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Optimal Thermal Environments for Plant Metabolic Processes (*Cucumis sativus* L.)

Light-Harvesting Chlorophyll *a/b* Pigment-Protein Complex of Photosystem II and Seedling Establishment in Cucumber

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Analysis of the temperatures providing maximal photosystem II fluorescence reappearance following illumination and thermal kinetic windows (TKWs), obtained from the temperature characteristics of enzyme apparent K_m values, have been proposed as indicators of the bounds of thermal stress in plants. In this study, we have evaluated the temperature optimum for the accumulation of the chlorophyll *a/b* light-harvesting complex of photosystem II (LHCP II), its mRNA, and the mRNA of the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in cucumber (*Cucumis sativus* L. cv Ashley) as a broader measure of metabolism than that provided by either the fluorescence reappearance or TKWs. The TKW for cucumber is between 23.5 and 39°C, with the minimum apparent K_m occurring at 32.5°C. The photosystem II variable fluorescence reappearance following illumination was maximal between 30 and 35°C. Maximum synthesis of the LHCP II occurred at 30°C. The light-induced accumulation of the LHCP II and the small subunit of Rubisco mRNAs showed similar temperature characteristics. Suboptimal temperatures delayed germination, altered cotyledonary soluble sugar content, and broadened the temperature range for chlorophyll accumulation. These results demonstrate an effect of seed reserve mobilization on the range of temperatures for chlorophyll accumulation, and suggest that metabolic temperature characteristics may be broadened by increasing available substrates for enzyme utilization. This study provides new information about the relationship between TKWs and cellular responses to temperature. In addition, the results suggest that the temperature range outside of which plants experience temperature stress is narrower than traditionally supposed.

Investigations of metabolic responses to temperature have identified a relationship between the average habitat temperature of native plant species and the apparent K_m of malate dehydrogenase and Glc-6-P dehydrogenase (Teeri and Peet, 1978; Simon, 1979; Teeri, 1980). An examination of populations from a cool environment exhibited a minimum apparent K_m at lower assay temperatures than plants from a warmer environment (Teeri and Peet, 1978; Simon, 1979; Teeri, 1980). Unlike native species, many crops have been moved from their native environments into environments with distinctly different temperature and rainfall patterns. Cultivation of these crops has been influenced more by insect, disease, and economic concerns than by an optimization of plant metabolism with the environment.

A concept of thermal stress in plants has been developed that links the biochemical characteristics of a plant with its optimal temperature range. The TKW concept uses the temperature characteristics of K_m to define the temperature range outside of which plants experience thermal stress. These measures are used as indicators of metabolic efficiency, not as absolute measures of it. The TKW has been shown to be a reliable indicator of the temperature range outside of which plants experience temperature stress and reduced plant performance (Burke et al., 1988; Burke, 1990; Mahan et al., 1990; Ferguson et al., 1991). TKWs have been identified for several crop species (Burke et al., 1988; Burke, 1990; Mahan et al., 1990; Ferguson et al., 1991), and have proven to be narrower than plant temperatures experienced on a seasonal or daily basis (Burke et al., 1988; Ferguson et al., 1991). Temperature extremes (for example, heat shock and chilling injury) are not required for a plant to experience thermal stress.

A second method for identifying optimal plant temperatures was developed in response to concerns raised about identifying optimum temperatures from the in vitro characteristics of a few enzymes (namely, the TKW). Comparison of the thermal response of PSII fluorescence reappearance with the temperature sensitivity of the apparent K_m of hydroxypyruvate reductase for NADH showed that temperatures providing maximal fluorescence reappearance corresponded with temperatures providing the minimum apparent K_m values (Burke, 1990; Ferguson et al., 1991). The fluorescence technique not only provided a useful tool for the analysis of plant temperature optima, but also strengthened the TKW concept.

Although similar temperature characteristics were identified for the TKW and the recovery of PSII variable fluorescence, the relationship between the temperatures defining the TKW and the temperature response of the myriad of

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Abbreviations: CAB, Chl *a/b* binding protein; DAP, days after planting; F_o, initial fluorescence; F_v, variable fluorescence; LHCP II, light-harvesting Chl *a/b* pigment-protein complex of PSII; SSU, small subunit of Rubisco; TKW, thermal kinetic window.

enzymes essential for metabolic coordination within cells remains unclear. The present study was designed to investigate the significance of the TKW concept at the cellular level. It compared the temperature sensitivity of the synthesis of the LHCP II with the temperature sensitivity of the reappearance of PSII F_v following illumination and the TKW. LHCP II comprises about half of the protein and pigment content of thylakoid membranes and functions as a light-harvesting antenna (Zuber, 1987). The LHCP II proteins are encoded by nuclear genes (Coruzzi et al., 1983; Dunsmuir et al., 1983; Lamppa et al., 1985), which are organized into gene families (Zuber, 1987). The precursor forms of LHCP II are synthesized in the cytoplasm and transported into the chloroplast where, following processing, the polypeptide is incorporated into the thylakoid membrane. Thus, this study of LHCP II synthesis encompasses several key processes in cellular function (transcription, translation, transport processes, and metabolite deposition), and as such can act as a comprehensive measure of the temperature characteristics of general plant metabolism. The temperature sensitivity of the synthesis of the LHCP II also allowed us to evaluate the effect of low temperature stress upon seedling establishment during germination by measuring the temperature responses of Chl accumulation on subsequent illumination. This offers a practical use of the concept of TKW in establishing the temperature range outside of which plants experience temperature stress and the consequences that create an impact on important agronomic factors when these limits are exceeded.

MATERIALS AND METHODS

Plant Material

In initial studies, cucumber (*Cucumis sativus* L. cv Ashley) seeds were planted in vermiculite and grown in the dark at 32.5°C. The thermal dependence of the synthesis of the LHCP II was measured in cotyledons 7 DAP. Cotyledons excised from etiolated seedlings were placed onto moistened 3 MM filter paper, positioned on 2- × 2.5-inch temperature blocks, and covered with Glad ClingWrap[1] (a CO_2-permeable polyethylene wrap). Eight temperature blocks were used, ranging from 10 to 45°C in 5°C increments. The cotyledon temperatures were within ±0.5°C of the set temperatures during the light treatment. Cotyledons were exposed to light levels of 300 μmol m^{-2} s^{-1} for 24 h. Chl determinations were made according to the procedure of Arnon (1949) following the 24-h illumination period. Temperature stress responses were evaluated in cucumber seedlings grown in dark incubators at 15, 20, 25, and 30°C. Cotyledons were excised from the seedlings at 2-d intervals from 2 to 14 DAP, placed on the temperature blocks, and exposed to light as described above.

Enzyme isolation and fluorescence analyses were performed on cucumber seedlings grown in flats containing vermiculite. The flats were placed in a greenhouse following sowing and were watered twice daily with an automatic misting system. Seedlings were grown for 12 to 14 d before enzyme or fluorescence analyses. Cucumbers used in the evaluation of the effects of available substrates on the temperature dependence of Chl accumulation were planted in moistened vermiculite and grown for 4 d at 32.5°C in the dark. One cotyledon was excised at d 4, and the plants were grown for an additional 5 d in the dark at 32.5°C. The temperature dependence of Chl accumulation was evaluated as described above in cotyledons from control (two cotyledons) and test seedlings (one cotyledon).

To determine the effect of variable soil water potential on the temperature sensitivity of Chl accumulation, cucumber seeds were sown into vermiculite moistened with different amounts of water to provide soil water potentials ranging from −0.01 to −0.37 MPa (Creelman et al., 1990). The seedlings were grown for 6 d at 25°C in the dark without additional water prior to illumination.

Determination of the TKW

The temperature response of the apparent K_m for NADH of NADH-hydroxypyruvate reductase from greenhouse-grown cucumber seedlings was determined with enzyme purified according to the procedure of Titus et al. (1983). TKWs were determined according to the procedure of Burke (1990).

Fluorescence Analysis

Fluorescence measurements were made according to the procedure of Peeler and Naylor (1988) with minor changes (Burke, 1990). Cotyledons from greenhouse-grown seedlings were placed on moistened 3 MM filter paper sitting on a wet sponge in a glass dish. The cotyledons were covered with transparent plastic film (Glad ClingWrap). A high-pressure sodium lamp emitting a light intensity of 650 μmol m^{-2} s^{-1} was used to illuminate the cotyledons before moving them to the dark. A 1-min illumination time was used initially; however, the length of illumination was adjusted if the F_v/F_o ratios taken immediately after placing the leaves in the dark were greater than 0.15. A constant illumination time was used for all treatments within an experiment. Following illumination, the cotyledons were transferred to eight temperature-controlled blocks preset to temperatures ranging from 10 to 45°C in 5°C increments. Cotyledon temperatures determined from thin-wire thermocouples were within ±0.5°C of the temperature set points. Fluorescence transients were obtained at 5-min intervals on each cotyledon with a Brancker SF-30 fluorometer (Richard Brancker Research, Ottawa, Canada) and were measured over a 10-s excitation period with a light intensity of 22 μmol m^{-2} s^{-1}. Each F_v reappearance curve was replicated three times. Results were expressed as the ratio F_v/F_o.

SDS-PAGE and Western Blot Analyses

Cotyledon proteins were separated by SDS-PAGE according to the procedure of Laemmli (1970) on 12 to 16% polyacrylamide gels with 5% polyacrylamide stacking gels. LHCP

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

II was identified by the western blot technique described by Mahan and Burke (1987). LHCP-II antiserum (immunoglobulin G) prepared as described in Mathis and Burkey (1987) was kindly provided by Dr. Kent Burkey (Raleigh, NC).

RNA Extraction and Analysis

RNA from cucumber cotyledons was isolated by a series of phenol extractions (Lane and Tumaitis-Kennedy, 1981). Total RNA was resolved by electrophoresis through a 1.2% agarose gel containing formaldehyde as described in Ausubel et al. (1989). The RNA was transferred onto Duralon-UV nylon membranes (Stratagene) using the Posiblot Pressure Blotter system (Stratagene) as detailed by the manufacturer. RNA was cross-linked to the nylon membranes by exposure to 120,000 μJ/cm^2 of UV radiation in a model 1800 Stratalinker (Stratagene).

The probe for CAB RNA was a cDNA fragment isolated by *Pst*I digestion of the plasmid pAB96 (Broglie et al., 1981), a kind gift from Dr. Nam-Hai Chua (The Rockefeller University). The cDNA was radiolabeled with [^{32}P]CTP by nick translation with a kit and protocol supplied by BRL. Hybridization was achieved in a solution containing 25 mM potassium phosphate at pH 7.4, 5× SSC, 5× Denhardt's solution, 40% formamide, and 50 μg/mL of sonicated salmon sperm DNA at 37°C overnight. Membranes were washed in 0.1× SSC, 0.1% SDS at 42°C prior to autoradiography.

The probe for SSU was a partial fragment of the cDNA clone rbcS-1 from *Mesembryanthemum* (De Rocher et al., 1991), received as a kind gift from Dr. Hans Bohnert (University of Arizona). The fragment was isolated by *Eco*RI digestion, labeled, and used under the hybridization conditions described above for CAB cDNA.

Loading inconsistences that may affect the analysis of RNA levels by northern blotting were detected by hybridization of the northern blots with a cDNA probe specific for plant 28S ribosomal RNA. Values obtained from this hybridization were used to normalize the data obtained from the use of the CAB and SSU probes. The ribosomal RNA probe used was a 350-bp cDNA (*Eco*RI released) derived from the 28S ribosomal RNA of jojoba, a kind gift from Dr. J. Velten (New Mexico State University). Labeling and hybridization conditions were as described above with the exception that washing of the northern blot was carried out at 50°C.

Integrated Intensity

Western blots stained via horseradish peroxidase and autoradiograms of northern blots were analyzed using a Bio Image Visage 2000 (Bio Image Products, Milligen/Biosearch Division of Millipore, Ann Arbor, MI) computer-aided image analysis system. Images were scanned and captured, and individual patterns were quantified using a comparative log software program. The results are reported as total integrated intensity in each band.

Soluble Sugar Determinations

The content of soluble sugars within etiolated cotyledons of seedlings grown at either 20 or 32.5°C were determined from ethanol extracts obtained according to the procedure of Rufty and Huber (1983). Extracts were obtained from cotyledons soaked in hot 80% ethanol. Following removal of the ethanol, the extracts (20 μL) were incubated with invertase (20 μL of a 1.5-mg/mL solution in 0.1 M sodium citrate, pH 6.0) for 30 min at 37°C. One hundred microliters of color reagent (Sigma 115A Glucose Assay Kit) were added to the sample and incubated at 37°C for 10 min, and Glc levels were determined at 490 nm on a Dynatech MR5000 Microtiter Plate reader.

RESULTS AND DISCUSSION

TKW Determination

In the present study, the TKW of cucumber was determined by the analysis of the temperature sensitivity of the apparent K_m of NADH-hydroxypyruvate reductase for NADH (Fig. 1). Analysis of the apparent K_m at 5°C intervals from 20 to 40°C showed a gradual decline in apparent K_m from 20 to 32.5°C. Apparent K_m values increased for temperatures above 32.5°C. Using the criterion of 200% of the minimum K_m for determining the TKW (Burke et al., 1988) resulted in a TKW encompassing temperatures from 23.5 to 39°C with a minimum apparent K_m value at 32.5°C. These results differ from those reported previously for cucumber NADPH-glutathione reductase in that the range of temperatures delineating the TKW is from 23.5 to 39°C rather than from 35 to 41°C (Mahan et al., 1990). The upper limits of the TKW in the two studies are similar; however, the lower limits differ by more than 10°C. A possible explanation for this difference is that NADH-hydroxypyruvate reductase and NADPH-glutathione reductase may have unique temperature characteristics within a plant. Alternatively, because the minimum apparent K_m values for NADPH-glutathione reductase are in the 1- to 2-μM range, even a small error in determination would have a significant impact on the TKW. If the reported 40°C apparent K_m of the NADPH-glutathione reductase (Mahan et al., 1990) was increased by as little as 1 μM, the TKW would change from 35 to 41°C to a range of 20 to 42°C. This TKW

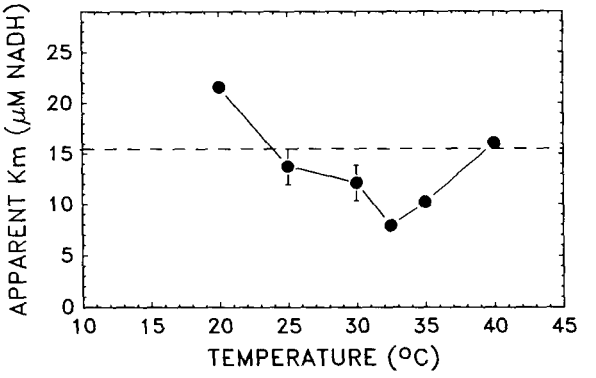

Figure 1. Temperature sensitivity of the apparent K_m for NADH of NADH-hydroxypyruvate reductase isolated from cucumber cotyledons. The dashed line indicates the upper limit of the values of the apparent K_m that were used in the determination of the TKW. SE bars are included when larger than the symbols.

for NADPH-glutathione reductase would be similar to that identified from NADH-hydroxypyruvate reductase in the present study. Because of the observed TKW differences, we evaluated the reappearance of PSII F_v following illumination to determine which temperatures were optimal.

Fluorescence Analyses

A strong correlation between the temperatures delimiting the TKWs of wheat, cotton, bell pepper, and petunia and the thermal dependence of the reappearance of the variable component of PSII fluorescence in the dark following illumination has been reported previously (Burke, 1990; Ferguson et al., 1991). Because of the difference in TKW between that determined for NADH-hydroxypyruvate reductase in the present study and that reported previously for NADPH-glutathione reductase (Mahan et al., 1990), we have used the fluorescence technique to further establish the bounds of the temperature optimum of cucumber.

Analysis of the recovery of PSII F_v following illumination in cucumber showed the highest F_v levels at 30 and 35°C (Fig. 2). The rate of F_v recovery increased as temperatures increased from 10 to 30°C and declined rapidly at temperatures above 35°C. In these experiments, both the magnitude and the rate of the reappearance of PSII F_v following illumination are used to determine the optimum plant temperatures (Burke, 1990). The temperature characteristics of the fluorescence reappearance in cucumber correlate closely with the TKW determined for NADH-hydroxypyruvate reductase in the present study. This finding demonstrates the usefulness of having more than one method to determine plant temperature optima.

LHCP II

The effects of pre-illumination temperatures, soil water deficits, and reduction in available seed reserves on the temperature sensitivity of Chl accumulation were evaluated in this study. The accumulation of Chl content and LHCP II accumulation were determined with monospecific antibodies to LHCP II polypeptides (Mathis and Burkey, 1987) following transfer to continuous illumination. In initial studies, cucumbers were grown at 32.5°C in the dark for 7 to 9 d prior to exposure to continuous illumination over the experimental range of temperatures. The 32.5°C chamber temperature was chosen because it was the temperature providing the lowest apparent K_m for NADH hydroxypyruvate reductase and was the midpoint between the two temperatures providing the maximum fluorescence reappearance following illumination.

The maximum accumulation of LHCP II in these cotyledons based on monospecific antibody staining was within the TKW (Fig. 3, top). Accumulation was reduced at both lower and higher temperatures with little or no synthesis of the complex at temperature extremes. Chl accumulation was maximum within the TKW (Fig. 3, middle) and, as expected, the relative Chl accumulation levels matched the intensity of antibody staining for LHCP II (Fig. 3, bottom). Because of the relationship between LHCP II content and Chl content, subsequent studies monitored only Chl accumulation in response to temperature changes.

To gain an insight into the effect of temperature on the control and coordination of the synthesis of LHCP II, the level of accumulation of the LHCP II mRNA in the illuminated cotyledons was determined across a range of temperatures (Fig. 4, top). Maximum accumulation of the LHCP II mRNA, determined by normalized probe signal strength, occurred in the cotyledons that were illuminated at 30°C. The accumulation was reduced at both lower and higher temperatures, with little or no accumulation of LHCP II mRNA at temperature extremes. To determine if this response was unique to the LHCP II mRNA accumulation or represented an accumulation pattern of other light-responsive systems, the temperature sensitivity of the accumulation of the mRNA for SSU was evaluated (Fig. 4, middle). SSU mRNA accumulation exhibited a similar temperature pattern to that of the LHCP II mRNA. Although not identical, the pattern of mRNA accumulation revealed by evaluating normalized integrated optical densities showed that maximal mRNA accumulation occurred within the TKW for cucumber (Fig. 4, bottom). In light of the marked impact of temperature on LHCP II mRNA and SSU mRNA accumulation, it is interesting to ponder what stage(s) of transcriptional or post-transcriptional events is routinely depressed by suboptimal and supraoptimal temperatures.

Temperature Characteristics of Chl Accumulation

The validation of TKW estimations by the study of the thermal characteristics of LHCP II synthesis and function offered a unique opportunity to extend the concept of TKW to establish the effect of temperature changes on an important agronomic factor, viz. germination and seedling establishment. This opportunity was afforded by the ability to follow LHCP II function by the simple means of observing the rate and magnitude of the greening process in cucumber cotyledons. Numerous studies have evaluated the developmental patterns of Chl accumulation in cucumber cotyledons and the interaction of growth regulators on the greening processes

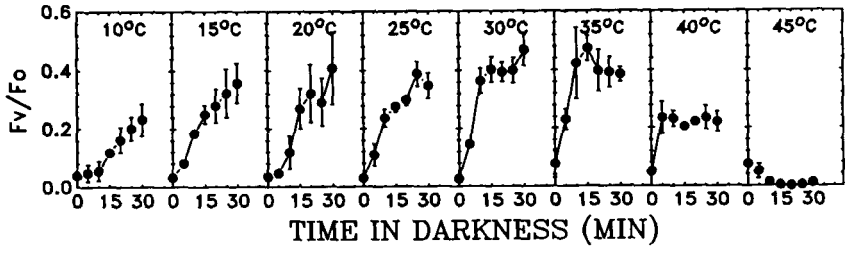

Figure 2. The temperature sensitivity of the reappearance of F_v (expressed as the ratio of F_v/F_o) in the cotyledons of cucumber. SE bars are included when larger than the symbols.

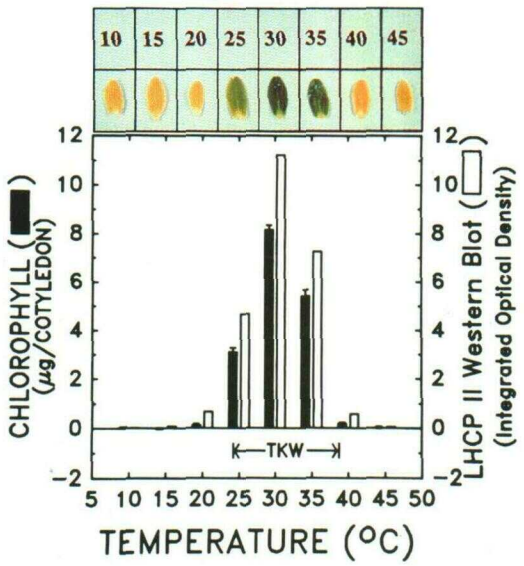

Figure 3. Temperature characteristics of the accumulation of the light-harvesting Chl *a/b* protein of PSII and Chl in etiolated cucumber cotyledons following 24 h of continuous illumination at different incubation temperatures. Top, LHCP II antiserum staining. Middle, Chl accumulation in isolated cucumber cotyledons. Bottom, A graph quantifying the accumulation of both LHCP II and Chl.

(Hardy et al., 1970; Moore et al., 1972; Choen and Arzee, 1984; Dei, 1984; Moran et al., 1990); however, the affect of different thermal environments on the greening process has not been evaluated fully. Because at the time of planting and germination low temperatures are more likely to be detrimental to seedling establishment, we concentrated on the effect of suboptimal temperatures (15, 20, and 25°C) on the temperature sensitivity of Chl accumulation on subsequent exposure to continuous illumination when compared with the same process occurring at 30°C (Fig. 5). Cucumbers germinated and grown in the dark at 15°C showed no Chl accumulation in cotyledons during the first 8 DAP when transferred to continuous illumination (Fig. 5A). Cotyledons from seedlings 10 DAP accumulated Chl at 25 and 30°C, and cotyledons 12 and 14 DAP accumulated Chl from 25 to 35°C. Cucumbers germinated and grown in the dark at 20°C showed no Chl accumulation in cotyledons during the first 6 DAP when transferred to continuous illumination (Fig. 5B). Cotyledons from seedlings 8 DAP accumulated Chl from 30 to 40°C, whereas cotyledons from seedlings 10 to 14 DAP accumulated Chl from 20 to 40°C. Cucumbers grown at 25°C prior to illumination accumulated Chl at 6 DAP (Fig. 5C). Chl accumulation occurred from 20 to 40°C; however, most accumulation occurred between 25 and 40°C. A similar temperature response was observed from 6 to 14 DAP in the seedlings grown at 25°C. Finally, cucumbers grown at 30°C exhibited Chl accumulation at 4 DAP (Fig. 5D). Chl accumulation occurred between 30 and 40°C, and no significant Chl accumulation occurred between 10 and 14 DAP.

The observation that with each 5°C increase in temperature from 15°C there is a 2-d reduction in the time after planting before Chl accumulation becomes evident suggested that perhaps there was an impact of low temperatures on the movement of water through the seed coat and that this may cause the delay in greening. To investigate this further, comparisons of the timing of Chl accumulation between control seeds, seeds with scarified seed coats, and seeds with the seed coat removed were made. Seedlings were germinated and grown for 6 d under two temperature regimens (15 and 25°C) prior to illumination at the temperatures spanning the experimental range. Following a 24-h light exposure beginning on d 6, no Chl accumulation was observed at any temperature in the cotyledons from the 15°C-grown seedlings. Chl accumulation in the cotyledons from the 25°C-grown seedlings was identical to that shown for 6 DAP in Figure 5C. The presence or absence of the seed coat did not alter the temperature range for Chl accumulation and did not alter the delay in accumulation observed with decreasing temperatures. These results suggest that the delay in cotyledon enlargement associated with water uptake may be related to the enzymic mobilization of nonosmotic reserves in sugars rather that the presence of a physical barrier.

It was also observed that there are differences in cotyledon expansion during growth in the dark depending on the particular temperature treatment. The cotyledons from the

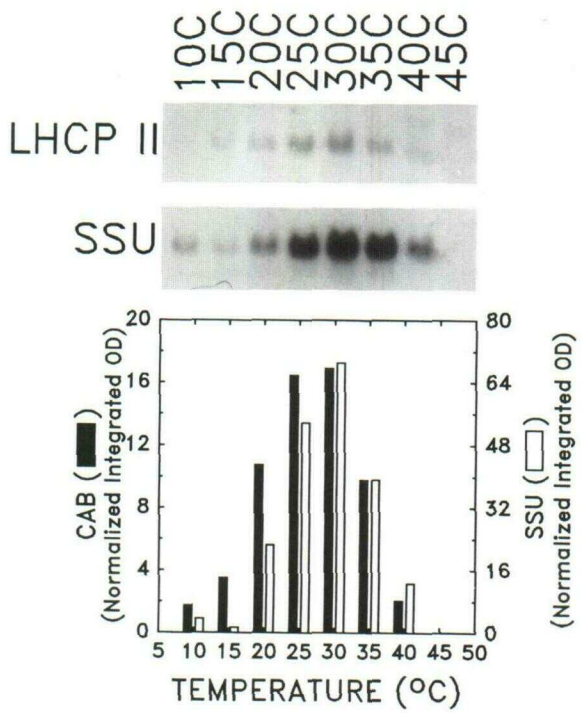

Figure 4. Temperature characteristics of the accumulation of LHCP II and SSU mRNA in etiolated cucumber cotyledons following a 24-h continuous illumination at different incubation temperatures. Accumulation patterns were quantified following normalization for loading inconsistencies by adjustment for differences in the hybridization of the northern blots for plant 28S ribosomal RNA densities. Top and middle, Northern analyses of LHCP II and SSU, respectively. Bottom, A graph quantifying the accumulation of LHCP II and SSU.

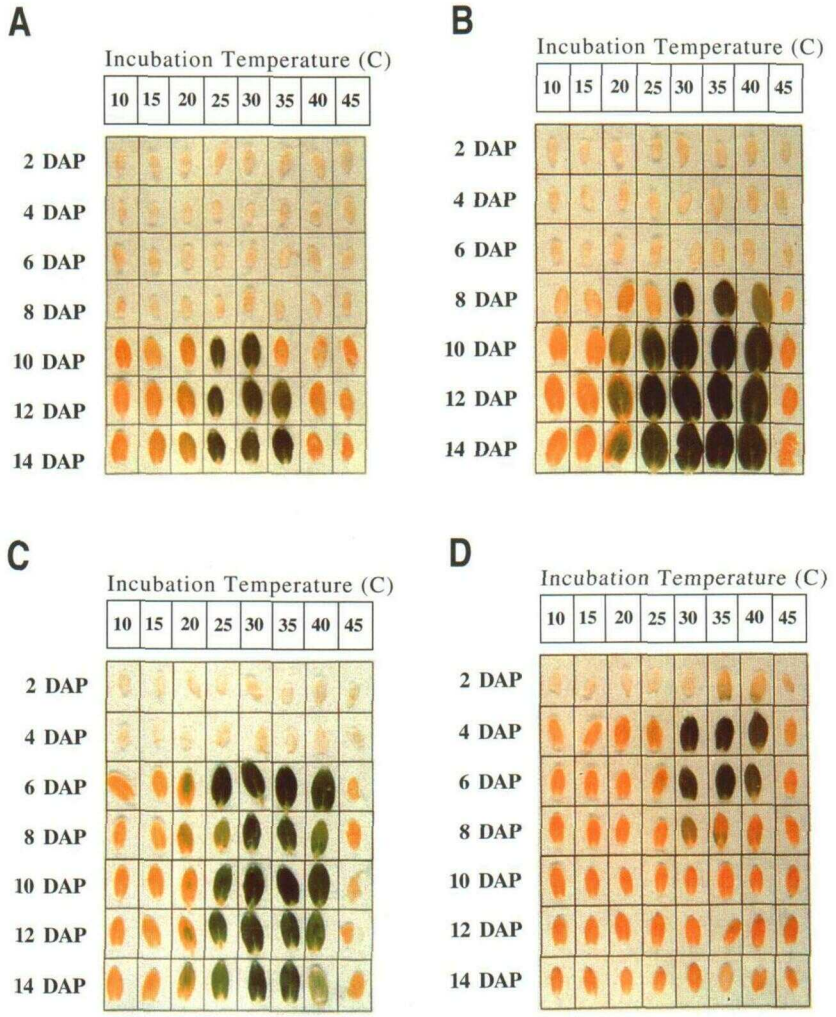

Figure 5. The effect of prior seedling growth temperatures on the sensitivity of Chl accumulation in cucumber cotyledons. Temperatures ranging from 10 to 45°C at 5°C intervals were evaluated from 2 to 14 DAP at 2-d intervals. Seedling growth temperatures of 15°C (A), 20°C (B), 25°C (C), and 30°C (D) were evaluated in this study.

20°C seedlings had enlarged more than those from any of the other treatments (see Fig. 5). These cotyledons also exhibited the broadest temperature range for Chl accumulation. This led us to hypothesize that there existed a greater amount of osmoticum, probably primarily as soluble sugars, within the cotyledonous tissues. This was confirmed by the analysis of soluble sugars (Glc and Suc), which revealed elevated sugar levels in the cotyledons from dark-grown seedlings at 20°C compared with the sugar levels in the cotyledons of the seedlings grown at 32.5°C (Fig. 6). Presumably, the 32.5°C plants use the mobilized reserves more rapidly than the 20°C seedlings, as exemplified by the reduced seedling sizes in the 20°C treatment compared with the 32.5°C seedlings. It appears that the conversion of lipids to sugars in the 20°C cotyledons occurs at a greater rate than the utilization of these sugars by the rest of the plant. On the other hand, the 32.5°C seedlings use these sugars more rapidly.

The possibility that the presence of elevated sugar levels within the cotyledons of the 20°C seedlings was associated with the observed broadening of the temperature response of Chl accumulation suggested that changing the availability of substrate pools might alter the temperature response of metabolic processes within the plant. To test this hypothesis, cucumber seedlings were grown for 4 d until seedling emergence, one of the two cotyledons was removed, and the seedlings containing one cotyledon were grown for an additional 3 to 5 d prior to illumination. This treatment reduced the availability of stored reserves because seedling growth after cotyledon removal was now dependent upon supplies from one cotyledon instead of two.

The temperature sensitivity of Chl accumulation in cotyledons from the control and single-cotyledon plants is shown in Figure 7. A differential Chl accumulation pattern was observed at 7 DAP with a drop-off in accumulation at 25 and 40°C in the single-cotyledon plants. This difference in Chl accumulation was accentuated at 8 DAP, and at 9 DAP no Chl accumulation occurred in the single-cotyledon plants. These data support the hypothesis that the availability of substrate pools has an impact on the range of temperatures in which specific metabolic processes occur. Modulation of available substrates should significantly affect the range of metabolically functional environmental temperatures. An everyday example of a problem related to these findings is the problem associated with seedling emergence through soil crusts. Commonly, rains following planting can cause soils to form a crust that restricts seedling emergence. In these

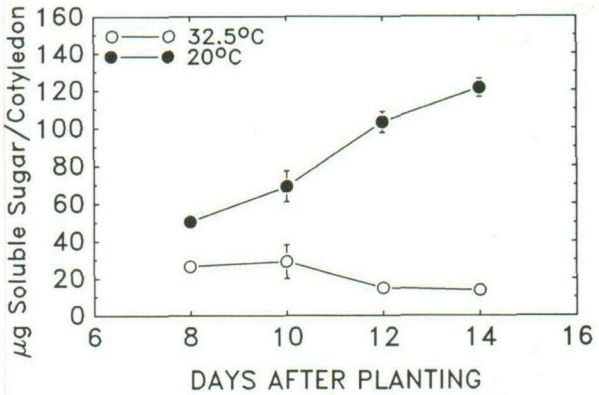

Figure 6. Soluble sugar (Glc and Suc) content within etiolated cucumber cotyledons from seedlings grown at either 20 or 32.5°C. SE bars are included when larger than the symbols.

cases, soil temperatures often fall within the optimal range of the seedling so that mobilization of seedling reserves occurs at a relatively fast rate. Because of the increased turgor required by the seedling to break through the crust, much of the seed reserves are transported to the hypocotyls to provide the osmoticum required for the needed turgor. As a result, a smaller portion of the seed reserves are available upon emergence through the crust for use in production of the photosynthetic machinery. From the present study, it is clear that

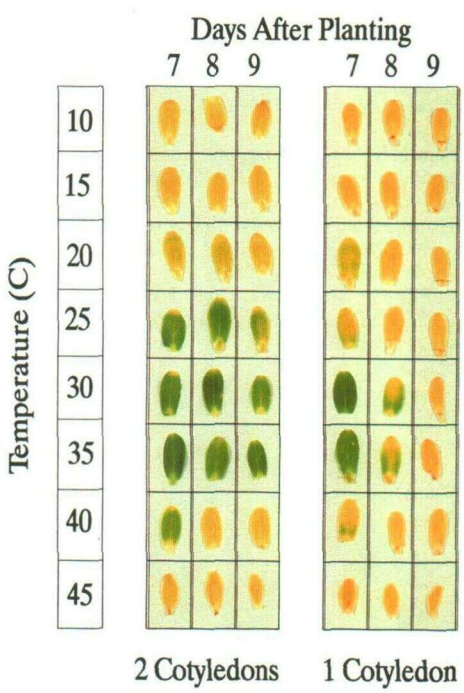

Figure 7. The effect of the availability of seed reserves on the temperature sensitivity of the accumulation of Chl in cucumber cotyledons. Temperatures ranging from 10 to 45°C at 5°C intervals were evaluated at 7, 8, and 9 DAP on cotyledons from control (two-cotyledon seedlings) and on the cotyledon remaining after excision of one cotyledon from seedlings at 4 DAP.

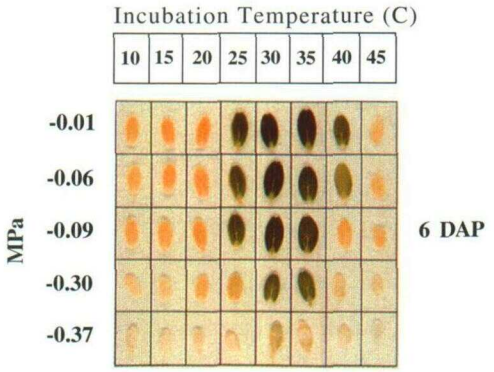

Figure 8. The effect of seedling growth at various soil water potentials on the sensitivity of Chl accumulation in cucumber cotyledons. Temperatures ranging from 10 to 45°C at 5°C intervals were evaluated on cotyledons from seedlings grown at 25°C for 6 d in soils having water potentials of −0.01, −0.06, −0.09, −0.30, and −0.37 MPa.

the range of temperatures supporting Chl accumulation in these seedlings will be much narrower than would have been experienced if the soil crust had not diverted needed energy supplies.

The effect of available soil water on the temperature sensitivity of Chl accumulation in cucumber cotyledons 6 d after planting in a 25°C chamber is shown in Figure 8. Chl accumulation in cotyledons from seedlings grown in vermiculite with an initial soil water potential of −0.01 MPa occurred from 25 to 40°C. A narrowing of the temperature range for Chl accumulation occurred at lower soil water potentials. Chl accumulation occurred at 30 and 35°C in the −0.37 MPa treatment; however, the level of accumulation was reduced relative to the −0.30 MPa sample. Seedling growth was reduced in the cucumbers experiencing low soil water potentials. The narrowing of the temperature range for Chl accumulation with decreasing soil water potential is similar to the patterns observed for cotyledons as the availability of stored reserves declines with time after planting (Fig. 7). It is possible that the increase in available soil water increases the availability of stored reserves for use in the synthesis and accumulation of Chl.

SUMMARY

This study investigated the temperature characteristics associated with three metabolic processes of cucumber seedlings. First, analysis of the temperature sensitivity of the interaction of NADH-hydroxypyruvate reductase with NADH showed that optimal temperatures for cucumber metabolism defined by the TKW were between 23.5 and 39°C. Second, investigation of the temperature sensitivity of the reappearance of PSII F_v following illumination showed optimal reappearance at 30 and 35°C. Third, the temperature characteristics of Chl accumulation upon exposure of etiolated seedlings to continuous illumination showed optimal Chl accumulation within the temperature delineated by the TKW. These studies also demonstrated the broader temperature response of Chl accumulation associated with the in-

creased availability of seed reserves. In conclusion, this study has provided methods for determining plant temperature optima and has demonstrated the impact of substrate availability on the temperature responses of cucumber metabolism.

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