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THE EFFECT OF TEMPERATURE OF THE RATE OF PHOTOSYNTHETIC ELECTRON TRANSFER IN CHLOROPLASTS OF CHILLING-SENSITIVE AND CHILLING-RESISTANT PLANTS

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SUMMARY

1. Photochemical activities as a function of temperature have been compared in chloroplasts isolated from chilling-sensitive (below approximately 12 °C) and chilling-resistant plants.

2. An Arrhenius plot of the photoreduction of $NADP^+$ from water by chloroplasts isolated from tomato (*Lycopersicon esculentum* var. Gross Lisse), a chilling-sensitive plant, shows a change in slope at about 12 °C. Between 25 and 14 °C the activation energy for this reaction is 8.3 kcal·mole^{-1}. Between 11 and 3 °C the activation energy increases to 22 kcal·mole^{-1}. Photoreduction of $NADP^+$ by chloroplasts from another chilling-sensitive plant, bean (*Phaseolus vulgaris* var. brown beauty), shows an increase in activation energy from 5.9 to 17.5 kcal·mole^{-1} below about 12 °C.

3. The photoreduction of $NADP^+$ by chloroplasts isolated from two chilling-resistant plants, lettuce (*Lactuca sativa* var. winter lake) and pea (*Pisum sativum* var. greenfeast), shows constant activation energies of 5.4 and 8.0 kcal·mole^{-1}, respectively, over the temperature range 3–25 °C.

4. The effect of temperature on photosynthetic electron transfer in the chloroplasts of chilling-sensitive plants is localized in Photosystem I region of photosynthesis. Both the photoreduction of $NADP^+$ from reduced 2,6-dichlorophenolindophenol and the ferredoxin–$NADP^+$ reductase (EC 1.6.99.4) activity of choroplasts of chilling-sensitive plants show increases in activation energies at approximately 12 °C whereas Photosystem II activity of chloroplasts of chilling-sensitive plants shows a constant activation energy over the temperature range 3–25 °C. The photoreduction of Diquat (1,1′-ethylene-2,2′-dipyridylium dibromide) from water by bean chloroplasts, however, does not show a change in activation energy over the same temperature range. The activation energies of each of these reactions in chilling-resistant plants is constant between 3 and 25 °C.

5. The effect of temperature on the activaton energy of these reactions in chloroplasts from chilling-sensitive plants is reversible.

6. In chilling-sensitive plants, the increased activation energies below approxi-

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; Diquat, 1,1′-ethylene-2,2′-dipyridylium dibromide.

mately 12 °C, with consequent decreased rates of reaction for the photoreduction of $NADP^+$, would result in impaired photosynthetic activity at chilling temperatures. This could explain the changes in chloroplast structure and function when chilling-sensitive plants are exposed to chilling temperatures.

INTRODUCTION

The inability of chilling-sensitive plants to maintain normal physiological function at chilling temperatures (approximately 0–12 °C) has been correlated with a marked increase in the activation energy of respiratory enzymes below a particular critical temperature[1]. This temperature is usually within the range 9–12 °C depending on the species. The increase in activation energy of the respiratory enzymes at chilling temperatures is not an intrinsic property of these enzyme systems in chilling-sensitive plants, but can be related to temperature-induced phase changes in the lipids of the mitochondrial membranes[2,3]. This phase change, detected by a change in mobility of nitroxide spin labels, occurs at precisely the same temperature as the change in the activation energy of the respiratory enzymes and is thought to induce conformational changes in the enzyme protein associated with the cristae membranes[3]. No change in activation energy was detected in the respiratory enzymes of mitochondria from chilling-resistant plants, nor was a phase change in the lipids detected by spin labelling[1,3]. The ability of these plants to withstand chilling temperatures is thus correlated with a more flexible state of the mitochondrial membranes at these temperatures. This difference in the physical properties of membrane lipids of mitochondria from chilling-sensitive and chilling-resistant plants can also be correlated with the relative proportion of saturated and unsaturated fatty acids of the membrane lipids[4].

The difference in the physical properties of membranes of chilling-sensitive and resistant plants might not be restricted to mitochondrial membranes. Measurements of the effect of temperature on the rate of CO_2 fixation by chilling-sensitive and resistant plants suggest that some change occurs in the kinetics of chloroplast enzyme systems of sensitive plants when the temperature is reduced below 12 °C (ref. 5). The effect of temperature on the activation energy of the photoreduction of $NADP^+$ from water and a number of other electron-transfer reactions of chloroplasts isolated from chilling-sensitive and resistant plants was therefore investigated. This paper shows that for chloroplasts from chilling-sensitive plants a sudden increase occurs in the activation energy of reactions associated with Photosystem I of photosynthesis when the temperature is reduced below approximately 12 °C. In contrast, no change in activation energy of these reactions was observed with chloroplasts from chilling-resistant plants.

MATERIALS AND METHODS

Preparation of chloroplasts

All plants used were grown in a greenhouse except lettuce which was purchased in the local market. The chilling-sensitive plants used had not been subjected to any chilling temperatures. Chloroplasts were prepared from two chilling-sensitive

plants, bean (*Phaseolus vulgaris* var. brown beauty) and tomato (*Lycopersicon esculentum* var. Gross Lisse) and from two chilling-resistant plants, pea (*Pisum sativum* var. greenfeast) and lettuce (*Lactuca sativa* var. winterlake.)

Chloroplasts were isolated from 7 g of washed, deribbed leaves by homogenizing (Omnimixer, Sorvall) with 50 ml of ice-cold medium containing 330 mM sorbitol, 30 mM Tris–HCl, pH 7.8, 1 mM EDTA (sodium salt), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 5 mM 2-mercaptoethanol and 2% (w/v) polyvinyl pyrrolidone (Polyclar)[6,7]. The homogenate was filtered through miracloth (Chicopee Mills, New York) and centrifuged at $270 \times g$ for 90 s. Chloroplasts were collected from the supernatant by centrifuging at $1000 \times g$ for 10 min. Chloroplasts from bean leaves were sedimented at $2000 \times g$ for 10 min. The chloroplasts were washed once and resuspended in a medium containing 0.33 M sorbitol, 1 mM $MgCl_2$, 10 mM phosphate buffer, pH 7.8, and 0.5% (w/v) bovine serum albumin (Fraction V, Calbiochem) and stored at 0 °C.

Chlorophyll was determined according to Arnon[8]. Swiss chard (*Beta vulgaris*) ferredoxin was prepared according to the method used by Losada and Arnon[9] for spinach.

Determinations of photochemical and enzyme activities at different temperatures

The photoreduction of 2,6-dichlorophenolindophenol (DCIP) and $NADP^+$ was followed spectrophotometrically using an Aminco–Chance dual-wavelength spectrophotometer at 575 *minus* 540 nm and 350 *minus* 370 nm, respectively. The $\Delta\varepsilon_{mM}$ values used for DCIP and $NADP^+$ reduction were 6.2 and 2.4, respectively. Saturating red actinic light at an intensity of 2×10^5 ergs·cm^{-2}·s^{-1} was obtained from a tungsten lamp filtered through a Corning 2-60 glass filter and a Corning 1-96 heat filter. Ferredoxin–$NADP^+$ reductase (reduced-NADP:ferredoxin oxidoreductase, EC 1.6.99.4) was measured by the reduction of DCIP by NADPH. The reaction was followed using the same wavelength combination as for DCIP photoreduction.

Temperature in the cell housing of the spectrophotometer was controlled by circulating water. Before each measurement the reaction mixture and cuvette were equilibrated in the water bath supplying the circulating water. The bath was initially cooled and then the temperature was increased in a step-wise fashion. Each sample required 1 min for analysis and about 90 min were needed to complete measurements over a temperature range of 3–27 °C. The temperature of each reaction mixture was determined with a thermocouple.

1,1′-Ethylene-2,2′-dipyridylium dibromide (Diquat) reduction was followed by determining O_2 uptake polarographically using an oxygen electrode (Rank Bros., Cambridge, Great Britain). Saturating actinic red light was provided by a quartz–iodide lamp of a slide projector filtered through a Corning 2-62 glass filter and heat filter. Samples at two different temperatures were run at the same time by using two electrodes and splitting the actinic light beam by a 50% transferring mirror. Light intensity in each reaction vessel was $7.5 \cdot 10^4$ ergs·cm^{-1}·s^{-1}.

RESULTS

Effect of temperature on photoreduction of $NADP^+$ from water

In Fig. 1, a comparison is made of the effects of temperature on the rate of

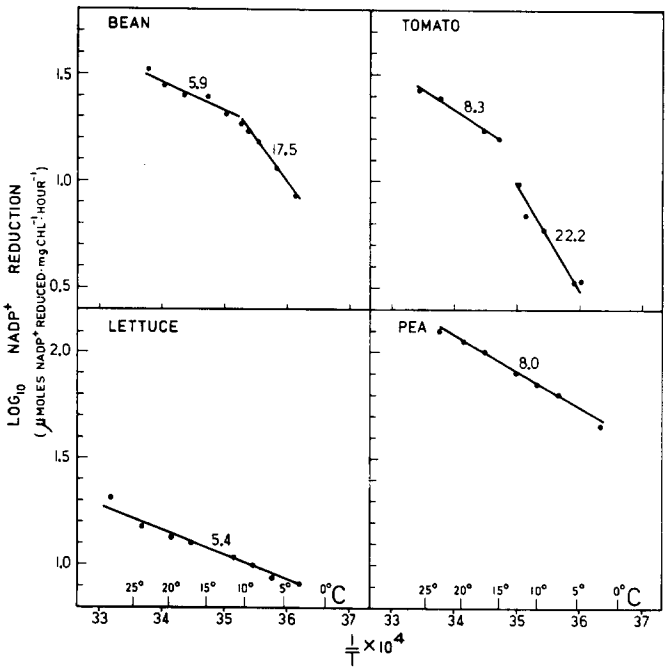

Fig. 1. Arrhenius plot of photoreduction of NADP+ from water by isolated chloroplasts of bean and tomato (chilling-sensitive) and pea and lettuce (chilling-resistant). The values adjacent to the plotted lines in this and the following figures are the calculated activation energies in kcal·mole^{-1}. The reaction mixture contained in a total volume of 1 ml in μmoles: phosphate at pH 8.0, 9; $MgCl_2$, 0.9; sorbitol, 27; NADP+, 0.25; a saturating amount of Swiss chard ferredoxin and chloroplasts containing 5 μg chlorophyll.

$NADP^+$ photoreduction catalysed by chloroplasts from bean and tomato as examples of chilling-sensitive plants and from pea and lettuce as examples of chilling-resistant plants. The data, presented as an Arrhenius plot, *i.e.* the logarithm of photoreduction *versus* the reciprocal of the absolute temperature, show that for chloroplasts of the chilling-sensitive plants there is a sharp change in slope at about 12 °C. This change in slope in the Arrhenius plot indicates a change in activation energy for the reaction. For bean chloroplasts the activation energy increased from 5.9 to 17.5 kcal·mole^{-1} below about 12 °C and for tomato chloroplasts from 8.3 to 22.2 kcal·mole^{-1}. This effect of chilling temperatures on the activation energy of $NADP^+$ photoreduction was reversible. Chloroplasts which exhibited an increase in activation energy at the chilling temperatures showed a return to the original activation energy when the temperature was increased above 12 °C.

No change was observed in the activation energy of the same reaction catalysed by chloroplasts of the chilling-resistant plants, pea and lettuce over the temperature range 3–25 °C. The activation energy was 5.4 kcal·mole^{-1} for pea chloroplasts and 8.0 kcal·mole^{-1} for lettuce chloroplasts. These values are similar to those observed for the chilling-sensitive plants at temperatures above 12 °C.

As the reduction of $NADP^+$ from water is a multi-component, electron-transport system[10,11], the change in activation energy observed in Fig. 1 could be due

to a change in activation energy of any one of the individual reactions. It is possible to separate the light-driven electron transport into partial reactions representing Photosystem I and Photosystem II by the use of artificial electron donors and acceptors[11]. The effect of temperature on the activation energy of these partial reactions was also measured.

Effect of temperature on Photosystem II and Photosystem I activities

The reduction of DCIP from water by isolated chloroplasts measures the activity of Photosystem II[12]. The Arrhenius plots of this activity in chloroplasts of a chilling-sensitive (bean) and a chilling-resistant plant (pea) are compared in Fig. 2.

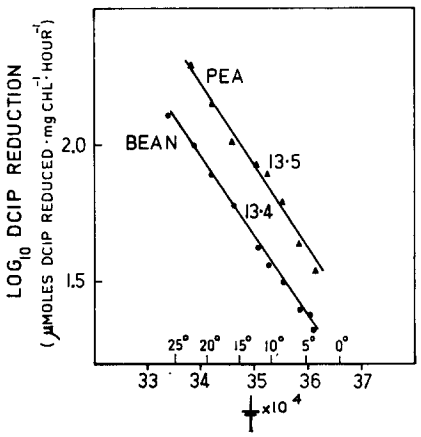

Fig. 2. Arrhenius plot of DCIP photoreduction by isolated chloroplasts of bean and pea. Reaction mixture was as for Fig. 1 except 20 nmoles DCIP replaced $NADP^+$ and ferredoxin.

Both showed constant and similar activation energies for the photoreduction of DCIP over the entire temperature range studied. Chloroplasts isolated from the leaves of maize, another chilling-sensitive plant, also exhibited constant activation energy in similar experiments. These data indicated that the temperature-induced change in activation energy found for $NADP^+$ photoreduction from water by chloroplasts from chilling-sensitive plants (Fig. 1) was not associated with Photosystem II. The activation energy for DCIP photoreduction is higher than that for $NADP^+$ photoreduction from water. A possible explanation for this observation is that the reaction between DCIP and the reductant produced in the light has a higher activation energy.

The reduction of $NADP^+$ from reduced DCIP, which involves only Photosystem I[13], exhibited a change in activation energy about 12 °C in the case of tomato chloroplasts as shown in Fig. 3. A similar result was obtained using bean chloroplasts. This effect of chilling temperatures on the activation energy of Photosystem I activity was not observed in tomato chloroplasts aged by storing at 0 °C for 6 h before starting the measurements as illustrated by the straight line in the same figure.

In contrast to freshly-prepared tomato chloroplasts, pea chloroplasts showed a constant activation energy for Photosystem I activity over the entire temperature range.

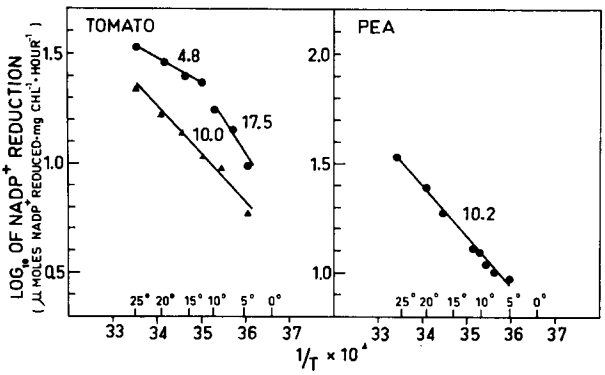

Fig. 3. Arrhenius plot of photoreduction of NADP+ from reduced DCIP by freshly-isolated tomato and pea chloroplasts and by aged tomato chloroplasts (kept at 0 °C for 6 h). The reaction mixture was as given for Fig. 1, except the following additions in μmoles: DCIP, 0.02; sodium ascorbate, 20; 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 0.01. ●—●, freshly isolated tomato chloroplasts; ▲—▲, aged tomato chloroplasts.

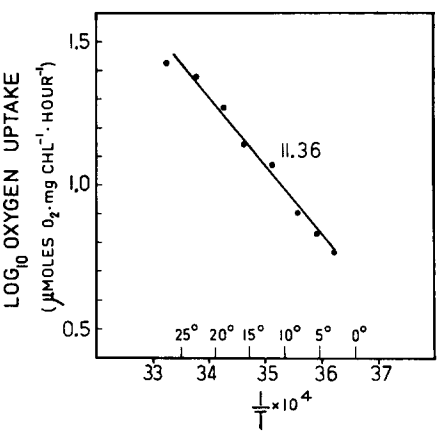

Fig. 4. Arrhenius plot of photoreduction of Diquat measured as O_2 uptake by isolated chloroplasts of bean. The reaction mixture in a total volume of 3 ml contained in μmoles: phosphate at pH 8.0, 27; $MgCl_2$, 2.7; sorbitol, 81; diquat, 0.1; NaN_3, 1.5 and chloroplasts containing 60 μg chlorophyll.

Photosystem I activity was also measured by the light-dependent reduction of diquat from water[11], a reaction which does not involve the ferredoxin–$NADP^+$ reductase or probably any other component of the electron-transport chain beyond the primary electron acceptor of Photosystem I[10]. As shown in Fig. 4 chloroplasts from the chilling-sensitive bean exhibit a linear Arrhenius plot for this reaction with an activation energy of 11.4 kcal·mole^{-1}. The marked difference in the response to chilling temperature of $NADP^+$ reduction from reduced DCIP and diquat reduction from water for chloroplasts of chilling-sensitive plants prompted an examination of the effect of temperature on the diaphorase activity of the terminal enzyme of the photosynthetic electron transfer pathway leading to $NADP^+$ reduction, namely, ferredoxin–$NADP^+$ reductase[10,14–17].

Effect of temperature on ferredoxin–NADP$^+$ reductase activity

Fig. 5 shows the Arrhenius plot for the reduction of DCIP by NADPH catalysed by the ferredoxin–NADP$^+$ reductase of isolated chloroplasts of tomato and pea[14–16]. The Arrhenius plot of this activity in tomato chloroplasts shows a change in activation energy at about 11 °C. This temperature is similar to the temperature at which a change in activation energy was observed for the photoreduction of NADP$^+$ from either water (Fig. 1) or reduced DCIP (Fig. 3) by tomato chloroplasts.

Fig. 5 also shows that the activation energy for ferredoxin–NADP$^+$ reductase of pea chloroplasts was constant for the whole temperature range.

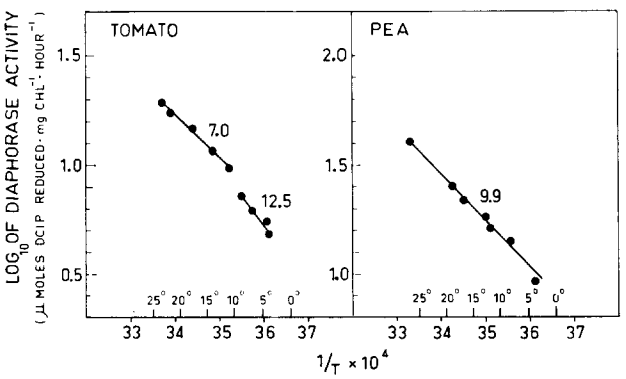

Fig. 5. Arrhenius plot of ferredoxin–NADP$^+$ reductase (measured as NADPH-diaphorase activity) of isolated chloroplasts of tomato and pea. The reaction mixture contained in a total volume of 1 ml in μmoles: phosphate at pH 8.0, 9; $MgCl_2$, 0.9; sorbitol, 27; DCIP, 0.02 and chloroplasts containing 5 μg chlorophyll. To initiate the reaction 0.25 μmole NADPH from a freshly prepared solution was added.

DISCUSSION

The results clearly demonstrate a major difference in the temperature response of the photosynthetic electron transport system in chilling-sensitive and chilling-resistant plants. Within the chilling-temperature range (10–12 °C) the electron transport system associated with the chloroplasts from chilling-sensitive plants exhibits an abrupt increase in activation energy at approximately 12 °C whereas no change in activation energy was observed with chloroplasts of chilling-resistant plants (Fig. 1).

This difference in temperature response resembles the differences previously observed in the effect of temperature on the kinetics of mitochondrial electron transport of these two groups of plants[1,2] and of homeothermic and poikilothermic animals[18]. The increase in activation energy for the photoreduction of NADP$^+$ from water by chloroplasts from tomato leaves (Fig. 1) and the increase in activation energy for succinate oxidation by mitochondria from tomato fruit (see ref. 1) occur at precisely the same temperature, 12 °C. Both of these processes are catalysed by enzymes closely associated with the membranes of these organelles and remain associated with the membrane during isolation of the organelles.

The change in activation energy for the overall photosynthetic electron transport process in chloroplasts of chilling-sensitive plants (Fig. 1) appears to be the

result of a temperature-induced increase in the activation energy of the terminal electron transfer reaction catalysed by ferredoxin–$NADP^+$ reductase. In the chloroplast, this enzyme is bound to the thylakoids[19]. Reducing the temperature does not alter the activation energy of the reactions associated with Photosystem II (Fig. 2) or the reactions from water to the primary electron acceptor in Photosystem I (Fig. 4). This is in contrast with the effect of temperature on the respiratory electron transfer system of mitochondria. In these organelles all of the reactions showed an increase in activation energy at the same temperature[2]. These observations are of particular significance to the question of the location of enzymes within the different organelles. For mitochondria the enzymes associated with the electron transfer system are integral components of the inner membrane and might therefore be expected to reflect changes in the conformation of the membrane. The fact that only the interaction of ferredoxin and $NADP^+$ in chloroplasts shows an increase in activation energy at chilling temperatures indicates either that this is the only enzyme-catalysed electron-transfer reaction in the system or that the enzyme is located in a region of the membrane which has physical properties different from the regions with which the other electron-transfer reactions are associated. These two possibilities result from the fact that an increase in the activation energy of an enzyme as a consequence of lowering the temperature is indicative of a phase change in the membrane[2,3,20].

As with the respiratory enzymes of mitochondria the temperature-induced increase in activation energy of the chloroplast enzyme is not an intrinsic property of the enzyme. It can be abolished by ageing the chloroplast (Fig. 3) in a similar manner to the abolition of the temperature-induced changes in mitochondrial enzymes by ageing and treatment with detergent[2]. Furthermore, the change in activation energies of the mitochondrial enzymes is a consequence of a thermal phase change in the lipids of the membranes to which the enzymes are bound[2,20]. Thermal phase changes in the lipids of mitochondrial membranes have been demonstrated by electron spin resonance of spin-labelled lipid analogues incorporated into the mitochondrial lipids. These lipid phase changes occur at the same temperature as changes in the activation energy of enzymes[3]. Using the same procedure, similar phase changes have also been observed in the membrane lipids of chloroplasts from chilling-sensitive plants and these occur at the same temperature as the increase in activation energy of the photoreduction of $NADP^+$ from water (Raison, J. K., unpublished). Thus the change in activation energy for the overall photosynthetic electron transport process as a result of lowering the temperature is most likely the consequence of a thermal phase change in lipid components of the chloroplasts membrane.

The presence of temperature-induced phase changes in membranes within the biological temperature range has been correlated with a relatively higher proportion of saturated fatty acids in the membrane lipids[3]. Mitochondria from chilling-sensitive plants[21] and homeothermic animals[18] contain a higher proportion of saturated fatty acids than mitochondria from chilling-resistant plants and poikilothermic animals and only in the two former groups of organisms have temperature-induced phase changes been detected[3]. With chloroplasts the high content of galactolipids, containing either one or two linolenic acid residues per mole[22], would suggest that thermal phase transitions in lipids and enzyme activity would only be evident well below 0 °C. The fact that they occur at approximately 12 °C suggests that the

galactolipids are not homogeneously distributed throughout the membranes. The loss of the temperature-induced increase in activation energy of $NADP^+$ photoreduction in aged tomato chloroplasts (Fig. 3) may be due to a loss of certain fatty acids from the lipids as incubation of spinach chloroplasts at room temperature results in a marked increase in the free fatty acid content of the chloroplasts[23].

The occurrence of thermal phase transitions in the membranes of both chloroplasts and mitochondria at temperatures below approximately 12 °C and the resulting increase in activation energy of enzyme systems associated with these membranes provides a biochemical explanation for the physiological manifestations of chilling injury. Below the temperature of the phase change electron transport in Photosystem I of chloroplasts of chilling-sensitive plants would be drastically reduced, in comparison to other electron transport reactions and reactions catalysed by "soluble" enzymes. The differential effect of chilling temperatures on these two enzyme systems causes imbalances in metabolism and the possible accumulation of metabolites toxic to the cell. The accumulation of ethanol and acetaldehyde in banana tissue as a result of storage at chilling temperatures has been reported[24] and is explicable in terms of the differential effect of chilling on enzymes associated with mitochondrial electron transport and the "soluble" enzymes of the glycolytic pathway. In addition disproportionate reduction in the rate of electron transfer at chilling temperatures in both chloroplast and mitochondria would result in a decrease in ATP production. This would most probably lead to a reduction in synthetic processes and in reactions essential for the maintenance of cellular integrity. For example a 40% loss of phosphatidylethanolamine from the mitochondrial membranes of sweet potato root (a chilling-sensitive plant) occurred upon storage at 0–1 °C for 14 days, consistent with a reduction in synthetic capacity[25]. Irreversible changes in chloroplast structure and function have also been noted in chilling-sensitive plants *Sorghum*, maize and *Paspalum* after 1.5–3 days exposure to mild chilling temperatures (10 °C) in the light[26]. These changes have been duplicated by artificially varying the pH gradients in chloroplasts and a decrease in the pH of thylakoid intraspace has been suggested as an explanation. While the data presented here neither support nor reject this view, changes in the kinetics of active transport of ions through membranes as a result of thermal-induced phase changes have been reported[27] and an alteration in the ion gradients across the thylakoid membrane would be consistent with a thermal phase change in the membrane lipids. Thus although it is not possible to designate the specific cause of the degeneration of chloroplast structure and function as a result of exposure to chilling temperatures the primary event appears to be a thermal phase transition in the membrane lipids, concominant changes in the physical properties of the membranes, and an increase in activation energy of the photosynthetic electron transfer system.

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