Plant, Cell and Environment (2000) **23**, 337–350

INVITED REVIEW

The ultrastructure of chilling stress

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ABSTRACT

Chilling injury to crop plants was first described 70 years ago and has been systematically investigated with electron microscopy since the late 1960s. Chloroplasts are the first and most severely impacted organelle. Thylakoids swell and distort, starch granules disappear, and a peripheral reticulum (vesicles arising from inner membrane of chloroplast envelope) appears. Chloroplast disintegration follows prolonged chilling. Mitochondria, nuclei and other organelles are less susceptible to chilling injury. Organellar development and ontogeny may also be disrupted. The inherent chilling sensitivity of a plant, as well as the ability of some species to acclimate to chilling, influence the timing and appearance of ultrastructural injury with the resulting outcome being mild, moderate, or severe. Other environmental factors that exacerbate injury are irradiance, chilling duration, and water status. The physiological basis for chloroplast swelling may be linked to chilling-stable starch-degrading enzymes that produce soluble sugars thus lowering stromal water potential at a time when chloroplast photosynthate export is reduced. Thylakoid dilation appears to be related to photo-oxidative conditions produced during chilling in the light. The peripheral reticulum is proposed to increase surface area of the transport-limiting membrane (chloroplast inner membrane) in response to the chilling-induced reduction in metabolite transport. Many of the ultrastructural symptoms appearing during moderate stress resemble those seen in programmed cell death. Future research directions are discussed.

Key-words: chilling stress; chloroplast ultrastructure; photosynthesis; programmed cell death; stress response.

INTRODUCTION

Low-temperature, or chilling, stress (damage caused by low, but above-freezing temperatures) has been recognized as a unique environmental impact on crop plant physiology for over 70 years. As early as 1926, Faris reported chlorotic bands on maize leaves that correlated to tissue damage caused by cold night time temperatures (Faris 1926). However, the phenomenon of chilling stress was not systematically investigated until the 1970s and 1980s with the many studies of van Hasselt in the Netherlands on cucumber (van Hasselt 1974), the comprehensive investigations by Taylor and colleagues in Australia on maize (Taylor & Craig 1971), and a series of papers by Kaniuga and colleagues in Poland on tomato (Gemel, Golinowski & Kaniuga 1986). All three pioneers investigated the impact of low temperatures on both the physiology and the ultrastructure of photosynthetic tissues.

Although there are a number of variables affecting the severity and time course of chilling injury, general ultrastructural symptoms are largely similar across species (Table 1). They include swelling and disorganization of both chloroplasts and mitochondria, reduced size and number of starch granules, dilation of thylakoids and unstacking of grana, formation of small vesicles of chloroplast peripheral reticulum, lipid droplet accumulation in chloroplasts, and condensation of chromatin in the nucleus (Jagels 1970; Taylor & Craig 1971; Nessler & Wernsman 1980; Murphy & Wilson 1981; Leddet & Geneves 1982; Wise, McWilliam & Naylor 1983; Musser *et al*. 1984; Gemel *et al*. 1986; Ma, Lin & Chen 1990; Ishikawa 1996; Yun *et al*. 1996). The present review will summarize what is known about the ultrastructural symptoms of chilling stress, focusing on the factors involved in the development of chilling injury and continuing with a discussion of the possible physiological bases of the injury. In order to do so, care must be taken to avoid confusing chilling injury with changes in plant cells that occur as a result of adaptations to repeated episodes of chilling temperatures, so-called 'cold hardening'. Hardening serves to protect the plant from subsequent seasonal freezing and is not generally considered to be injurious to the plant. It must also be noted that the inherent sensitivity of the plant to chilling affects the time course, extent and symptomology of injury, therefore wherever possible, species discussed in this review will be identified as will their chilling sensitivity.

THE SYMPTOMOLOGY OF CHILLING-INDUCED ULTRASTRUCTURAL INJURY

Chloroplast

Given the centrality of photosynthesis to plant physiology and its sensitivity to chilling stress (Taylor & Rowley 1971), it is not surprising that the chloroplast is commonly the earliest visible site of ultrastructural chilling injury in the plant cell (Kimball & Salisbury 1973). Under permissive temperatures, chloroplasts have numerous starch granules and well-developed granal stacks interconnected by stromal

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Table 1. A compilation of factors and symptoms observed in ultrastructural studies of chilling stress

Aspect of chilling stress	Symptom or observation	Reference
Chilling in the dark	Starch is depleted. Extent of chloroplast swelling and thylakoid dilation dependent upon chilling sensitivity.	Jagels 1970; Wise *et al*. 1983
Chilling in the light	Light exacerbates injury: Starch depletion, chloroplast swelling, thylakoid dilation, formation of peripheral reticulum and intraleaf light intensity gradient noted.	Jagels 1970; Murphy & Wilson 1981; Musser *et al*. 1984; van Hasselt 1974; Taylor & Craig 1971; Terishima & Inoue 1985
Concomitant water stress	Chloroplast swelling, lipid droplet accumulation, chloroplast disintegration seen. High relative humidity protects.	Wise *et al*. 1983
Duration of chilling	First stage: distorted thylakoid membranes, decreased starch, formation of peripheral reticulum. Second stage: disintegration of chloroplast envelope, lipid droplet accumulation. Third stage: unstacking of grana, dilation of thylakoid intraspaces.	van Hasselt 1974; Wise *et al*. 1983; Taylor & Craig 1971; Wu *et al*. 1997
Acclimation to chilling	Thylakoid membrane architecture (smaller grana stacks), leaf mesophyll cytoplasm, and phospholipid: free sterol ratio in leaf cell membranes all change.	Garber & Steponkus 1976; Huner *et al*. 1993; O'Neil *et al*. 1981
Recovery from chilling	Ability to recover is species-dependent; regreening may be impossible in extremely chilling-sensitive plants.	Jagels 1970; Murphy & Wilson 1981
Inherent chilling sensitivity	Mitochondrial swelling seen only in extremely chilling-sensitive plants. Plasmolysis occurs only in chilling-sensitive species; chilling stress alone may not be sufficient to damage highly chilling-resistant species.	Ishikawa 1996; Leddet & Geneve 1982; Murphy & Wilson 1981; Nessler & Wernsman 1980; Taylor & Craig 1971; Wise & Naylor 1987; Wise *et al*. 1983
Ontogenetic effects of chilling	Chloroplast development is retarded in chilling -resistant species.	Karpilova *et al*. 1981; Humbeck *et al*. 1994

thylakoids, and the two membranes of the chloroplast envelope are intact (Fig. 1a). Typically, the first manifestations of chilling injury are chloroplast swelling, a distortion and swelling of thylakoids, a reduction in the size and number of starch granules (Fig. 1b), and the formation of small vesicles of the chloroplast envelope (Figs 1c, 1g & 2a), called the peripheral reticulum (Wise *et al*. 1983). Prolonged chilling leads to accumulations of lipid droplets (Fig. 1d), a darkening of the stroma (Fig. 1e), unstacking of grana, and disintegration of the chloroplast envelope, the stromal contents mixing with the cytoplasm. Starch granules continue to diminish with time and, in plants that are chilling resistant, disappear completely (Fig. 1f) (Jagels 1970; Taylor & Craig 1971; van Hasselt 1974; Murphy & Wilson 1981; Musser *et al*. 1984). In addition to the above, other studies have reported a disorientation of grana with respect to one

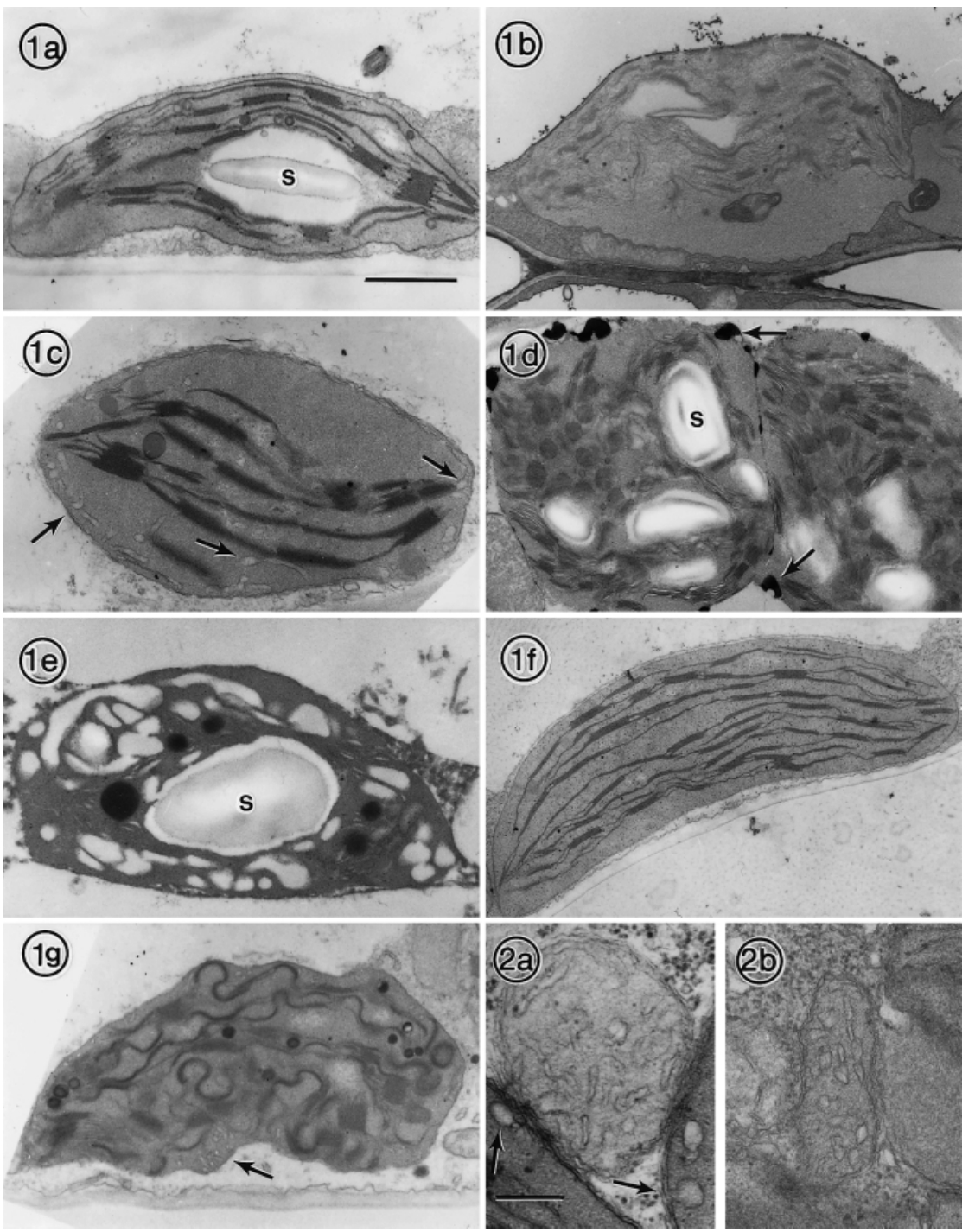
Figure 1. Symptoms of chilling injury in chloroplasts. Figure 1a, spinach (*Spinacia oleracea*, cv. Bloomsdale) chloroplast from a plant grown under permissive conditions (6 weeks of 12 h days at 300 μmol photons m^2 s^{-1} 23 °C and 12 h nights 21 °C) showing well developed grana and a single, large starch granule (s). Figure 1b, swollen cucumber (*Cucumis sativus*, cv. Ashley) chloroplast from a mature leaf treated for 9 h at 5 °C and 1000 μmol photons m^2 s^{-1}. Note also distorted thylakoids, and small starch granules. Figure 1c, cotton (*Gossypium hirsutum*, cv. Stoneville 213-Delta Pine) chloroplast treated for 72 h in the dark at 5 °C and 70% relative humidity (RH) showing several vesicles of the peripheral reticulum (arrows). Figure 1d, bean (*Phaseolus vulgaris*, cv. Blue Lake Bush Bean) chloroplast treated for 4 h at 5 °C, 500 μmol photons m^2 s^{-1}, and 70% RH. Note lipid accumulations (arrows) and tilted grana stacks. Figure 1e, bean (*Phaseolus vulgaris*, cv. Blue Lake Bush Bean) chloroplast treated for 144 h in the dark at 5 °C and 100% RH with swollen thylakoids and dark stroma. Figure 1f, collard (*Brassica oleracea*, var. *acephela*) chloroplast after 6 d in the dark at 5 °C and 70% RH. Except for lack of starch, the plastid from this chilling-resistant species appears normal. Figure 1g, 'serpentine' chloroplast from a mature bean (*Phaseolus vulgaris*, cv. Blue Lake Bush Bean) plant treated for 144 h at 5 °C, 16 h of 500 μmol photons m^2 s^{-1}, and 100% RH. Note also peripheral reticulum (arrow). Scale bar in Figure 1a equals 1·0 μm and applies to all of Figure 1.

Figure 2. Mitochondria from a chilling-resistant (pea) and a chilling-sensitive (cucumber) species. Figure 2a, pea (*Pisum sativum*, cv. Early Alaska) mitochondrion treated for 12 h at 5 °C and 1000 μmol photons m^2 s^{-1}. Vesicles of PR can be seen in adjacent chloroplasts (arrows). Figure 2b, cucumber (*Cucumis sativus*, cv. Ashley) mitochondrion treated for 6 h at 5 °C and 500 μmol photons m^2 s^{-1}. Scale bar in Figure 2a equals 0·5 μm and applies to all of Figure 2.

another and warping and bending of grana stacks (Fig. 1g), followed by the disruption and subsequent disappearance of stroma lamellae (Nessler & Wernsman 1980; Wise *et al*. 1983; Gemel *et al*. 1986; Yun *et al*. 1996). Curiously, chloroplasts in maize leaf mesophyll cells are more susceptible to chilling-induced injury than are the chloroplasts in adjacent bundle sheath cells (Slack, Roughan & Bassett 1974).

Mitochondrion

In contrast to chloroplasts, mitochondria appear to be more resistant to chilling temperatures (Fig. 2). The ultrastructure of mitochondria in leaves of *Nicotiana tabacum* L. was not noticeably affected by exposure to chilling temperatures, even during advanced stages of chloroplast degeneration



(Nessler & Wernsman 1980). The same was true of cucumber (Wise & Naylor 1987) and maize (Taylor & Craig 1971) mitochondria during chilling. On the other hand, Murphy & Wilson (1981) reported swelling and disorganization of mitochondria in *Episcia reptans* Mart., an extremely chilling-sensitive plant, after chilling for 6 h at 5 °C. Leddet & Geneves (1982) also observed dilated mitochondria in the chilling-sensitive *Ephedra vulgaris* (Richt.) after chilling for 24 h at 2 °C. Additionally, vacuolation and enlarged cristae were noted by Ishikawa (1996) in mitochondria of chilled mung bean cells (*Vigna radiata* L., var. Wilczek) – also chilling-sensitive. It would appear that only mitochondria in plants that are hypersensitive to cold are visibly affected by chilling.

Nucleus and other cellular structures

Effects of chilling temperatures on the nucleus are not as widely reported in the literature, either because visible changes rarely occur or they are not pronounced. However, condensation of chromatin was observed in the nucleus of chilled tissues of *Saintpaulia ionantha* (cv. Ritali, Yun *et al*. 1996) as were changes in the appearance of the nucleolus in *Triticum aestivum* L. (cv. Ducat, Gazeau 1985). Ishikawa (1996) reported swollen nuclei with fragmented chromatin and nucleoli, and bundles of microfilaments and other dense material in the nucleus and cytoplasm of cultured cells of chilled *V. radiata*. In addition, enlargement of Golgi vesicles and dilation of the endoplasmic reticulum were observed just prior to chilling-induced swelling of membranes. A similar vesiculation of *E. reptans* cytoplasmic membranes was reported by Murphy & Wilson (1981).

FACTORS INVOLVED IN THE DEVELOPMENT OF CHILLING-INDUCED ULTRASTRUCTURAL INJURY

Environmental stresses in plants rarely occur individually; chilling stress is no exception. There are many factors such as light intensity, relative humidity and the inherent sensitivity of the plant to chilling that interact and may either enhance the effect or act as protection against injury. Indeed, it is arguably next to impossible to control for all of these factors, and any treatment of the subject would be incomplete without consideration of their contribution to ultrastructural chilling injury.

Sensitivity to chilling

Some plants, by nature, are more resistant to chilling stress than others (Table 2). Therefore, an accurate discussion of ultrastructural chilling injury must include an identification of the species that is being described. Generally speaking, the more sensitive a plant is to chilling, the sooner and more extensive are the ultrastructural changes (Kimball & Salisbury 1973; Nessler & Wernsman 1980; Wise & Naylor 1987;

Table 2. Relative chilling sensitivity of selected species and observed chilling-induced symptoms

Species type	Observation	Reference
Extremely chilling-sensitive		
Ephedra vulgaris *Episcia reptans* *Gossypium hirsutum* *Saintpaulia ionantha* *Vigna radiata*	Only group in which chilling-induced injury to mitochondria has been observed. Rapid chilling injury results in cell plasmolysis. Re-greening not possible.	Ishikawa 1996; Leddet & Geneve 1982; Murphy & Wilson 1981; Wise *et al*. 1983; Yun *et al*. 1996
Less chilling-sensitive		
Cucumis sativum *Fragaria virginiana* *Glycine max* *Lycopersicon esculentum* Maize *Nicotiana tabacum* *Paspalum dilatatum* *Phaseolus vulgaris* *Pisum sativum* *Sorghum* spp.	Exhibit delayed response to chilling. Chloroplast swelling, thylakoid dilation, randomly tilted grana stacks, formation of peripheral reticulum, serpentine-like thylakoids, and accumulation of lipid droplets in the stroma all observed. Chloroplasts disintegrate with prolonged chilling.	Gemel *et al*. 1986; Karpilova *et al*. 1981; Taylor & Craig 1971; van Hasselt 1974; Wise *et al*. 1983
Chilling-resistant		
Arabidopsis thaliana *Brassica oleracea* *Cephaloziella exiliflora* *Hordeum vulgare* *Secale cereale* *Selaginella* spp. *Spinacia oleracea* *Triticum aestivum*	No chilling injury observed unless another stress factor is simultaneously imposed. Plastids remain intact. Re-greening only becomes impossible in advanced stages of chilling injury.	Garber & Steponkus 1976; Humbeck *et al*. 1994; Huner *et al*. 1993; Jagels 1970; O'Neil *et al*. 1981; Post & Vesk 1992; Wu *et al*. 1997

Ma *et al*. 1990). Some highly resistant plants may show no discernible effects from chilling, even for long periods of time, unless another stress factor is simultaneously imposed.

Wise *et al*. (1983) treated three species of differing chilling sensitivities to various combinations of chilling, irradiance and water stress. The most chilling-sensitive species (*Gossypium hirsutum* L., cv. Stoneville 213-Delta Pine) suffered damage within 24 h, with the response being total disruption of the chloroplast envelope and cell plasmolysis. A less chilling-sensitive plant (*Phaseolus vulgaris* L., cv. Blue Lake Bush Bean) showed a delayed response (within 144 h) characterized by chloroplast swelling, thylakoid dilation, randomly tilted grana stacks, and accumulation of lipid droplets in the stroma. The most chilling resistant of the three species (*Brassica oleracea* L., var. *acephela*) showed no signs of chilling injury until exposed to prolonged chilling, light, and water stress simultaneously. Even then, plastids remained intact.

Irradiance

The most obvious confounding factor and one that has received significant attention is the effect of light on the development of chilling injury. This is logical because it is the chloroplast, the light-harvesting organelle, whose ultrastructure is first affected by chilling stress (see above). Irradiance during chilling greatly exacerbates the injury due to chilling stress alone (Wise *et al*. 1983). In complete darkness, growth of chilled *Selaginella* spp. was halted but the plants remained green and, except for starch depletion, chloroplasts appeared normal (Jagels 1970). However, during chilling in the light, chlorophyll was bleached, lipid droplets accumulated, and the thylakoids degenerated (Jagels 1970). In *Sorghum* (hybrid NK 145) leaves there was a gradient in the degree of chloroplast ultrastructural damage during the early stages of light-induced chilling stress, with injury most pronounced near the surface of the leaves exposed directly to light (Taylor & Craig 1971). These effects were attributed to light intensity gradients within the leaf, the presence of which were subsequently well described by Terashima & Inoue (1985). On the other hand, chloroplasts in the phloem parenchyma of *Paspalum dilatatum* Poir underwent ultrastructural changes before chloroplasts in the lower mesophyll (Taylor & Craig 1971). In this latter instance, the presence of starch was postulated by the authors as a 'translocatable retardant' of chilling stress.

Low temperature also accelerates chromoplast formation under moderate to high light conditions, the ramifications of which are species-dependent. Chloroplast to chromoplast conversion is in many species, depending upon the plant tissue, part of the normal process of senescence or fruit ripening. However in *Selaginella* spp., chromoplast formation was found to be a dynamic process in which repeated chloroplast to chromoplast interconversion occurs in response to changes in environmental conditions (Jagels 1970). Chromoplast formation was also observed in the Antarctic liverwort *Cephaloziella exiliflora* (Tayl.) Steph. during adaptation to environmental conditions (Post & Vesk 1992).

Duration of chilling

Ultrastructural chilling injury develops progressively with time (Wise *et al*. 1983; Ma *et al*. 1990). The longer a plant is exposed to chilling conditions, the more extensive and irreversible is the injury. For ease of discussion, some researchers have arbitrarily organized the chilling-induced changes into discrete developmental stages (Taylor & Craig 1971; van Hasselt 1974).

The earliest stage of chilling-induced injury can be characterized by swelling and distortion of chloroplast thylakoid membranes, decreased number and size of starch granules, and formation of small vesicles of the chloroplast envelope called the peripheral reticulum (van Hasselt 1974; Wise *et al*. 1983). Ishikawa (1996) also observed whorls of rough endoplasmic reticulum (RER) surrounding clear regions of cytoplasm, markedly rough vacuolar membranes, plastids and mitochondria with vacuoles, enlargement of Golgi vesicles, and dilation of the RER in chilled cells of *V. radiata*. The next stage involves a continued disintegration of the envelope and accumulation of lipid droplets with increased staining of the stroma (Wise *et al*. 1983). More advanced stages of chilling result in unstacking of grana and marked dilation of thylakoid intraspaces (Taylor & Craig 1971; van Hasselt 1974; Wu *et al*. 1997).

Vapour pressure deficit

Much work has been done on the effects of water stress alone on plant cell ultrastructure (Fellows & Boyer 1978). However, relative humidity is a factor that is frequently ignored in studies of chilling injury. High relative humidity (100%) was found to be protective to chloroplasts in both cotton (chilling-sensitive) and bean (chilling-resistant), and the protective effect was enhanced by treatment in the dark (Wise *et al*. 1983). Injury was still possible, however, in a highly chilling-sensitive species like the tropical *E. reptans*, even in a saturated atmosphere (Murphy & Wilson 1981).

Ontogenetic effects

Chilling can affect the growth and development of plants and their internal structures. Karpilova *et al*. (1980) looked at the effect of non-freezing, chilling temperatures on the ultrastructure of organelles in *Cucumis sativus* (var. Klinskie Mnogoplodyne) at different stages of development. They found an increased number of thylakoids and starch grains in chloroplasts of chilled plants as compared with control plants during the initiation of visible leaf growth. The cytoplasm and other organelles were not appreciably affected at this stage. During the cell elongation phase of growth, an increase in the number of lipid droplets was observed in chloroplasts of chilled plants. The third growth phase, identified by the cessation of leaf growth and the start

of senescence, was characterized by a more well-developed lamellar granal structure in chloroplasts of chilled plants as compared with control plants; the stromal lamellae were especially prominent. Karpilova *et al*. (1980) interpreted these developmental changes as adaptive in nature and connected them to the general retardation in growth processes observed in plants grown under chilling conditions. In this regard, they also observed that the export of photosynthate in plants grown under favourable conditions reached its peak from leaves that had completed their growth; whereas under chilling conditions, peak photosynthate export was reached in leaves whose area comprised only about 60% of maximum. Humbeck, Melis & Krupinska (1994) also reported retardation of chloroplast development in leaves of *Hordeum vulgare* (cv. Carina) grown at 5 °C, evidenced by a higher photosynthetic capacity per unit chlorophyll, a smaller chlorophyll content per fresh weight and decreased efficiency of Photosystem II centres. Humbeck *et al*. (1994) noted that others have found the opposite effect in chilling-sensitive plants such as maize and tomato. This reinforces the importance of the differing ability of species to acclimate to low temperatures, i.e. to harden.

Acclimation/hardening

Acclimation, or hardening, may be defined as changes that occur in a plant in response to chilling temperatures which confer subsequent tolerance to the cold (Huner 1985; Huner *et al*. 1993). It is the very ability of plants to undergo such changes that accounts for major differences in chilling susceptibilities between species and protects against irreversible chilling-induced injury. The exact mechanism by which hardening occurs is as yet unknown and is likely to differ somewhat by species (Senser & Beck 1984).

Cellular membranes have been the focus of much attention in studies of the mechanism of cold hardening. Garber & Steponkus (1976) investigated the effects of cold hardening temperatures on thylakoid membranes of *Spinacia oleracea*, a cold-hardy species (see Table 1). They reported the formation of a paracrystalline array of proteins in thylakoid membranes of cold-acclimated plants, as observed by freeze-fracture electron microscopy. They also noted a decreased particle concentration on the inner fracture face of the thylakoid membranes and a homogenization of particle size as compared to particles in membranes of non-acclimated thylakoids, which are of two sizes. It is generally accepted that membranes split along the hydrophobic region when fractured in the frozen state. These differences therefore could be attributed to an alteration in the hydrophobic region of thylakoid membranes following cold acclimation. The identity of the particles was not investigated, but it was suggested that this phenomenon may, in part, be due to increases in membrane lipid unsaturation.

Huner *et al*. (1984) also observed a loss in the bimodal nature of particle size distribution and an increase in particle size in membranes of freeze-fractured thylakoids of *Secale cereale* (cv. Puma), a winter-hardy species. They did not notice a difference, however, in particle concentration between acclimated and non-acclimated plants. In addition, they found cytological differences between leaf mesophyll cells of acclimated versus non-acclimated plants: cold-hardening temperatures caused the formation of both univacuolate and multivacuolate mesophyll cells in acclimated plants. Non-acclimated mesophyll cells were all univacuolate. Chloroplast ultrastructure was affected by cold hardening as shown by an increase in smaller granal stacks. Chlorophyll, β-carotene, and xanthophyll content increased at cold-hardening temperatures, but photosynthetic unit size remained the same.

Phospholipid content increased two-fold in leaf membranes of the cold-sensitive *Fragaria virginiana* Duchesne, with a proportionately smaller increase in free sterols during cold hardening (O'Neil, Priestly & Chabot 1981). Those authors attributed these findings to the proliferation of the endoplasmic reticulum in cold hardened plants as evidenced by an increase in vesiculated smooth endoplasmic reticulum and tonoplast membrane in electron micrographs of cold-hardened leaf cells (see also Pomeroy & Andrews 1978). In this way, they hypothesized, freeze tolerance was increased because there was more substrate available for vesicle formation and subsequent extension of the plasma membrane and tonoplast. This protected the plant from freezing presumably by allowing the plant to adapt to the reduction in membrane surface area that occurs during freeze-induced dehydration and subsequent rehydration during thawing. Ristic & Ashworth (1993), likewise, reported significant invaginations of the plasma membrane and fragmented endoplasmic reticulum (ER) during cold acclimation of *Arabidopsis thaliana*.

Recovery

Another factor to consider in the etiology of ultrastructural chilling injury is the ability of plants to recover after exposure to chilling temperatures, and at what point recovery becomes impossible. As is the case with other factors, recovery from chilling injury is species-dependent and probably related to a plant's inherent chilling sensitivity. For example, in the chilling-resistant *Selaginella* spp., it was not until the advanced stages of chilling injury, in which swollen grana were observed, that re-greening of the leaves became impossible (Jagels 1970). In contrast, the chilling-sensitive plant *Episcia reptans* exhibited swelling and disorganization of chloroplasts and mitochondria, and vesiculation of cytoplasmic membranes after only a few hours at 5 °C; rewarming these plants after 5–6 h of chilling resulted in further damage to chloroplast and mitochondrial structure (Murphy & Wilson 1981).

THE PHYSIOLOGY OF CHILLING-INDUCED ULTRASTRUCTURAL INJURY

Unlike other environmental stresses such as drought, high light or air pollution, the imposition of chilling represents a large, instantaneous, and simultaneous change in the thermodynamic microclimate of every molecule in every cell of

a plant. Enzymatic reactions will be instantly slowed due to decreases in substrate diffusion rates. Membrane properties will be immediately altered, although the hypothesis that a membrane phase change might be the overriding 'primary sensor' of chilling temperatures (Wolfe 1978; Lyons, Graham & Raison 1979) has not survived in its simplest form (Martin 1986). Finally, transport processes across membranes would be interrupted by lowered temperatures as well. Any ultrastructural damage therefore is likely to be a direct or indirect consequence of these primary effects.

Transmission electron microscopy (TEM) is, by its very nature, an excellent tool for describing the appearance or location of a structure, but the amount of information TEM can yield on how or why a structure looks that way (i.e. mechanistic information) is rather more limited. In spite of this, and with the recognition of the various symptoms as described above, it is possible to make some conjecture regarding the various physiological mechanisms of chilling-induced ultrastructural injury. Because the chloroplast is the first and most severely impacted organelle during chilling injury, and because more studies have been done of chloroplasts than any other organelle during chilling injury, it will be the focus of the following discussion.

Chloroplast swelling during chilling

Chloroplast swelling is an almost universal symptom in studies of the effects of chilling temperature on cellular ultrastructure (see above). (As an interesting corollary, we are unaware of any reports of chloroplast shrinkage during chilling stress.) This leads to the conclusion that an increase in stromal osmolytes accompanies chilling stress.

There are several lines of evidence to implicate starch degradation products as being the osmotically active agents involved in chloroplast swelling:

1. The disappearance of starch granules during chilling stress has been repeatedly noted (Jagels 1970; Taylor & Craig 1971; van Hasselt 1974; Murphy & Wilson 1981; Wise *et al*. 1983; Musser *et al*. 1984).
2. Starch-degrading activities in potato tubers (Deiting, Zrenner & Stitt 1998), tomato leaves (Bruggemann, Kooij & van Hasselt 1992), and xylem ray parenchyma cells (Sauter & Kloth 1987; Sauter & van Cleve 1991) remain high at low temperatures. Unfortunately, very little is known about the enzymes involved in starch degradation in photosynthetic tissues (Caspar *et al*. 1991), but it may be safe to conclude that chloroplastic amylases are likewise cold-tolerant, even in chilling-sensitive species.
3. Transport of photosynthate out of the chloroplast may be reduced by chilling temperatures. Whether or not triose phosphate export from the chloroplast is directly inhibited has yet to be determined. However, protein uptake by chloroplasts, an energy-requiring process, is inhibited in the cold due to the lack of a sufficient trans-envelope proton motive force (Leheny & Theg 1994). It is therefore probable that photosynthate export from the chloroplast, another energy-requiring process, may likewise be inhibited. Photosynthate export may also be inhibited indirectly by a chilling-induced decrease in phloem loading (Gamalei *et al*. 1994) or phloem transport (Mitchell & Madore 1992). Leaf soluble sugars tend to increase during chilling (Strand *et al*. 1997) but the compartmentation of the sugars has, to our knowledge, not been directly investigated.

Thylakoid dilation due to photo-oxidative conditions during chilling

Chilling-sensitive plants are known to experience severe photo-oxidation during chilling in the light (see Wise 1995 for a review), and thylakoid dilation is a common symptom under such environmental conditions (see above). Thylakoid dilation is also seen upon the application of photo-oxidative herbicides such as paraquat (Harvey & Fraser 1980) and atrazine (Ashton, Gifford & Bisalputra 1963). This suggests a link between the thylakoid dilation seen during chilling and the presence of photo-oxidation induced by chilling. As of yet, that link remains to be mechanistically proven.

Development of the chloroplast peripheral reticulum during chilling

The chloroplast peripheral reticulum (PR) is an oddity of chloroplast biology that has received rather limited attention but is seen to occur in a variety of non-stressed tissues (Wise & Harris 1984). It was first recognized as a discrete structure by Shumway & Weier (1967) and in 1968 Laetsch proposed that it might be involved in rapid transport of metabolites into or out of the chloroplast. The transport role is supported by direct connections of the PR with the inner membrane of the chloroplast envelope (Shumway & Weier 1967; Laetsch 1968), the membrane known to control metabolite transport (Heldt & Saur 1971). In addition, freeze fracture TEM showed the PR and inner chloroplast membrane to have similar particle sizes and distributions (Sprey & Laetsch 1978). Therefore, the idea that the PR is involved in transport gains support from structural studies. The only mechanistic study of PR function of which we are aware (Mosejev & Romanovskaya 1988) demonstrated that the PR plays a role in the sequestration of Ca^{+2} ions, an idea that is not at odds with the transport hypothesis.

The chloroplast PR frequently appears as a consequence of chilling injury (Musser *et al*. 1984) and does so in a temperature-dependent fashion (Morré *et al.* 1991). Wise *et al*. (1983) found that a PR developed in *Phaseolus vulgaris* and *Gossypium hirsutum* plants that were chilled in the light and at a high humidity. Those conditions were sufficient to impose a significant stress on the plants but not so severe that the plants died immediately. They concluded that the PR develops to increase inner chloroplast membrane surface area during a chilling-induced decrease in transport capacity (see above for a discussion how chilling temperatures may effect photosynthate export from the

chloroplast). Morré *et al*. (1991) came to an alternative conclusion in their study of chilling stress in leaf discs of tobacco, pea, spinach, and soybean. They proposed that the PR (called the 'stromal low temperature compartment' in their study) serves to increase transport of lipids and thylakoid constituents from the envelope to the thylakoid during acclimation to the low temperatures. Given that the PR also appears during tissue senescence (Greening, Butterfield & Harris 1982), a time during which transport of components out of the chloroplast is high and thylakoid membrane synthesis is low, the transport function for the PR originally proposed by Laetsch (1968) seems more likely than the one proposed by Morré *et al.* (1991).

The use of mutants in studies of chilling and ultrastructure

Mutants have seen only limited use in studies of the ultrastructure of chilling sensitivity. A study on maize by Millerd, Goodchild & Spencer (1969) noted a loss of chloroplastic ribosomes and therefore subsequent repair as the major lesion which imparted chilling sensitivity to the mutant. Wernsman and colleagues (Matzinger & Wernsman 1973; Nessler, Long & Wernsman 1980; Nessler & Wernsman 1980) found the chilling sensitivity of a tobacco mutant to be maternally inherited and linked the ultrastructural damage to non-specific losses of chloroplast function (i.e. decreased pigments and Rubisco activity). A sweetclover (*Melilotus alba*) mutant that exhibited a loss of grana stacking under chilling conditions (Bevins, Madhavan & Markwell 1993) was found to be lacking the light-harvesting complexes of PSII (LHC-II). Because thylakoid stacking is known to be almost entirely dependent on binding interactions between light-harvesting complexes (Allen 1992), the chilling-induced loss of the LHC-II led to agranal chloroplasts. Browse and colleagues have extensively studied a series of *Arabidopsis thaliana* mutants with specific defects in leaf lipid composition (Browse & Somerville 1991; Somerville & Browse 1991), some of which have an increased sensitivity to low temperatures. However, after investigating numerous aspects of the physiology, biochemistry, and ultrastructure of these mutants, they concluded that 'although the phenotypes that have been described show some parallels to the physiology of chilling-sensitive plants, a detailed characterization of the mutants does not recommend that these mutants be used as models for the processes involved in chilling injury' (Wu *et al*. 1997 and references therein). Thus, to our knowledge, a true chilling-sensitive *Arabidopsis thaliana* mutant has yet to be described.

CAN CHILLING STRESS INDUCE PROGRAMMED CELL DEATH?

There are essentially two ways a cell can die. One is as a result of severe traumatic injury, as in exposure to high levels of a toxin or by freezing. Referred to as necrotic cell death, it is typically characterized by swelling of the cell and subsequent lysis resulting in leakage of cellular contents. The other way a cell can die is by the activation of a genetically encoded suicide pathway that begins with the expression of unique proteins involved in the systematic dismantling of the cell. Referred to as programmed cell death (PCD), this type of cellular death can occur both as a response to environmental stress and as a way to regulate the growth and development of an organism. It is characterized by a controlled disassembly of the cell and, in animal cells, results in the compartmentalization of cellular contents and transport of those contents out of the cell.

Programmed cell death in animals has been studied intensively (Jacobson, Weil & Raff 1997; Nagata 1997; Nicholson & Thornberry 1997). This phenomenon, in animals called apoptosis, is defined by condensation of chromatin and fragmentation of DNA in the nucleus, shrinkage of the cytoplasm, vesiculation of cellular membranes with formation of 'apoptotic bodies', and subsequent blebbing of the plasma membrane and phagocytosis of the cell. It requires the involvement of Ca^{2+}-dependent signal transduction pathways, the production of reactive oxygen species (ROS), and the activation of a cascade of proteolytic enzymes. Although not as well characterized, there is strong evidence that programmed cell death also occurs in plants (Greenberg 1996; Jones & Dangl 1996; Pennell & Lamb 1997; Buckner, Janick-Buckner & Gray 1998). PCD in plants can occur as a normal developmental process, such as in terminal tracheary element differentiation or as in the seasonal or environmentally induced senescence of older leaves. It may also occur as a response to an environmental stress such as pathogen attack, physical wounding, or exposure to low levels of toxins (see Buckner *et al*. 1998 for a review of PCD in maize). Although an increasing number of ultrastructural and biochemical markers of PCD appear to be shared between animal and plant cells (Mittler *et al*. 1997; Del Pozo & Lam 1998; D'Silva, Poirier & Heath 1998; Wang *et al*. 1998; Xu & Heath 1998; Yen & Yang 1998), it is unlikely that the process is identical between kingdoms because of inherent organismal differences in structure and function. Indeed, both the cause of cell death and therefore the utility to the organism can vary with the species and depend upon the circumstances leading to death. Inherent in the process, however, is the requirement for active cell metabolism and the transcription and translation of key regulatory proteins that will direct the fate of the cell. A number of studies have hinted at the possibility of such a process at work in the cells of cold-stressed plants (Ishikawa 1996 ; Yun *et al*. 1996; Wu *et al*. 1997). However, to our knowledge, no one has attempted to identify the ultrastructural or biochemical markers of PCD in the cells of chilled plants. Yet the ultrastructural evidence for the existence of this phenomenon in cold-stressed plants remains compelling.

Ultrastructural evidence for PCD during chilling stress

Yun *et al*. (1996) reported rapid (within 3 s) ultrastructural changes only in the palisade cells of the chilling-sensitive

plant *Saintpaulia* under conditions of low temperature and high humidity. These cells exhibited a disordered arrangement of the thylakoid intergranal lamellae, shrinkage of the cytoplasm away from the cell wall, and condensation of the chromatin in the nucleus. Physiologically the cells remained active, although a reduction in chlorophyll fluorescence and decreased photosynthetic activity was observed. The prominent physical symptom of injury in *Saintpaulia* under these conditions was cell death in limited areas of the leaf, called leaf spot.

Ishikawa (1996) also observed condensation and fragmentation of chromatin in chilled cultured cells of the chilling-sensitive mung bean. Chilling injury in these cells proceeded in an orderly and predictable fashion: early observations (within 6 h) included vesiculation of cellular membranes, including the ER and tonoplast, and vacuolation of plastids and mitochondria. Enlarged Golgi vesicles and dilated ER arranged in parallel arrays surrounding clear areas of the cytoplasm preceded the swelling of organelles. After 72–96 h, the cytoplasm condensed, and vesicles fused with vacuolar membranes releasing their contents. Cells in later stages of chilling exhibited shrinking of the cytoplasm away from the cell wall and digestion of organelles and ultimately of the cell itself. Physical symptoms, in this case, were irrelevant as the cells were grown in culture.

Arabidopsis thaliana is a chilling-resistant plant. Nevertheless, Wu *et al*. (1997) demonstrated a similar sequence of events in temperature-sensitive Arabidopsis mutants chilled at 2 °C for 28 d. Although more chilling-sensitive than their wild-type counterparts, these plants were not permanently damaged by chilling treatment. (Despite a slower growth rate, the plants were able to complete their life cycle over the entire 28-day chilling process.) Interestingly however, by day 14, chloroplasts had become disorganized with broken envelopes, starch grains disappeared, and thylakoid membranes became serpentine-like in appearance, reminiscent of those reported by Wise *et al*. (1983) in *P. vulgaris* stressed by chilling in the light. The chloroplasts in the Arabidopsis mutant were associated with small membrane-bound vacuoles at their periphery. In all other respects, the cells appeared normal. After 21 d at 2 °C, the chloroplasts had completely disappeared from the Arabidopsis mutant – the leaves appearing chlorotic. The authors referred to these changes as a form of autophagy, in which there is a regulated metabolic breakdown of chloroplasts, providing access to stored reserves that may sustain the plant until more favourable conditions prevail. Consistent with their hypothesis, the mutant plants retained the ability to recover completely upon transfer to permissive temperatures.

The above examples of ultrastructural chilling injury to plant cells show remarkable similarity to the ultrastructural changes that occur during demonstrated programmed cell death in plants grown at permissive temperatures, i.e. disintegration of chloroplasts and vesiculation of cellular membranes (Bleeker & Patterson 1997; Buchanan-Wollaston 1997), vacuolation and shrinkage of the cytoplasm (Mittler *et al*. 1997), and nuclear DNA condensation and fragmentation (Mittler *et al*. 1997; Wang *et al*. 1998; Yen & Yang 1998). In addition, the physiological response of plant cells exposed to low, above-freezing temperatures also resembles that seen in putative programmed cell death.

Physiological evidence for PCD during chilling stress

An early sign of impending cell death in some forms of plant PCD is the generation of reactive oxygen species (ROS) (MacRae & Ferguson 1985; Levine *et al*. 1994) and superoxide (Jabs, Dietrich & Dangl 1996), in some cases resulting in an increase in free cytoplasmic Ca^{+2} (Levine *et al*. 1996; Xu & Heath 1998). Ca^{+2} is a secondary messenger commonly used by plants in signal transduction (Snedden & Fromm 1998). ROS are known to be generated by chilling plants in the light (Wise 1995). Although a primary sensor of low temperature in plants has yet to be established, a redistribution of intracellular Ca^{+2} in response to chilling was reported by Woods, Polito & Reid (1984b) as an early event. Subsequently, a theory arose in support of a role for Ca^{+2} in the transduction of the events leading to chilling-induced injury (Minorsky 1985).

One consequence of elevated free Ca^{+2} in the cells of plants undergoing PCD at permissive temperatures is the activation of a cascade of proteolytic enzymes involved in the ultimate cleavage of key proteins, which in turn induces the systematic death of the cell. An example is the Ca^{+2}-dependent endonuclease responsible for the fragmentation of nuclear and chloroplastic DNA (Mittler & Lam 1995; D'Silva *et al*. 1998). Other substrates for these proteases include actin, tubulin, and other cytoskeletal proteins (Mittler, Simon & Lam 1997). The fragmentation of DNA in the nucleus of chilled plant cells has been discussed previously (see above). The effect of chilling temperatures on cytoskeletal proteins was investigated by Woods, Reid & Patterson (1984a), who found that prolonged exposure of plant cells to low above freezing temperatures disrupts cytoskeletal structure. The molecular triggers for these events in chilling stress remain to be demonstrated.

A proposed mechanism for the initiation and propagation of PCD during chilling stress

Although evidence for the role of PCD in the observed chilling-induced injury of plant cells is admittedly preliminary, it may be possible to propose a mechanism by which PCD may be induced in cold-stressed plants (see Fig. 3). That is the goal of the following section. Our hope is to stimulate further investigation into the molecular and cellular events that may occur during this phenomenon.

As previously mentioned, chilling-induced injury is first observed in the chloroplast. We suggest that the generation of ROS in the chloroplast, induced by conditions that result in the delayed response of plants to chilling, triggers an increase in free Ca^{+2} ions. Ca^{+2} ions induce changes in protein phosphorylation and act as second messengers to

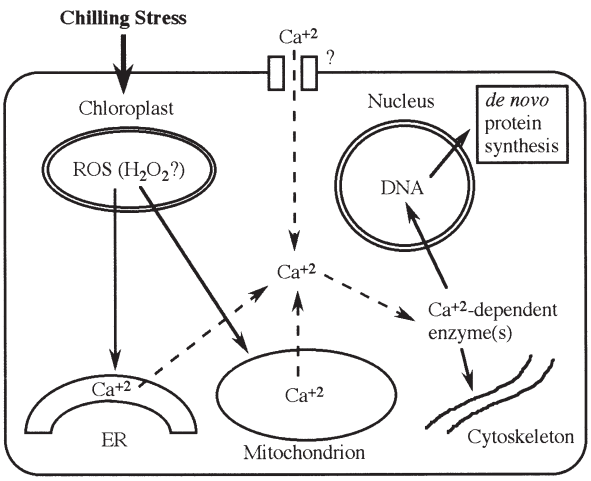


Figure 3. Initial steps in the proposed mechanism of PCD in chilling-induced injury. According to the model, the delayed response of plants to chilling, the proposed condition for the onset of PCD in chilled plant cells, is triggered by the ROS-induced influx of Ca^{+2} from intracellular and possibly extracellular stores (denoted by broken arrows). This leads to changes in calcium concentrations in both the cytoplasm and organelles. Free Ca^{+2} ions bind to calcium-dependent proteins, which interact with other cellular processes and structures, including the cytoskeleton. These Ca^{+2}-bound proteins may also result in changes in protein phosphorylation and stimulate a cascade of proteolytic enzymes that cleave key proteins and thereby regulate the process. Ca^{+2} might also regulate gene expression by acting as a second messenger in a signal transduction process, leading to the translation of novel proteins that further regulate the delayed response. Later effects include the Ca^{+2}-mediated fragmentation of DNA molecules – a key indicator of programmed cell death.

stimulate a variety of processes (Snedden & Fromm 1998), including a cascade of proteolytic enzymes (such as caspases) that selectively cleave key cellular proteins. Free Ca^{+2} may also be involved in the fragmentation of both chloroplast and nuclear DNA, by way of Ca^{+2}-dependent endonucleases that cleave DNA molecules into shorter fragments for more efficient packaging. Free Ca^{+2} ions affect membrane permeability and therefore the transport of metabolites into and out of the chloroplast by way of the PR.

Interaction with some cellular processes may be mediated by Ca^{+2} binding to calmodulin or other calcium-modulated proteins, in turn activating a variety of transcription factors resulting in the expression of genes and leading to the *de novo* synthesis of proteins involved in regulation of the cell-death process. Ca^{+2}-bound proteins and changes in protein phosphorylation are also implicated in the chilling-induced inhibition and disappearance of cytoskeletal proteins (Woods *et al*. 1984a), which is accompanied by a marked vesiculation of the cytoplasm (Woods *et al*. 1984b). The loss of integrity of the cytoskeleton may be responsible for the shrinkage of the cytoplasm away from the cell wall that is characteristic of later stages of chilling-induced injury (see Fig. 4 for a summary.) At this stage, vacuoles form in organelles and in the cytoplasm of the cell (Ishikawa 1996; Wu *et al*. 1997). Vesicles, filled with cellular contents, fuse with the vacuoles. The chloroplasts and other organelles are digested and ultimately form one large vacuole, which contains the essential elements of the cell including carbon, nitrogen, and nucleic acids. The cell, at last, phagocytoses, and its contents are transported to other less vulnerable parts of the plant.

The effect of this process is the rescue of the cell's

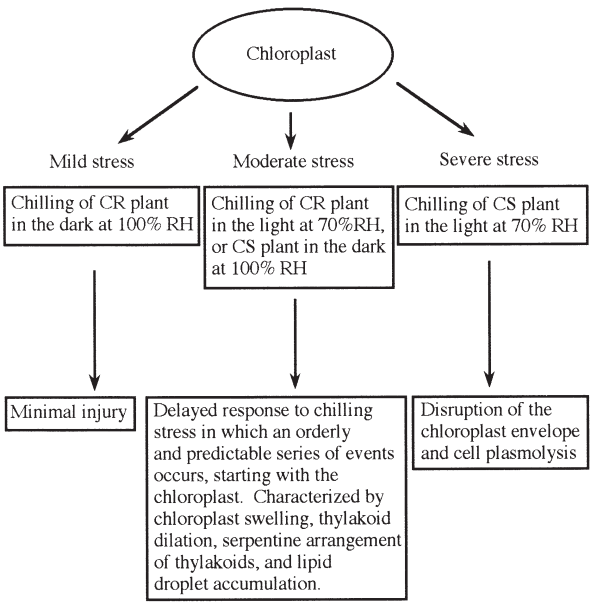


Figure 4. Interrelationship between environmental conditions and inherent chilling sensitivity on the course of chilling injury to the chloroplast. The chloroplast is the earliest visible site of ultrastructural chilling injury. The severity of the stress to the chloroplast is influenced both by the inherent sensitivity of the plant to chilling and by the conditions under which the plant is chilled. It is a dynamic relationship in that a chilling-sensitive (CS) plant, like cotton, will experience severe injury under multiple stresses but may be protected by high relative humidity (RH) or dark conditions. Conversely, a less chilling-sensitive plant, like bean, may experience moderate injury with chilling alone but severe injury with the introduction of multiple stresses. Some plants, like collard, are so resistant to chilling that even prolonged chilling has no visible effect unless multiple stresses are applied. Even then they are generally reversible upon return to ambient conditions. The delayed response to chilling, triggered by moderate stress to the plant, is proposed to be a likely candidate for the initiation of a regulated sequence of events leading to the systematic dismantling of the chloroplast and then of the cell, in some cases leading to cell death. It is hypothesized that, under less than optimal conditions (as defined by the inherent chilling sensitivity of the plant), some plants may have developed a tactic for coping with temporary non-permissive environmental conditions. These plants may undergo a genetically programmed metabolic process which has the effect of salvaging and packaging valuable resources, like carbon and nitrogen, for maintenance of metabolic activities in the short term or for transport to less vulnerable parts of the plant under stress of longer duration. CR, chilling-resistant.

resources under non-permissive environmental conditions in order to maintain basic metabolic processes, or in extreme cases the self-destruction of the cell, resulting in a surplus of valuable resources that may carry the remaining plant cells through the duration of the physical stress.

Induction of PCD during chilling stress

The delayed response to chilling is the most likely situation in which a plant might undergo a cell suicide process. The sequence of events and the ultrastructural changes that characterize the delayed response are remarkably like those of other cell-death mechanisms. Also, the plant species involved and the conditions under which they exhibit this response lend themselves to such a process. Extreme conditions, or species that are particularly sensitive to subtle changes in temperature would, by nature, result in a rather quick demise of the whole plant. On the other hand, chilling-resistant plants, or less resistant plants affected by mild chilling stress would have little to gain by undergoing such an energetically wasteful process. We propose three hypothetical paths that can be taken by a plant exposed to chilling stress:

1. One path is characteristic of extremely chilling-resistant species, like collard or spinach (Table 2). On this path, even prolonged chilling compounded by multiple stresses, like high light and/or high vapour pressure deficit, produce minimal injury; the effects are generally reversible. Such plants respond by initiating hardening processes instead of PCD.
2. A second path characterizes extremely chilling-sensitive plants, like *E. reptans* (Table 2). Plants that exhibit this path are generally of tropical origin that have had no selective pressure to develop such an adaptive process because they are rarely exposed to temperatures below 15 °C. When artificially exposed to such conditions, injury is rapid and irreversible.
3. The third path is the delayed response. It is characterized by a predictable sequence of events that is encoded in the genome of plants that live in highly unpredictable environments. By its very nature (it happens over a period of time), it involves cell metabolism and the active synthesis of new proteins. Conditions under which this response is exhibited are significant but not severe enough to cause immediate death. Species that exhibit this response (Table 2) may vary, dependent upon their inherent chilling sensitivity and whether they are protected by moderating conditions (darkness or low vapour pressure deficit) or exposed to multiple environmental insults (high light and/or high vapour pressure deficit) (see Fig. 4 for a detailed description.)

Further investigations of PCD and chilling stress

Much work needs to be done in order to demonstrate a cell-death mechanism in cold-stressed plants. Although there is no one definitive marker of programmed cell death, by convention nuclear DNA fragmentation and ultrastructural evidence have been used to suggest that such a pathway may be in place (Mittler *et al*. 1997). Ultrastructurally, most of the work to date on chilling stress has focused on the chloroplast and has been descriptive in nature. Future studies might look beyond the chloroplast and investigate key physiological phenomena occurring throughout the cell when the delayed response to chilling is observed. The key is to look for evidence that cell metabolic processes have been maintained and that novel proteins are being expressed.

It would be useful to find a temperature-sensitive mutant that undergoes necrotic cell death under the same conditions that trigger the delayed response in the wild-type plant. Cells that exhibit the delayed response could be identified ultrastructurally, or by TUNEL (TdT/UTP nick end labelling) staining to test for fragmentation of DNA. Cells experiencing necrotic cell death exhibit rapid cellular swelling and lysis. In this way, the gene(s) that protect the plant from necrosis could be identified, cloned, and potentially used to stimulate PCD in other cell lines.

Caspases are highly conserved proteases that are thought to regulate PCD by inducing many of the morphological changes that are characteristic of PCD. The involvement of caspases is considered essential to the cell-death process (Nicholson & Thornberry 1997). Caspase-specific inhibitors have been used to arrest the PCD process in plants (Del Pozo & Lam 1998). If a caspase-inhibitor were found to suppress the symptoms of the delayed response, it would be compelling evidence for a programmed cell death mechanism in the cells of chilling-stressed plants.

Other questions remain regarding the possibility of PCD in chilling-stressed plants: If it is true that ROS are the stimuli for the Ca^{+2} influx, is there a light-dependence to the chilling-induced injury characteristic of the delayed response? Or is the converse true – that is, the redistribution of intracellular Ca^{+2} stimulates the generation of ROS? The role of hormones such as ABA, likewise, remains to be investigated.

CONCLUSIONS AND FUTURE DIRECTIONS

The ultrastructural symptomology of chilling injury to plant tissues is well established in the literature: Chloroplasts are frequently affected first (and in many different ways) while other organelles show injury only later. Factors such as a species inherent sensitivity to chilling, concomitant irradiance, duration of chilling, plant water status, and acclimation may interact to exacerbate or mitigate the injury. What remains much less understood is the precise physiological mechanism(s) underlying a particular ultrastructural lesion. We have attempted in this review to provide some possible mechanistic explanations for some of the symptoms; others remain to be investigated. It is clear, however, that ultrastructural studies have contributed greatly to our understanding of the location, timing, and physiology of chilling injury.

Future research may well focus on differentiating between primary and secondary symptoms. In other words, are the observed symptoms strictly the result of a direct effect of temperature, or are they caused indirectly by programmed genetic mechanisms that are activated by the initial environmental insult? Chilling stress induces changes in gene expression (Guy 1990) that may be related to protective pathways (Sabehat, Lurie & Weiss 1998) or to degradative pathways (von Kampen, Nielander & Wettern 1995). In this regard, it may be prudent to ask to what extent the ultrastructural changes occurring during chilling injury may be related to similar symptoms seen in mammalian cells during apoptosis. Whether or not the plant version of programmed cell death bears any mechanistic relationship to the animal version remains to be investigated.

ACKNOWLEDGMENT

This work was supported, in part, by USDA grant 96–35311–3694 to R.R.W.

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Received 26 April 1999; received in revised form 29 November 1999; accepted for publication 29 November 1999