybrid	Treatment	Net photosynthetic rate mg CO_2 dm^{-2} hr^{-1}	Net photosynthetic rate mg CO_2 mg Chl hr^{-1}	Leaf chlorophyll content mg Chl dm^{-2}	PSU size[1] total Chl/P700	PSU density nmoles P700 dm^{-2}
DK22	I	59	11	5.3	280	21.3
	II	65	13	4.9	285	19.3
	III	43	15	2.8	290	10.9
	IV	31	12	2.6	305	9.6
XL43	I	55	10	5.5	230	27.2
	II	62	12	5.0	325	17.0
	III	36	9	3.8	350	12.2
	IV	33	12	2.8	375	11.3
XL95	I	62	12	5.3	250	23.9
	II	62	14	4.5	275	18.5
	III	43	14	3.0	345	9.8
	IV	31	10	3.1	360	9.6

[1]PSU size determined for mesophyll cells only.

TABLE IV. Correlation analysis of the measured physiological variables with net photosynthetic rate and shoot dry weight.

Dependent variable	Independent variable	r[a]	r^2[b] (%)
Net photosynthetic rate (area basis)	mg Chl/dm^2	.874	76.4
"	Stomatal conductance to CO_2	.833	69.5
"	PSU/dm^2	.806	65.0
Shoot dry weight	Stomatal conductance to CO_2	.822	67.6
"	Net photosynthetic rate (area basis)	.763	58.2
"	mg Chl/dm^2	.730	53.3
"	PSU/dm^2	.657	43.2

[a]All r values significant at 0.05 level.

[b]r^2 = coefficient of determination.

genotypes to low temperature stress. It is clear that the relationships among the potentially rate-limiting parameters of photosynthesis can and do change during stress treatments (3, 17). The nature and degree of change are determined by the specific kind and duration of environmental treatment, and the genetic constitution of the test plant. Different metabolic processes within a leaf are known to have widely differing abilities to recover their activity following exposure to low temperature (10). Although over-all differences in dry matter production were correlated with differences in photosynthetic rate, the differences observed in net photosynthetic rate of a single leaf were not correlated with among-genotypes differences in shoot dry weight within any of the treatments. This was true regardless of whether the photosynthetic rate was expressed on a leaf area or Chl basis. The lack of correlation may be due to the fact that single leaf measurement under one set of conditions is not a good estimate of whole plant net photosynthesis under all conditions of the experiment. Or, it could be because metabolic events other than net photosynthesis are controlling the translation of photosynthate into plant growth (17). Evans (14) and Wareing and Patrick (25) have pointed out the importance of translocation and distribution of assimilates in considerations of growth. A previous study on grass subjected to low night temperatures showed that starch was not mobilized but continued to accumulate to such an extent that structural damage to the chloroplast resulted (18).

Under the standard environmental conditions there were no large differences in total leaf diffusive resistance among treatments I, II, and III for any of the genotypes. The data suggest that either 8 cool days early in development, or 4 cool days early followed by 4 cool days late in development were not sufficient to induce changes in the ability of the stomatal apparatus to recover subsequently to the warm control regime. However, all three hybrids from treatment IV had significantly higher total leaf resistances under the standard measurement conditions. This suggests that a long period (23 days) of cool temperature has reduced the ability of the stomatal apparatus to respond quickly upon return to a warmer environment. In TIV, if total r_{leaf} is the primary limiting variable, then the smaller r_{leaf} in XL95 may represent a greater level of stress that is not compensated for in other ways. This could explain the resulting greater reduction in photosynthetic rate relative to r_{leaf} in XL95 than in the other two genotypes under the most extreme low temperature regime.

The relationship between photosynthetic rate and the density of PSUs/unit leaf area in all three genotypes is in agreement with previous findings on barley (6), peanut (5), and conifers (7). In particular, the southern and northern genotypes (XL95 and DK22, respectively) show a reduced photosynthetic rate which is accompanied by a reduction in leaf Chl and a reduction in the number of energy-transducing sites (PSUs). It is likely that the majority of Chl lost in response to low temperature in these maize lines can be attributed to the decrease in the number of PSUs, and hence a reduction in the availability of ATP and reducing power for carbon metabolism. The northern genotype shows a very stable PSU size in the different temperature regimes, while the midrange and southern genotypes show progressive increases in PSU size with increased periods of low temperature. This suggests that PSU size is very plastic in these two genotypes and is extremely responsive to environmental modulation. The increased PSU size and consequent increase in light-harvesting Chl, shown to be principally attributable to an increase in the light-harvesting Chl *a*/*b*-protein (8, 13), may perform a protein storage function in addition to its role in photosynthesis to meet the future changing energy and amino-nitrogen needs of the plant (7). Such an adaptive mechanism may provide these plants with one means of compensating for stress without severely limiting photosynthetic potential. It is

important to note that the PSU data provided here are for mesophyll cells only. Inasmuch as the mesophyll chloroplasts constitute about 80% of the total leaf Chl (16), and that in response to another stress (water stress), it is mesophyll chloroplasts which are by far the most responsive (2), we feel that changes in the lamellar characteristics of maize mesophyll are generally reflective of changes of the whole leaf at least in terms of the parameters measured (*cf.* 22). In addition the PSU density is calculated only from mesophyll chloroplast preparations, thus the values given are minimum values. If none of the treatments significantly influences the number of PSUs in the bundle sheath portion, the relative difference among the genotypes and treatments would remain the same. If some of the treatments affected PSU density in the bundle sheath of any of the genotypes, this most likely would enhance the temperature and genetic differences. These environmental simulations did not include any periods of water stress; however, it is known (1, 2, 12) that periods of moderate water stress greatly influence several chloroplast and photosynthetic parameters.

It is evident that a single variable such as temperature can have dramatic and increasing effects on plant metabolism over the range of that stress likely to occur in nature. Similarly, it is clear that different genotypes of a single species can have very different responses to such a variable, depending on when during its development the stress is imposed. Studies designed to examine the interaction of environmental factors using controlled environments are needed and should provide significant insight into the influence on plant growth of natural among-day environmental variations typical of natural and agricultural ecosystems.

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