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Correlation between the Circadian Rhythm of Resistance to Extreme Temperatures and Changes in Fatty Acid Composition in Cotton Seedlings

Arnon Rikin*, Jack W. Dillwith, and Douglas K. Bergman

Departments of Botany (A.R.) and Entomology (J.W.D., D.K.B.), Oklahoma State University, Stillwater, Oklahoma 74078

Fluctuations in fatty acid composition were examined in cotton (*Gossypium hirsutum* L. cv Deltapine 50) leaves during light-dark cycles of 12:12 h and under continuous light and were correlated to the rhythmic changes in chilling (5°C) resistance (CR) and heat (53°C) resistance (HR). The chilling-resistant and chilling-sensitive phases developed in the dark or the light period, respectively, and this rhythm persisted under continuous light for three cycles. The heat-resistant phase developed in the light period and an additional peak of HR occurred in the middle of the dark period. Under continuous light, only one peak of HR developed, lasting from the middle of the subjective night to the middle of the subjective day. The amounts of palmitic and oleic acids were constant during the light-dark cycle and under continuous light, but those of linoleic and linolenic acids fluctuated, attaining a high level in the middle of the dark period or the subjective night, and a low level in the middle of the light period or the subjective day. A low temperature of 20°C induced CR and affected changes in fatty acid composition similar to those that occurred during the daily CR phase. A high temperature of 40°C induced HR but did not affect changes in fatty acid composition. The results in their entirety show that the CR that develops rhythmically as well as the low-temperature-induced CR coincide with increased levels of polyunsaturated fatty acids. No correlation is found between changes in fatty acid composition and the HR that develops rhythmically or the high-temperature-induced HR.

The development of CR and HR in plants is regulated by environmental stimuli (Levitt, 1980) and endogenously by a circadian rhythm (Schwemmle and Lange, 1959; Couderchet and Koukkari, 1987; McMillan and Rikin, 1990; Rikin, 1991, 1992a, 1992b). In many plants a gradual decrease or increase in temperature induces CR and HR, respectively (Levitt, 1980). Circadian rhythms of CR and HR have been described in several plants. The circadian rhythm of CR regulates the development of a chilling-resistant phase starting at the end of the day and lasting most of the night (McMillan and Rikin, 1990; Rikin, 1991, 1992a), and the circadian rhythm of HR regulates the development of a heat-resistant phase usually in the middle of the day (Schwemmle and Lange, 1959; Rikin, 1992b). These circadian rhythms are not affected by a wide range of environmental conditions, thus ensuring the development of the chilling- and heat-resistant phases when chilling and heat are expected to start or to reach their maximal levels.

* Corresponding author; fax 1–405–744–7673.

Temperature-induced changes in membrane lipids are a key factor in the regulation of CR and HR. In many chilling-sensitive plants, the thermal-phase transition of the membrane lipids correlates with the lower limit for survival (Raison, 1986). This transition depends on the level of fatty acid unsaturation, especially of the molecular species of phosphatidylglycerol at both the *sn*-1 and *sn*-2 position (Murata and Yamaya, 1984; Tasaka et al., 1990). Induction of CR by low temperatures in many cases coincides with an increase in unsaturation (Somerville and Browse, 1991) and a decrease in the transition temperature (Raison, 1986; Orr and Raison, 1990). A cause-and-effect relationship between CR and membrane composition has been demonstrated by genetic manipulation of fatty acid unsaturation in prokaryotes (Wada et al., 1990) and higher plants (Murata et al., 1992). Introduction of the fatty acid ω6 desaturase gene from *Synechocystis* PCC6803 into the chilling-sensitive species *Anacystis nidulans*, which does not contain polyunsaturated fatty acids, results in accumulation of 18:2 in membrane lipids and in CR (Wada et al., 1990). In *Nicotiana tabacum*, the level of fatty acid unsaturation of phosphatidylglycerol and the degree of CR can be changed by transformation with cDNA for glycerol-3-phosphate acyltransferases from squash and *Arabidopsis*. In the transgenic tobacco plants, higher levels of fatty acid unsaturation in phosphatidylglycerol resulted in a higher degree of CR (Murata et al., 1992).

The acquisition of HR in several plants is accompanied by a decrease in fatty acid unsaturation (Kee and Nobel, 1985), resulting in a decrease in lipid fluidity and an increase in the transition temperature (Raison, 1986). Further support that lipid unsaturation is a component of HR is derived from work with *Arabidopsis* mutants (Somerville and Browse, 1991). A mutant deficient in the activity of the chloroplast fatty acid ω9 desaturase accumulates high amounts of 16:0 and shows an overall reduction in the level of unsaturation of chloroplast lipids. This decrease in unsaturation shifts the plant growth temperature range upward (Kunst et al., 1989).

In the present study, we examined the correlation between the endogenous rhythmic changes in CR and HR and changes in lipid unsaturation. Also, we compared changes in lipid unsaturation between the endogenously developed and the temperature-induced CR and HR.

Abbreviations: CR, chilling resistance; HR, heat resistance; LDC, light-dark cycle of 24 h; 16:0, palmitic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Cotton seeds (*Gossypium hirsutum* L. cv Deltapine 50, obtained from Delta and Pine Land Co., Scott, MS; harvested in 1988) were sown in plastic pots (10 cm diameter, 8.5 cm high) filled with a mixture of peat and vermiculite (Terra-lite, Redi-Earth; W.R. Grace & Co., Cambridge, MA). Four days after sowing, seedlings were thinned to three per pot. They were grown in growth chambers at 33°C, 85% RH under LDCs of 12:12 h. The light (250 μmol m^{-2} s^{-1}) source was a combination of fluorescent (F48T18-CW-VHO; Sylvania, Danvers, MA) and incandescent lamps. The seedlings were fertilized 4 d after sowing with 60 mL of 3 g/L 20N-20P-20K soluble fertilizer (Peters; W.R. Grace & Co.) and irrigated as needed with water. All experiments were started with 14-d-old seedlings.

Exposure to Extreme Temperatures, Evaluation of Injury, and Acclimation Treatments

Chilling treatment was given by exposing seedlings to low temperatures as specified in each experiment for 1.5 d under 85% RH. Heat treatment was given by exposing seedlings to high temperatures as specified in each experiment for 40 min at 40% RH. After the chilling or heat exposure, the seedlings were returned to 33°C for 3 or 4 LDCs of 12:12 h. At the end of this period, CR or HR was evaluated by measuring the fresh weight of the shoot above the cotyledons.

Acclimation to chilling was induced by transfer of seedlings to 20°C, 85% RH for 6 h. Acclimation to heat was induced by transfer of seedlings to 40°C, 85% RH for 6 h. Each treatment was conducted with at least nine seedlings in three pots. Each experiment was repeated at least three times.

Fatty Acids Analysis

Fatty acid amounts and compositions were determined in lipid extracts from the first true leaf. Four leaf discs (1.2 cm diameter) were cut from each leaf and were immediately weighed. Lipids were extracted according to the method of Bligh and Dyer (1959). The lipid extracts were separated into neutral lipid, glycolipid, and phospholipid fractions using a column containing 2 g of Bio-Sil A (Bio-Rad Laboratories, Richmond, CA) according to the method of Lynch and Thompson (1986). Neutral lipids were eluted with 30 mL of chloroform, glycolipids with 30 mL of acetone, and phospholipids with 30 mL of chloroform:methanol (1:1, v/v). Methylheptadecanoate was added to each isolated lipid fraction as an internal standard. Lipids were saponified with 5% KOH in methanol (w/v) for 1 h at 60°C and fatty acid methyl esters were formed by adding 14% BF_3 in methanol followed by heating at 60°C for 1 h in a closed vial (Ryan et al., 1982). The methyl esters were purified using a small column (0.5 × 6 cm) of Bio-Sil A washed first with 3 mL of hexane and were eluted with 3 mL of 5% diethyl ether in hexane (v/v). The methyl esters were analyzed by GC using a DB-225 column (30 m × 0.25 mm, 0.15-μm film thickness, J & W Scientific, Folsom, CA) temperature programmed as follows: 120°C for 2 min, 10°C/min to 200°C, 5°C/min to 225°C, hold for 4 min. Fatty acids in plant extracts were identified by comparison of chromatographic behavior to that of authentic standards. Fatty acid methyl esters were quantified by comparison of peak areas to the internal standard.

Plant material for each treatment was collected from four seedlings in four pots. Each experiment was repeated three times. The results shown are the mean ± SE.

RESULTS

Rhythmic Changes in CR and HR

Cotton seedlings grown under LDCs of 12:12 h showed circadian changes in both CR and HR (Fig. 1). The seedlings were most sensitive to chilling during the light period, then developed CR toward the end of the light period and remained resistant until the end of the dark period, when they again became sensitive. HR reached its maximal level at the middle of the light period, then declined to its minimal level at the end of the light period. In the middle of the dark period, HR reached another peak. Under continuous light for three cycles, the rhythm of CR persisted similarly to the rhythm under the regular LDC. The rhythm of HR also persisted under continuous light for three cycles, but unlike under LDC, only one peak of HR was observed. This resistant phase developed in the middle of the subjective night, then began to decline in the middle of the light period and reached its minimal level at the end of the light period (Fig. 1).

The rhythmic changes in CR and HR described in Figure 1 were determined in relation to one low (5°C) or one high (53°C) temperature. To find whether these rhythmic changes occurred because of rhythmic changes in the critical temperature that inflicted damage, seedlings grown under normal temperature (33°C) were exposed to a wide range of low temperatures from 0°C to high temperatures of up to 61°C (Fig. 2). Seedlings were exposed to decreasing temperatures starting in the middle of the light period and starting in the middle of the dark period, i.e. the times of minimal and maximal CR, respectively. Other seedlings were exposed to increasing temperatures starting in the middle of the light

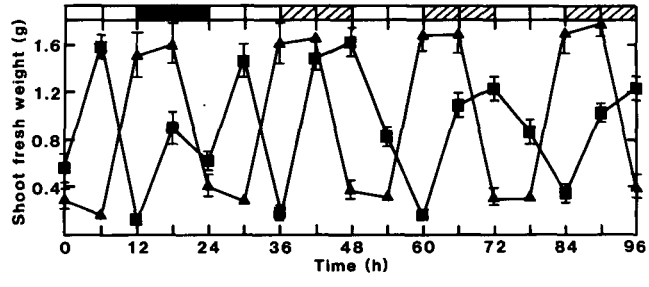

Figure 1. Rhythmic changes in the resistance of cotton seedlings to chilling and heat. Seedlings were grown at 33°C for 14 LDCs of 12:12 h after sowing. Then, during the 15th LDC and additional three cycles of continuous light, at 6-h intervals seedlings were exposed to chilling (5°C, 1.5 d) or heat (53°C, 40 min). After the chilling or heat exposure, the seedlings were returned to 33°C for 4 LDCs and at the end of this period shoot fresh weight was determined. ▭, Light period; ■, dark period; ▨, subjective night; ▲, chilled; ■, heated.

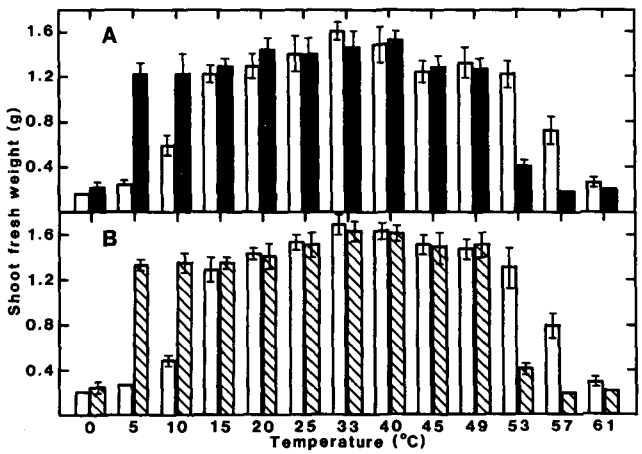

Figure 2. Rhythmic changes in the critical temperature of chilling or heat injury in cotton seedlings. Seedlings were grown at 33°C for 14 LDCs of 12:12 h after sowing. In the 15th LDC (A), seedlings were exposed to 0, 5, 10, 15, 20, and 25°C for 1.5 d in the middle of the light period (□), or the middle of the dark period (■); or to 40, 45, 49, 53, 57, and 61°C for 40 min in the middle of the light period (□) or 2 h after the beginning of the dark period (■). After the 15th LDC, another set of seedlings was kept under continuous light for two cycles. In the second cycle (B), in the subjective day (□) and the subjective night (▨), seedlings were exposed to the same temperature treatments and at the same times as in the 15th LDC. After all the temperature treatments, the seedlings were returned to 33°C for three LDCs and at the end of this period shoot fresh weight was determined.

period and 2 h after the beginning of the dark period, i.e. the times of high and low HR, respectively. Seedlings exposed to decreasing temperatures starting at the chilling-sensitive phase began to be damaged by a temperature of 10°C, whereas an exposure started at the chilling-resistant phase inflicted damage only at a temperature as low as 0°C. Seedlings exposed to increasing temperatures during the heat-sensitive phase began to be damaged by a temperature of 53°C. An exposure during the heat-resistant phase started to cause damage at a temperature as high as 57°C. However, at 57°C the damage inflicted was less for seedlings at the heat-resistant phase than that inflicted to seedlings during the heat-sensitive phase (Fig. 2A). Seedlings kept under continuous light for two cycles showed the same pattern of rhythmic changes in the critical temperatures of chilling or heat injury as seedlings maintained under the regular LDC (Fig. 2B).

Fluctuations in Fatty Acid Composition under LDCs and under Continuous Light

Fatty acid composition in leaves was analyzed in the middle of the light period and the middle of the dark period (Fig. 3, A–C). The major fatty acids in cotton leaves from greater to lesser abundance were 18:3, 16:0, 18:2, and 18:1. The amounts of 16:0 and 18:1 were unchanged, whereas the amounts of 18:2 and 18:3 fluctuated throughout the LDC. The amounts of 18:2 and 18:3 were higher in the middle of the dark period than in the middle of the light period (Fig. 3A). Lipids in which the 18:2 content fluctuated were exclusively phospholipids (Fig. 3B), and lipids in which the 18:3 content fluctuated were exclusively glycolipids (Fig. 3C). In seedlings kept under continuous light for two cycles, a significant increase occurred in total lipid content, but the rhythmic changes in 18:2 and 18:3 content persisted similarly to the changes in seedlings under the regular LDC (Fig. 3, D–F).

Acclimation to Chilling and Heat

The seedlings were acclimated to chilling or heat by treatment with an intermediary temperature before the exposure to the damaging extreme temperature (Fig. 4). Thus, a temperature of 20°C induced CR and the seedlings became resistant to chilling throughout the LDC. A temperature of 40°C induced HR and the seedlings became heat resistant throughout the LDC. The degree of CR induced by low temperature was approximately the same as the CR developed in the daily chilling-resistant phase. The degree of HR induced by high temperature was higher than the HR developed in the daily heat-resistant phase (Fig. 4). A possible

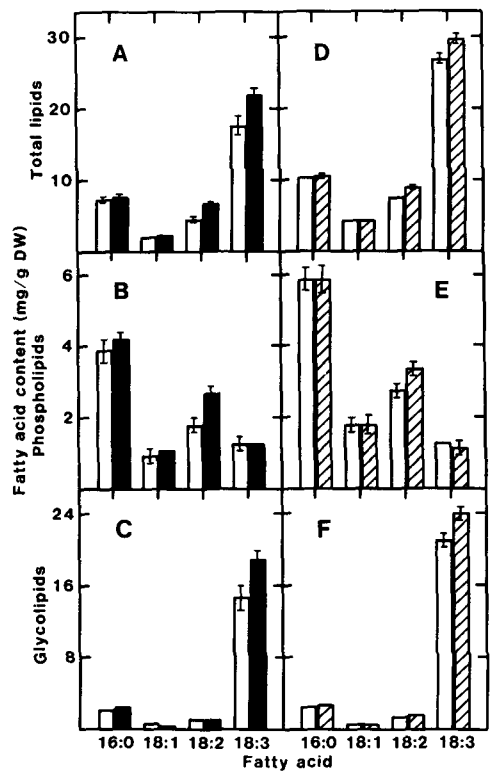

Figure 3. Rhythmic changes in fatty acid composition in leaves of cotton seedlings. Seedlings were grown at 33°C for 14 LDCs of 12:12 h after sowing. Fatty acid composition of total lipids (A), phospholipids (B), and glycolipids (C) was determined in the 15th LDC in the middle of the light period (□) or in the middle of the dark period (■). After the 15th LDC, the seedlings were kept under continuous light for two cycles. In the second cycle, fatty acid composition of total lipids (D), phospholipids (E), and glycolipids (F) was determined in the middle of the subjective day (□) and in the middle of the subjective night (▨).

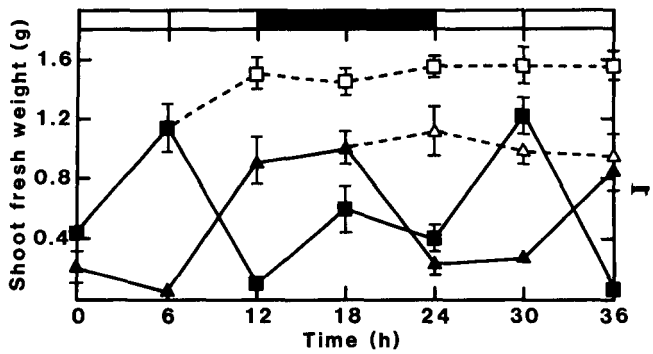

Figure 4. Acclimation of cotton seedlings to chilling and heat. Seedlings were grown at 33°C for 14 LDCs of 12:12 h after sowing. Then, in the 15th LDC in the middle of the light period, acclimation to heat was started by transfer of seedlings to 40°C; in the middle of the dark period acclimation to chilling was started by transfer of another group of seedlings to 20°C. Throughout the experiment, at 6-h intervals seedlings were exposed to heat of 53°C for 40 min, or to chilling of 5°C for 1.5 d. After the heat or chilling exposure, the seedlings were returned to 33°C for 4 LDCs, and at the end of this period shoot fresh weight was determined. ▭, Light period; ▬, dark period; ■, heated, nonacclimated; □, heated, acclimated; ▲, chilled, nonacclimated; △, chilled, acclimated.

effect of acclimation on the critical temperature of injury was examined by exposing nonacclimated and acclimated seedlings to a series of potentially damaging low and high temperatures (Fig. 5). Seedlings acclimated to chilling by a low temperature (20°C) withstood low temperatures down to 5°C, similar to nonacclimated seedlings during the chilling-resistant phase. At 0°C, both nonacclimated and acclimated seedlings were severely damaged (Fig. 5A). Seedlings acclimated to heat by high temperature (40°C) withstood high temperature up to 53°C and became slightly damaged at 57°C. Nonacclimated seedlings during their heat-resistant phase were less resistant to 53 and 57°C than those acclimated to heat. At 61°C, both nonacclimated and acclimated seedlings were severely damaged (Fig. 5B).

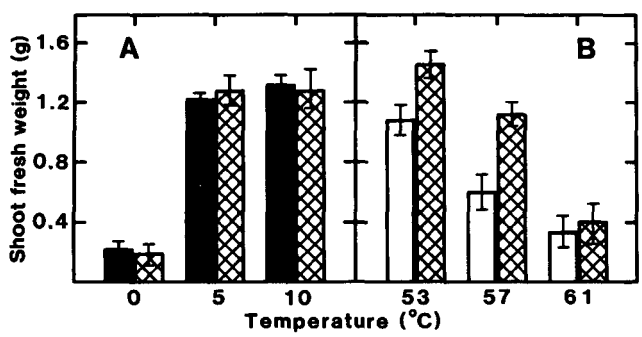

Figure 5. Changes in the critical temperature of chilling or heat injury by acclimation. Seedlings were grown at 33°C for 14 LDCs of 12:12 h after sowing. During the 15th LDC, in the middle of the dark period (A) nonacclimated seedlings (■) and seedlings acclimated to chilling (▩) were exposed to 0, 5, and 10°C for 1.5 d. In the middle of the light period (B) nonacclimated seedlings (□) and seedlings acclimated to heat (▩) were exposed to 53, 57, and 61°C for 40 min. Acclimation was induced by treating the seedlings for 6 h prior to chilling or heat exposure with 20 or 40°C, respectively. After the exposure to the extreme temperatures, the seedlings were returned to 33°C for 4 LDCs, and at the end of this period shoot fresh weight was determined.

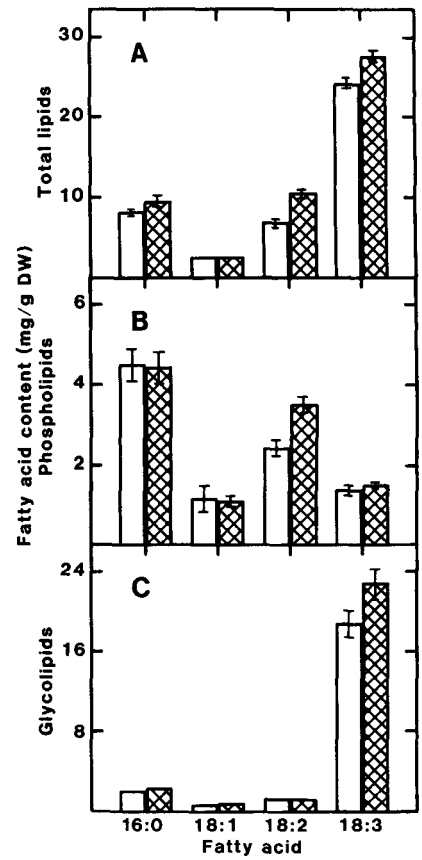

Figure 6. Effect of acclimation to chilling on fatty acid composition in leaves of cotton seedlings. Seedlings were grown at 33°C for 14 LDCs of 12:12 h after sowing. Fatty acid composition of total lipids (A), phospholipids (B), and glycolipids (C) was determined in the 15th LDC in the middle of the light period, in nonacclimated (□) and acclimated (▩) seedlings. Acclimation to chilling was induced by treating the seedlings for 6 h with 20°C.

Changes of Fatty Acid Composition by Acclimation to Chilling and Heat

Induction of CR by a low temperature for 6 h was associated with changes in fatty acid composition. The content of 18:2 and 18:3 was higher in acclimated seedlings when compared with nonacclimated seedlings in their chilling-sensitive phase. The content of 16:0 and 18:1 was not changed in seedlings acclimated to chilling (Fig. 6A). Lipids in which 18:2 changed were exclusively phospholipids (Fig. 6B), and lipids in which 18:3 changed were exclusively glycolipids (Fig. 6C). Induction of HR by high temperature for 6 h was not associated with any pronounced changes in fatty acid composition when acclimated seedlings were compared

with nonacclimated seedlings during their heat-sensitive phase (Fig. 7).

DISCUSSION

Changes in membrane lipids play a major role in the adaptive response of plants to extreme temperatures (Murata and Yamaya, 1984; Raison, 1986). In the present study, we show that certain changes in fatty acid composition correlate with the sensitive and resistant phases of the CR rhythm. These changes in fatty acid composition and their correlation to CR persist under continuous light, indicating their circadian characteristics, i.e. they are regulated endogenously, irrespective of the immediate environmental conditions. During both the LDC and continuous light, an increase in 18:2 in phospholipids and 18:3 in glycolipids corresponds to a higher degree of CR. This increase in polyunsaturated fatty acids during the acquisition of CR increases the overall unsaturation of the membrane lipids and probably decreases the temperature of phase transition as found in many plant systems (Raison, 1986; Somerville and Browse, 1991). This possibility is supported by the finding that the development

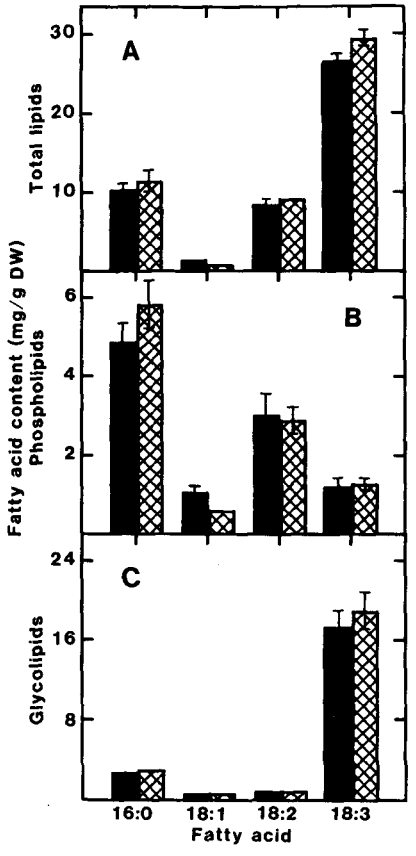

Figure 7. Effect of acclimation to heat on fatty acid composition in leaves of cotton seedlings. Seedlings were grown at 33°C for 14 LDCs of 12:12 h after sowing. Fatty acid composition of total lipids (A), phospholipids (B), and glycolipids (C) was determined in the 15th LDC, 2 h after the beginning of the dark period, in nonacclimated (■) and acclimated (▩) seedlings. Acclimation to heat was induced by treating the seedlings for 6 h with 40°C.

of the daily chilling-resistant phase is accompanied by a decrease in the critical temperature of chilling injury. Although changes in polyunsaturated fatty acids are an important factor in CR and probably also in rhythmic changes in CR, other factors may be involved in these processes (King et al., 1988).

Several lines of circumstantial evidence from a previous work (McMillan and Rikin, 1990) indicate that a similar mechanism is involved in the induction of CR by low temperatures and the development of the daily resistant phase that is regulated by a circadian rhythm. First, the magnitude of CR induced by low temperature at the daily sensitive phase and that of the maximal daily CR are approximately the same. Second, CR induced by low temperature and the daily CR are nonadditive. Third, the time required to induce CR by low temperature is similar to the time of transition from the daily sensitive to the daily resistant phase (McMillan and Rikin, 1990). The present study shows that similar changes in fatty acid composition, i.e. an increase in the levels of 18:2 and 18:3, occurs during the induction of CR by low temperatures and during the development of the daily resistant phase. The increase in fatty acid unsaturation resulting from the daily sensitive to the daily resistant phase (McMillan and Rikin, 1990). The present study shows that similar changes in fatty acid composition, i.e. an increase in the levels of 18:2 and 18:3, occur during the induction of CR by low temperatures and during the development of the daily resist- phase and in the low-temperature-induced CR is approximately 18% of the total lipids. This change is probably sufficient for the observed decrease in the critical temperature of chilling injury. Small changes in lipid unsaturation that are associated with low-temperature-induced CR have been documented in many chilling-sensitive plants. In oleander, the thermal phase transition at low temperature involves only about 5% of the lipids and is initiated by the solidification of dipalmitoylphosphatidylglycerol (Raison, 1986).

The changes in lipid membrane composition that are associated with increased HR are expected to be the opposite of those for CR, i.e. a decrease in fatty acid unsaturation (Somerville and Browse, 1991). Thus, acclimation of oleander to growth at high temperature (45°C) decreases lipid fluidity, resulting in higher transition temperature and lower CR. When these plants are acclimated to low temperature, the opposite trends are detected (Raison, 1986). However, in cotton seedlings the rhythmic development of HR as well as the high-temperature-induced HR do not correlate with decreased lipid unsaturation. Similar instances of acquisition of HR without marked changes in fatty acid unsaturation or composition have been reported for several other plants (Kee and Nobel, 1985). The lack of correlation between the changes in HR and fatty acid composition indicate a HR mechanism without a major involvement of membrane lipids. Alternatively, although there are no detectable gross changes in fatty acid composition, it is possible that very small changes do occur in the degree of unsaturation of a specific lipid or a particular membrane that is crucial for HR. The HR induced by high temperature and the HR developed in the daily rhythm are additive. Thus, the HR induced by high temperature during the daily resistant phase is higher than the HR in the daily resistant phase alone. Therefore, it is possible

that more than one mechanism is involved in the regulation and development of HR in cotton seedlings.

Several models suggest that at least some components of the circadian clock are located in membranes (Njus et al., 1974). Therefore, the rhythmic lipid changes in cotton leaves may be related to the basic mechanism of the circadian clock or may represent the "hands" of the clock. In *Chenopodium rubrum* plants, 18:1 in phosphatidylcholine and phosphatidylethanolamine fluctuates rhythmically in synchrony with the circadian rhythm of stem elongation. The rhythmic changes in 18:1 stop when growth is completed (Lecharny et al., 1990). These rhythmic fluctuations in 18:1 are probably linked to the process of elongation and thus represent the hands of the clock. A circadian rhythm of fatty acid composition of phospholipids operates in *Neurospora crassa* (Roeder et al., 1982). Circumstantial evidence indicates that this rhythm is associated with the circadian clock. The *cel* (chain elongation) mutant, which is partially blocked in the synthesis of fatty acids, has lost the temperature compensation of its conidiation rhythm (Mattern et al., 1982) and has a period sensitive to supplemental fatty acids (Mattern, 1985). Also, several mutants with abnormal phospholipid fatty acid composition show altered properties of their rhythms (Coté and Brody, 1987a, 1987b). In cotton seedlings, the higher degree of CR achieved during the daily resistant phase or induced by low temperature correlates with prevention or shortening of phase delay by low temperatures (Rikin, 1991). This prevention or decrease of phase shifting may be regarded as acclimation of the circadian clock to withstand damaging low temperatures, thus ensuring that rhythms with adaptive values will occur on time when needed. The mechanism that induces CR also confers greater ability for prevention of phase shifting by low temperatures in circadian rhythms (Rikin, 1991). This mechanism involves an increase in the level of the polyunsaturated fatty acids 18:2 and 18:3. These changes affect the operation of the circadian clock under low temperatures and, therefore, may be a component of, or a factor closely related to, the circadian clock.

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LITERATURE CITED

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem **37:** 911–917

Coté GG, Brody S (1987a) Circadian rhythms in *Neurospora crassa:* membrane composition of a mutant defective in temperature compensation. Biochim Biophys Acta **898:** 23–36

Coté GG, Brody S (1987b) Circadian rhythms in *Neurospora crassa:* a clock mutant, *prd-1,* is altered in membrane fatty acid composition. Biochim Biophys Acta **904:** 131–139

Couderchet M, Koukkari WL (1987) Daily variations in the sensitivity of soybean seedlings to low temperature. Chronobiol Int **4:** 537–541

Kee SC, Nobel PS (1985) Fatty acid composition of chlorenchyma membrane fractions from three desert succulents grown at moderate and high temperatures. Biochim Biophys Acta **820:** 100–106

King AI, Joyce DC, Reid MS (1988) Role of carbohydrates in diurnal chilling sensitivity of tomato seedlings. Plant Physiol **86:** 764–768

Kunst L, Browse J, Somerville C (1989) Enhanced thermal tolerance in a mutant of *Arabidopsis* deficient in palmitic acid unsaturation. Plant Physiol **91:** 401–408

Lecharny A, Tremolières A, Wagner E (1990) Correlation between the endogenous circadian rhythmicity in growth rate and fluctuations in oleic acid content in expanding stems of *Chenopodium rubrum* L. Planta **182:** 211–215

Levitt J (1980) Responses of Plants to Environmental Stresses. I. Chilling, Freezing and High Temperature Stresses. Academic Press, New York

Lynch DV, Thompson GA Jr. (1986) Analysis of phospholipid molecular species by gas chromatography and coupled gas chromatography-mass spectrometry. *In* HF Linskens, JF Jackson, eds, Modern Methods of Plant Analysis, Vol 3. Springer-Verlag, Berlin, pp 100–120

Mattern DL (1985) Unsaturated fatty acid isomers: effects on the circadian rhythm of a fatty-acid-deficient *Neurospora crassa* mutant. Arch Biochem Biophys **237:** 402–407

Mattern DL, Forman LR, Brody S (1982) Circadian rhythms in *Neurospora crassa:* a mutation affecting temperature compensation. Proc Natl Acad Sci USA **79:** 825–829

McMillan KD, Rikin A (1990) Relationship between circadian rhythm of chilling resistance and acclimation to chilling in cotton seedlings. Planta **182:** 455–460

Murata N, Ishizaki-Nishizawa O, Higashi S, Hayashi H, Tasaka Y, Nishida I (1992) Genetically engineered alteration in chilling sensitivity of plants. Nature **356:** 710–713

Murata N, Yamaya J (1984) Temperature-dependent phase behavior of phosphatidylglycerols from chilling-sensitive and chilling-resistant plants. Plant Physiol **74:** 1016–1024

Njus D, Sulzman FM, Hastings JW (1974) Membrane model for the circadian clock. Nature **248:** 116–120

Orr GR, Raison JK (1990) The effect of changing the composition of phosphatidylglycerol from thylakoid polar lipids of oleander and cucumber on the temperature of transition related to chilling injury. Planta **181:** 137–143

Raison JK (1986) Alterations in the physical properties and thermal response of membrane lipids: correlations with acclimation to chilling and high temperature. *In* JB St John, E Berlin, PC Jackson, eds, Frontiers of Membrane Research in Agriculture. Rowman and Allanheld, Totowa, NJ, pp 383–401

Rikin A (1991) Temperature-induced phase shifting of circadian rhythms in cotton seedlings as related to variations in chilling resistance. Planta **185:** 407–414

Rikin A (1992a) Temporal organization of chilling resistance in cotton seedlings: effects of low temperature and relative humidity. Planta **187:** 517–522

Rikin A (1992b) Circadian rhythm of heat resistance in cotton seedlings. Synthesis of heat shock proteins. Eur J Cell Biol **59:** 160–165

Roeder PE, Sargent ML, Brody S (1982) Circadian rhythms in *Neurospora crassa:* oscillations in fatty acids. Biochemistry **21:** 4909–4916

Ryan RO, de Renobales M, Dillwith JW, Heisler CR, Blomquist GJ (1982) Biosynthesis of myristate in an aphid: involvement of a specific acylthioesterase. Arch Biochem Biophys **213:** 26–36

Schwemmle B, Lange OL (1959) Endogen-tagesperiodische Schwankunger der Hitzeresistenz bei *Kalanchoë blossfeldiana.* Planta **53:** 134–144

Somerville C, Browse J (1991) Plant lipids: metabolism, mutants, and membrane. Science **252:** 80–87

Tasaka Y, Nishida I, Higachi S, Beppu T, Murata N (1990) Fatty acid composition of phosphatidylglycerols in relation to chilling sensitivity of woody plants. Plant Cell Physiol **31:** 545–550

Wada H, Gombos Z, Murata N (1990) Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. Nature **347:** 200–203