BIOLOGIA PLANTARUM 51 (4): 690-698, 2007

Ultrastructural alterations in mesophyll and bundle sheath chloroplasts of two maize cultivars in response to chilling at high irradiance

A.S. SAROPULOS* and D.S.H. DRENNAN

Department of Agricultural Botany, the University of Reading, Reading RG6 2AU, UK

Abstract

Maize (*Zea mays* L.) seedlings of two cultivars (cv. Bastion adapted to W. Europe, and cv. Batan 8686 adapted to the highlands of Mexico), raised in a glasshouse (19 - 25 °C), were transferred to 4.5 or 9 °C at photon flux density (PPFD) of 950 μmol m^{-2} s^{-1} with 10-h photoperiod for 58 h and then allowed to recover at 22 °C for 16 h (14 h dark and 2 h at PPFD of 180 μmol m^{-2} s^{-1}). The ultrastructural responses after 4 h or 26 h at 4.5 °C were the disappearance of starch grains in the bundle sheath chloroplasts and the contraction of intrathylakoid spaces in stromal thylakoids of the mesophyll chloroplasts. At this time, bundle sheath chloroplasts of cv. Batan 8686 formed peripheral reticulum. Prolonged stress at 4.5 °C (50 h) caused plastid swelling and the dilation of intrathylakoid spaces, mainly in mesophyll chloroplasts. Bundle sheath chloroplasts of cv. Batan 8686 seedlings appeared well preserved in shape and structure. Batan 8686 had also higher net photosynthetic rates during chilling and recovery than Bastion. Extended leaf photobleaching developed during the recovery period after chilling at 4.5 °C. This was associated with collapsed chloroplast envelopes, disintegrated chloroplasts and very poor staining.

Additional key words: low temperature, photooxidation, photosynthesis, *Zea mays*.

Introduction

The combination of chilling, non-freezing temperatures (0 - 15 °C) and high photon flux density (PPFD) can cause damage to the photosynthetic apparatus of chilling-sensitive plants by inactivating or damaging the photosystem 2 reaction centres, a process termed chilling-dependent photoinhibition of photosynthesis (Baker *et al*. 1988, Long *et al*. 1994). Prolonged exposure to chilling photoinhibitory conditions can further lead to a chilling-, light-, and oxygen-dependent bleaching of chloroplasts (Wise 1995) and to chloroplast disintegration (Kratsch and Wise 2000). The modifications to the chloroplast ultrastructure of leaf tissues chilled at high PPFD have been examined in several studies. Typical mesophyll chloroplasts of the chilling-sensitive *Sorghum bicolor*, *Paspalum dilatatum* (Taylor and Craig 1971), *Episcia reptans* (Murphy and Wilson 1981), *Phaseolus vulgaris*, *Gossypium hirsutum* (Wise *et al*. 1983), *Glycine max* (Musser *et al*. 1984), *Cucumis sativus* (Wise and Naylor 1987), *Vigna radiata* (Ma *et al*. 1990) and *Zea mays* (Pinhero *et al*. 1999, Sopher *et al*. 1999) seedlings were grossly swollen with dilated intrathylakoid spaces and broken envelopes, after exposure to chilling at moderate to high PPFD (500 - 1300 μmol m^{-2} s^{-1}) for less than 36 h. In contrast, typical mesophyll chloroplasts of chilled *Pisum sativum* and *Brassica oleracea* var. *acephala* (chilling-tolerant) seedlings were greatly swollen but with intact chloroplast envelopes, and exhibited negligible lipid peroxidation (Wise *et al*. 1983, Wise and Naylor 1987).

Maize is a C_4 species of the NADP-malic enzyme class with a distinctive leaf anatomy comprising two different kinds of photosynthetic cells: mesophyll and bundle sheath cells (Laetsch 1974, Hatch 1977). The susceptibility of maize to chilling stress during the early growing season in temperate climates and its poor photosynthetic performance at low temperatures are well known (Baker and Nie 1994, Haldimann 1999). There is evidence that field-grown maize exhibits an increased photosynthetic electron transport to CO_2 assimilation ratio as well as increased antioxidant enzyme activities during periods of low temperature (Fryer *et al*. 1998). Fryer *et al*. (1998) suggested that the linear photo-

Received 23 November 2005, *accepted* 8 August 2006.

Abbreviations: C_4 - species that produce four-carbon acids as the primary initial CO_2 fixation products; P_N - net photosynthetic rate; PAR - photosynthetic active radiation; PPFD - photosynthetic photon flux density; TEM - transmission electron microscopy.

Acknowledgements: We thank Dr. L. Bonner for her guidance and assistance in the TEM studies.

* Corresponding author; present address: Cherianon 7, Kalamaria GR-55133, Greece; fax: (+30) 23710 39207; e-mail: saropulos@the.forthnet.gr

synthetic electron flux is switched to O_2 reduction via the Mehler reaction in chilled maize leaves. Furthermore, the antioxidant defence system in maize leaves is not uniformly distributed between the mesophyll and bundle sheath cells (Doulis *et al*. 1997, Pastori *et al*. 2000). Bundle sheath proteins have been shown to be more sensitive to oxidative damage than those of the mesophyll when maize leaves were exposed to paraquat or sub-optimal growth temperatures (Kingston-Smith and Foyer 2000). Damage to bundle sheath chloroplasts advanced also faster than that observed in mesophyll chloroplasts in a mutant of maize, which is deficient in plastoquinone, α-tocopherol and carotenoids (Wise and Cook 1998). Kingston-Smith and Foyer (2000), proposed that this differential distribution of antioxidants and the increased susceptibility of bundle sheath chloroplasts to oxidative stress at low temperatures could contribute to the extreme chilling-sensitivity of maize seedlings.

On the other hand, ultrastructural studies in C_4 species have revealed that low night temperatures inhibit mesophyll chloroplast development whereas chloroplast development in adjacent bundle sheath cells remains unaltered, thus causing the formation of longitudinal chlorotic bands on emerging leaves (Slack *et al*. 1974). In addition, chilling-induced photooxidation in sorghum leaves caused more damage to the upper mesophyll chloroplasts than to the bundle sheath chloroplasts (Taylor and Craig 1971). This gradient in degree of damage was attributed to the gradient of PPFD inside the leaf. Sopher *et al*. (1999) and Pinhero *et al*. (1999), reported damage to mesophyll chloroplasts in chilled maize seedlings without giving any reference to ultrastructural modifications in bundle sheath chloroplasts. Moreover, Gomez *et al*. (2004), found a specific increase in the potential contribution of the bundle sheath cells to glutathione synthesis during chilling.

This study examines the chloroplast ultrastructure of maize leaves, in order to investigate what structural alterations in mesophyll and bundle sheath cells are associated with the chilling-dependent photoinhibition of photosynthesis and the severe photobleaching that can be observed in chilled maize leaves (Saropulos and Drennan 2002). The changes in chloroplast ultrastructure were followed from the beginning of the chilling treatment till the appearance of severe photobleaching in the recovery period. Electron microscopical studies on chloroplasts of maize leaves subjected to milder chilling photoinhibitory conditions that did not lead to visible photodestruction of chlorophyll, were also conducted for comparison purposes. The effect of chilling on chloroplast ultrastructure was compared between an early maturing maize cultivar adapted for cultivation in Western Europe (cv. Bastion) with a cold tolerant experimental maize cultivar bred at CIMMYT from a population with 80 % Mexican highland germplasm and 20 % US germplasm (cv. Batan 8686 VE).

Materials and methods

Plants and growth conditions: Seedlings of the maize (*Zea mays* L.) cultivars Bastion and Batan 8686 VE were used in the experiments. The plants were raised in seed trays under glasshouse conditions with temperature ranging between 19 and 25 °C and PPFD at the leaf level ranging between 550 and 1100 μmol m^{-2} s^{-1}. Maize seeds were sown in a mixture of peat, loam and sand (1:1:1, v/v/v) enriched with nutrients. All seedlings were transplanted into plastic pots (10 × 10 cm) containing the enriched mixture of peat, loam and sand, as soon as they could be handled.

Chilling treatment: The pots were placed inside a modified growth chamber (Saropulos 1995) at the beginning of the photoperiod when the third maize leaf was the youngest fully expanded leaf. Photoperiod was 10 h. Small squares from the middle part of the third maize leaf (1 - 2 mm wide) were cut after 4 h, 26 h (2 h after the commencement of the 2nd photoperiod) and 50 h (2 h after the commencement of the 3rd photoperiod) of chilling at 4.5°C with PPFD of 950 ± 150 μmol m^{-2} s^{-1} at leaf height (wilted leaves were held horizontally by tapes), and after 58 h of treatment followed by 14 h recovery (at 22 °C) in the dark and 2 h recovery under low PPFD (180 μmol m^{-2} s^{-1}). Specimens were also collected after 58 h of mild chilling at 9 °C with PPFD 950 ± 150 μmol m^{-2} s^{-1} (10-h photoperiod) followed by 16 h recovery (14 h in the dark and 2 h at low PPFD) as well as from unchilled maize plants (controls). At each sampling time, samples from five replicate plants were collected and used for TEM. Different batches of plants (five replicates) were used for the photosynthetic measurements.

Electron microscopy: Immediately after excision, the specimens were fixed under vacuum in Karnovsky's fixative (4 % paraformaldehyde and 3 % glutaraldehyde in 0.05 M Sorensen's phosphate buffer, pH 7) for 4 h at room temperature. Specimens were subsequently buffer-washed four times at 15 min intervals and post-fixed in 1 % (m/v) aqueous osmium tetroxide (OsO_4) solution for 3 h at room temperature. The samples were then washed three times in glass distilled water and dehydrated through a graded acetone:water series (30, 50, 70, 90, 95 and twice in 100 % acetone), leaving the material for 30 min in each solution. Then, acetone was replaced by propylene oxide through a series of 25, 50 and 75 % propylene oxide in acetone and twice in 100 % propylene oxide, allowing 20 min for each change. Specimens were embedded in *EPON* (*Shell*, London, UK) via a series of resin:propylene oxide mixtures (20, 40, 60 and 80 % resin), followed by two changes of 100 % *EPON*, allowing 12 - 24 h for each change. The resin was

polymerised at 60 °C for 24 h. Silver-gold sections (50 - 100 nm) were cut on a *Reichert-Jung* (Nussloch, Germany) *Ultracut* ultramicrotome, using a diamond knife. The sections were cut in transverse direction so that both layers could be seen together on the same section. They were double-stained using an *LKB 2168 Ultrostainer* (Bonner 1988), first with 4 % uranyl acetate in 50 % ethanol for 1 h at 40 °C and then with lead citrate for 5 min at room temperature. Transmission electron microscopy was performed on a *Hitachi H-800* (Wokingham, UK) TEM operating at 75 kV.

Measurement of net photosynthetic rate: An open gas-exchange system (*LCA-2*, *Analytical Development Co*., Hoddesdon, UK) including an infrared gas analyser (*LCA*), a pump (*ASU*), a Parkinson leaf chamber (*PL2-B*) and a data logger (*DL2*) were used for the measurement of net photosynthesis of the central part of the third maize leaf. PPFD used to compare changes in net photosynthetic rate, P_N, was 500 μmol m^{-2} s^{-1} and the air temperature inside the chamber during the readings was 25 ± 1 °C. Measurements were taken after an equilibration period of 30 min.

Results

Chloroplast ultrastructure and photosynthesis of unchilled maize seedlings: Bundle sheath chloroplasts from unchilled maize cv. Batan 8686 seedlings were characterised by mainly granaless thylakoids with only a few thylakoid connections, abundant starch grains, well-stained stroma and an extended peripheral reticulum (Fig. 1*A*,*B*). Unchilled mesophyll chloroplasts had numerous granal stacks but they generally lacked starch grains (Fig. 2*A*,*B*). The osmiophilic droplets which were observed in various numbers in all chloroplasts (Fig. 1*B*, 2*B*) are described as plastoglobuli functioning as stores of lipids and pigments (Tevini and Steinmüller 1985). There were no differences between the two maize cultivars in the chloroplast ultrastructure of unchilled plants. Unchilled seedlings of both cultivars had also similar P_N (Table 1).

Chloroplast ultrastructure and photosynthesis of maize seedlings chilled at 4.5 °C: After chilling (4.5 °C) for only four hours at PPFD of 950 μmol m^{-2} s^{-1}, the starch grains of bundle sheath chloroplasts were substantially decreased in number and size (Fig. 1*C*,*D*). The formation of a double layer of peripheral reticulum was observed in the majority of bundle sheath chloroplasts from cv. Batan 8686 seedlings (Fig. 1*D*). There was a complete absence of starch grains in mesophyll chloroplasts. Upper mesophyll chloroplasts, which were exposed directly to the light, exhibited a parallel thylakoid orientation and a contraction of the intrathylakoid space (lumen), mainly in stromal thylakoids and in some of the outermost, stroma-facing granal thylakoid membranes (Fig. 2*C*,*D*). An increase in size of peripheral reticulum in Batan 8686 mesophyll chloroplasts was also detected (Fig. 2*D*). Lower mesophyll chloroplasts were identical to unchilled ones in both cultivars.

Twenty-six hours of chilling caused a disappearance of starch grains in the bundle sheath chloroplasts of both cultivars (Fig. 1*E*,*F*). A further increase in the number of layers and size of the peripheral reticulum was observed in bundle sheath chloroplasts of cv. Batan (Fig. 1*F*). This increase was most noticeable on the side of the chloroplast which was adjacent to the cell wall. The intrathylakoid space of upper mesophyll chloroplasts remained contracted and the stroma was lighter stained in upper mesophyll chloroplasts of cv. Bastion (Fig. 2*E*,*F*). No symptoms of photobleaching had been developed at this stage but the P_N of the leaves (measured at 25 ºC) were dramatically reduced by 90 % (of control level) in Bastion and by 80 % in Batan seedlings (Table 1). It should be reported that intercellular CO_2 concentrations, which were calculated according to the equations described by Von Caemmerer and Farquhar (1981), were elevated by more than 10 % above control level in all chilling treatments, thus precluding any stomatal limitation artificially caused by the temperature change (from 4.5 or 9 to 25 ºC).

Table 1. Net photosynthetic rates [μmol(CO_2) m^{-2} s^{-1}] of unchilled and chilled maize cv. Batan and cv. Bastion seedlings. Measurements were taken from the middle part of the 3rd leaf at 25 ºC and PPFD of 500 μmol m^{-2} s^{-1}. Means ± SE, n = 5.

Treatment	cv. Bastion	cv. Batan
Unchilled leaves	14.36 ± 0.28	14.68 ± 0.52
26 h at 4.5 °C	1.48 ± 0.57	2.88 ± 0.90
50 h at 4.5 °C	0.16 ± 0.30	0.58 ± 0.28
58 h at 4.5 and 16 h at 22 ºC	-0.36 ± 0.10	-0.42 ± 0.11
58 h at 9.0 and 16 h at 22 ºC	8.06 ± 1.80	11.42 ± 0.94

The first minor symptoms of chlorophyll photodestruction appeared after 50 h of chilling and they were associated with marked destructive changes in the mesophyll chloroplast ultrastructure (Fig. 3*C*). Many mesophyll chloroplasts of cv. Bastion were swollen and the thylakoid membranes were dilated, having an increased intraspace (Fig. 3*A*). The envelope of some mesophyll chloroplasts of cv. Batan had collapsed and the stroma components were mixed with the cytoplasm (right hand-side chloroplast, Fig. 3*E*). In broken chloroplasts the thylakoids were separated from each other but still connected by the few grana that remained (mesophyll chloroplast, Fig. 3*F*). This pattern of damage was observed in both upper and lower mesophyll cells (Fig. 3*C*,*D*). There was considerable variation in damage both between cells and within cells. Batan showed a higher frequency of mesophyll chloroplasts with broken

envelope, while the majority of the mesophyll chloroplasts in Bastion were grossly swollen.

In contrast, the bundle sheath chloroplasts of cv. Batan 8686 seedlings appeared well preserved in shape and structure (Fig. 3*D*,*F*). Bundle sheath chloroplasts in Bastion exhibited more structural damage than in Batan. Many of them were swollen, with expanded intrathylakoid space and the peripheral reticulum had nearly disappeared (Fig. 3*B*). Maize leaves with such ultrastructural damage proved to be quite incapable of photosynthesis when returned to normal conditions but some net CO_2 gain was still evident (Table 1).

Leaf tissues of both maize cultivars collected in the recovery phase, after 58 h of chilling, were severely photobleached. Electron micrographs from these tissues showed low contrast and poor staining (Fig. 4*A*,*B*). Both mesophyll (Fig. 4*B*) and bundle sheath chloroplasts (Fig. 4*A*) exhibited disintegrated envelopes and thylakoids, and a mixing of cytoplasmic and stromal material. A large number of osmiophilic droplets with reduced electron-opaqueness, which may be accumulations of degrading lipids, were detected in some mesophyll chloroplasts. Photobleached tissues exhibited net CO_2 loss when measured at 25 ºC (Table 1).

Chloroplast ultrastructure and photosynthesis of maize seedlings chilled at 9 °C: The mild stress imposed on maize seedlings by the 9 °C treatment did not cause any visible sign of chlorophyll photodestruction even in the recovery phase. Bundle sheath chloroplasts from

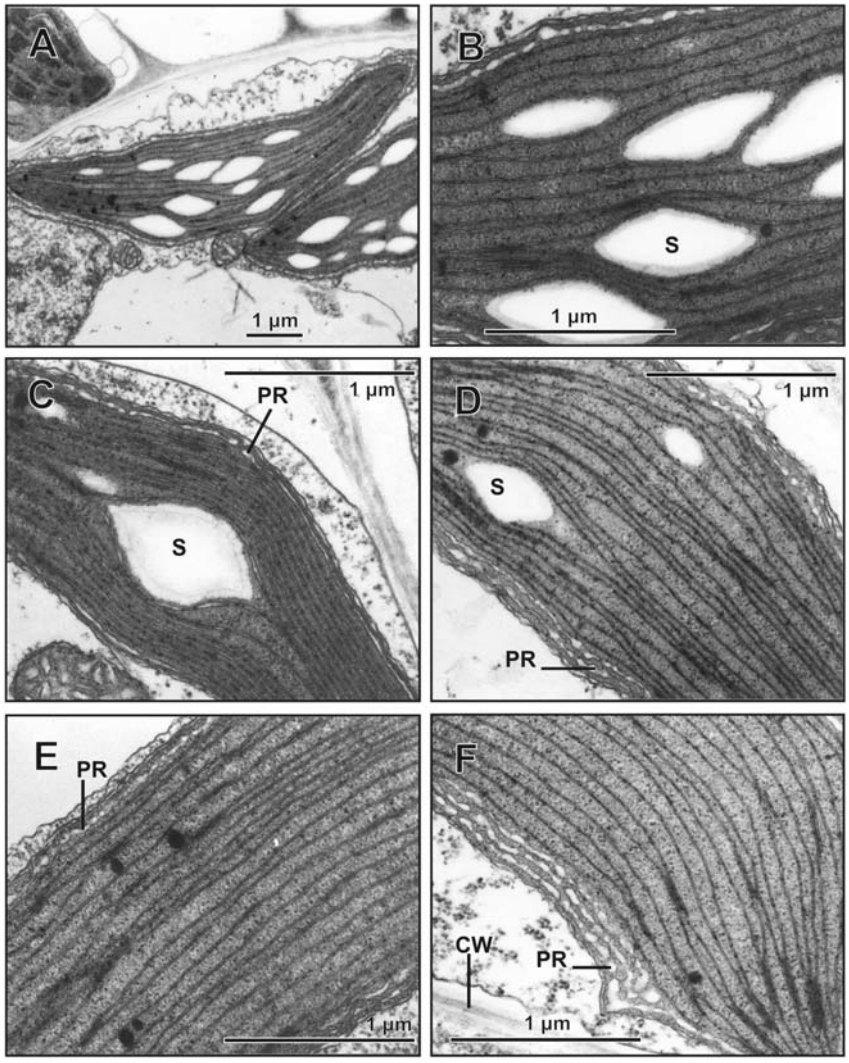

Fig. 1. Electron micrographs of maize bundle sheath chloroplasts from unchilled maize plants (*A*, *B*) and after 4 h (*C*, *D*) or 26 h (*E*, *F*) at 4.5 °C with PPFD = 950 µmol m^{-2} s^{-1}. Photoperiod was 10 h. *A* - bundle sheath chloroplasts of cv. Batan 8686 seedling; *B* - magnified part of micrograph *A*; *C*, *E* - bundle sheath chloroplasts of cv. Bastion; *B*, *D*, *F* - bundle sheath chloroplasts of cv. Batan 8686; CW - cell wall; PR - peripheral reticulum; S - starch grain.

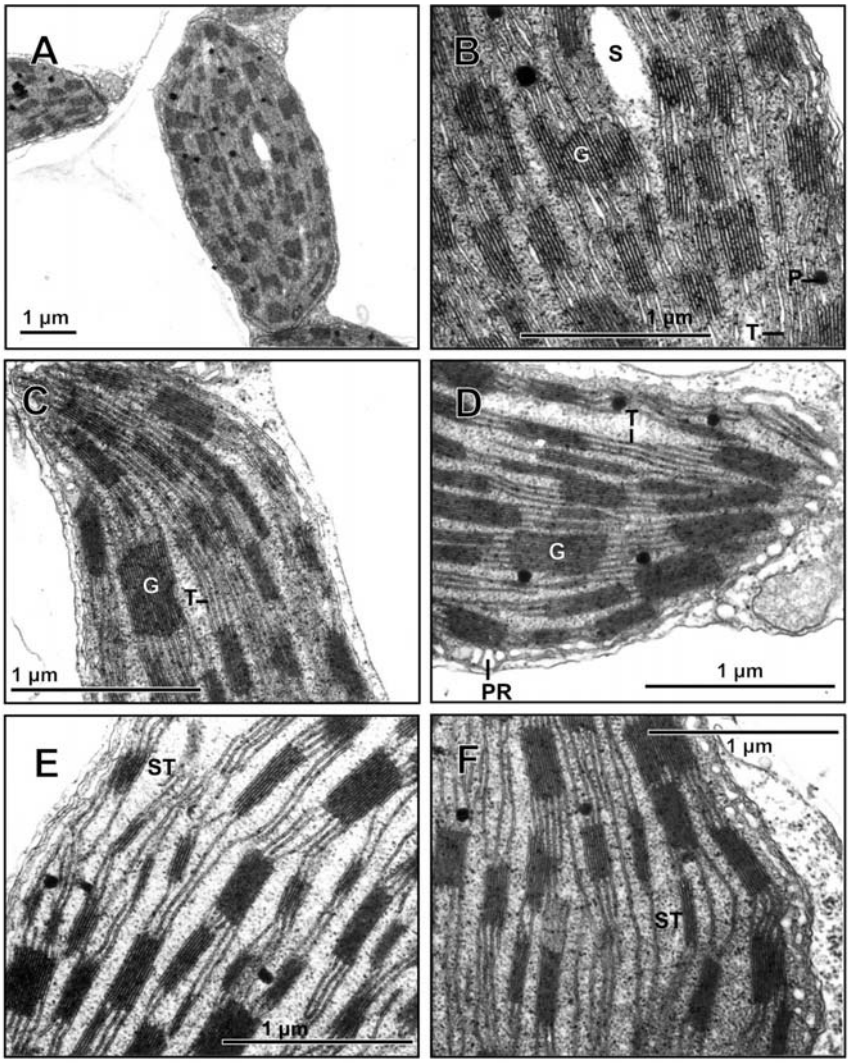

Fig. 2. Electron micrographs of maize mesophyll chloroplasts from unchilled maize plants (*A*, *B*) and after 4 h (*C*, *D*) or 26 h (*E*, *F*) at 4.5 °C with PPFD = 950 µmol m^{-2} s^{-1}. Photoperiod was 10 h. *A* - mesophyll chloroplasts of cv. Batan 8686 seedlings; *B* - magnified part of micrograph *A*; *C*, *E* - upper mesophyll chloroplasts of cv. Bastion; *D*, *F* - upper mesophyll chloroplasts of cv. Batan 8686; G - granum; P - plastoglobulus; PR - peripheral reticulum; S - starch; ST - stroma; T - stromal thylakoid.

leaves which were collected after 16-h recovery appeared to be partly swollen and lacked starch grains (Fig. 4*C*,*D*). The envelopes remained intact and the cells were well compartmentalised. Bundle sheath chloroplasts from Batan seedlings contained multiple layers of peripheral reticulum (Fig. 4*D*). The only ultrastructural changes observed in mesophyll chloroplasts in the recovery phase, was a decrease in the stromal staining and a contraction of the intrathylakoid space (lumen) in stromal thylakoids of the upper mesophyll chloroplasts (Fig. 4*E*,*F*). Photosynthesis of chilled maize leaves after 16-h recovery was still inhibited compared to that of unchilled leaves (Table 1).

Discussion

Injury of chloroplast ultrastructure developed progressively with chilling time. Major structural alterations were observed in electron micrographs of both upper and lower mesophyll chloroplasts of maize leaves when subjected to 4.5 °C for 50 h (PPFD 950 µmol m^{-2} s^{-1} with 10-h photoperiod). At that time, maize leaves started to show symptoms of photooxidative bleaching of pigments. Taylor and Craig (1971) reported a light-dependent gradient of damage across sorghum leaves when subjected to low-temperature (10 °C) for 2.5 d under

PPFD 170 W m^{-2} (approximately 780 μmol m^{-2} s^{-1}) with 12-h photoperiod. Such a gradient was not found in maize after 50 h of treatment, as the severe stress resulted in extended chloroplast damage in mesophyll cells throughout the leaf. Kratsch and Wise (2000) suggested that the stromal swelling, which was observed in mesophyll chloroplasts at this stage, can arise from an accumulation of soluble sugars, because starch hydrolysis remains active when the membrane-bound Pi-triose translocators are inactivated by chilling. However, this hypothesis cannot be valid for the maize mesophyll chloroplasts, which lack starch grains. An increased trans-thylakoid pH gradient caused by excessive light can result in loss of organic acids (Murakami and Packer 1970a) and Ca^{2+} (Long *et al*. 1994) from the thylakoid intraspace, thus affecting the osmotic potential of both the stroma and the lumen. Lipid peroxidation could also modify the permeability of the membranes and result in swelling (Halliwell 1984). Therefore, excess light could also cause the damage through lipid peroxidation and abnormal ΔpH.

Surprisingly, bundle sheath chloroplasts of both maize cultivars showed less damage than lower mesophyll chloroplasts despite the fact that bundle sheath chloroplasts were more exposed to the light. Mesophyll cells are also more prone to damage than bundle sheath cells in water stressed maize leaves (Giles *et al*. 1974). Bundle sheath cells of NADP-ME C_4 species like maize are often thick-walled and rather gas-impermeable, with chloroplasts relatively deficient in photosystem 2, and hence have much lower oxygen concentrations and non-cyclic electron transport flow which can lead to the

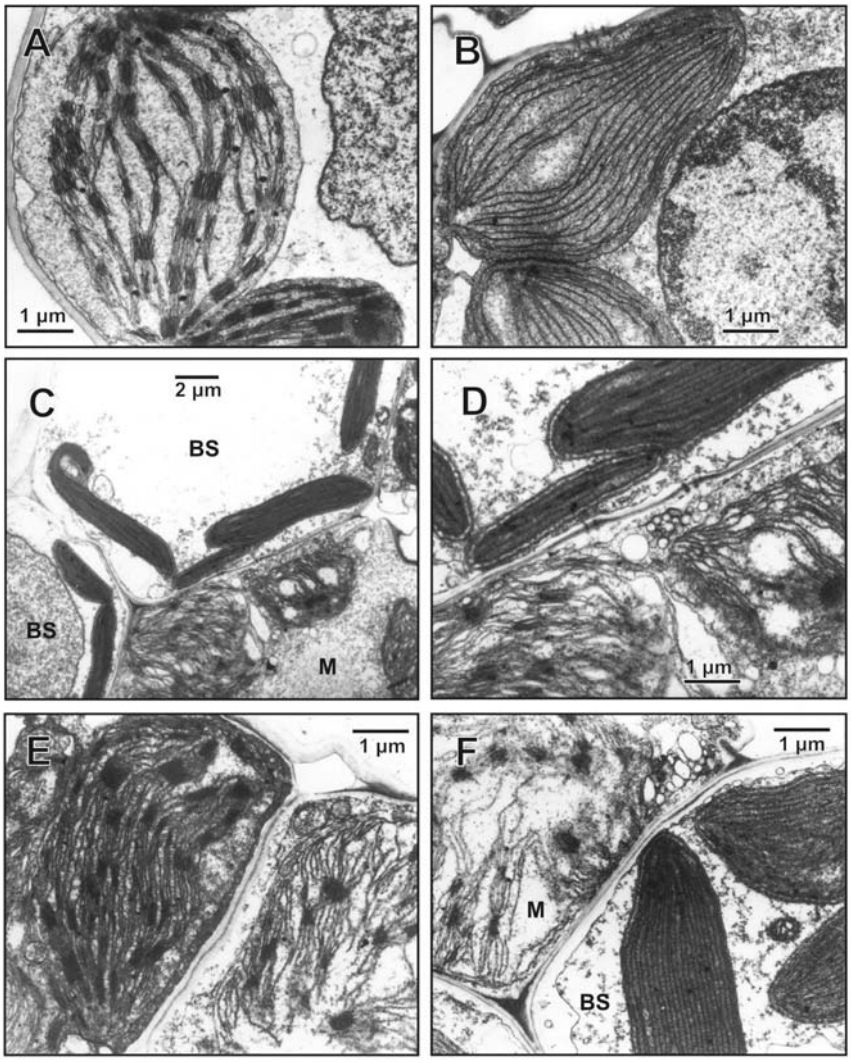

Fig. 3. Electron micrographs of maize chloroplasts after 50 h at 4.5 °C with PPFD = 950 μmol m^{-2} s^{-1}. Photoperiod was 10 h. *A* - mesophyll chloroplasts of cv. Bastion; *B* - bundle sheath chloroplasts of cv. Bastion; *C* - chloroplasts in adjacent bundle sheath and lower mesophyll cells of cv. Batan 8686; *D* - magnified part of micrograph *C*; *E* - mesophyll chloroplasts of cv. Batan 8686; *F* - bundle sheath chloroplasts next to disintegrated mesophyll chloroplast of cv. Batan 8686; BS - bundle sheath cell; M - mesophyll cell.

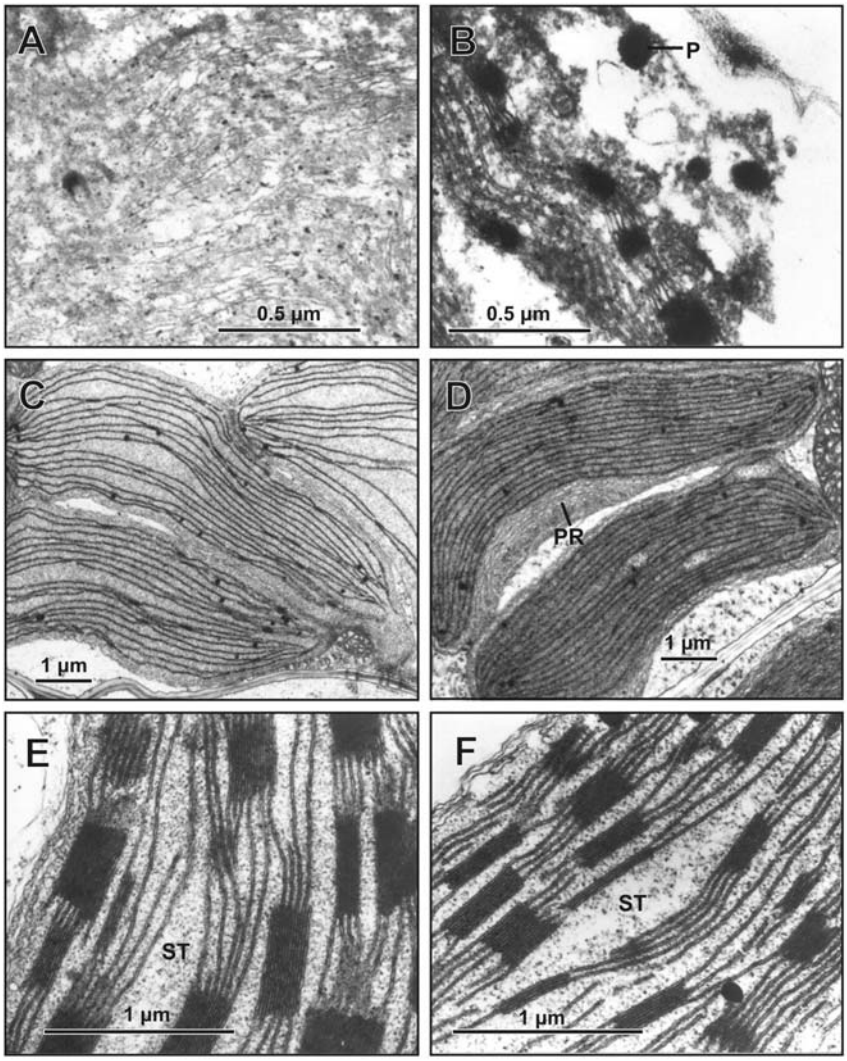

Fig. 4. Electron micrographs of maize chloroplasts after 58 h at 4.5 ºC (*A*, *B*) or 9 °C (*C* - *F*) with PPFD = 950 µmol m^{-2} s^{-1} followed by 16-h recovery at 22 °C. Photoperiod was 10 h. *A* - disintegrated bundle sheath chloroplast of cv. Bastion; *B* - disintegrated mesophyll chloroplast of cv. Bastion; *C* - bundle sheath chloroplasts of cv. Bastion; *D* - bundle sheath chloroplasts of cv. Batan 8686; *E* - upper mesophyll chloroplast of cv. Bastion; *F* - upper mesophyll chloroplast of cv. Batan 8686; P - plastoglobulus; PR - peripheral reticulum; ST - stroma.

formation of oxygen free radicals (Fryer *et al*. 1998). Moreover, they are well equipped with the antioxidant enzymes superoxide dismutase and ascorbate peroxidase for the scavenging of reactive oxygen species (Doulis *et al*. 1997, Pastori *et al*. 2000). Accumulation of H_2O_2 could be damaging in bundle sheath cells if the transport of reduced ascorbate from disintegrated mesophyll cells is impaired (Kingston-Smith and Foyer 2000), and such a mechanism of oxidative damage could be responsible for the structural damage observed in the bundle sheath during recovery. Another advantage of bundle sheath cells could be their proximity to the bundle and the development of multiple layers of peripheral reticulum in cv. Batan 8686, which could allow the bundle sheath chloroplasts to survive any osmotic imbalances. The reasons for the sensitivity of mesophyll cells to chilling injury at high PPFD and the possible role of the differential distribution of antioxidants between the two cell types need further investigation.

The appearance of visible photobleaching of chlorophyll in chilled maize leaves was associated with major ultrastructural modifications and negligible photosynthetic capacity. Leaf tissues with no visible signs of photooxidative damage (chilled at 9 ºC or chilled at 4.5 ºC for less than 50 h) showed minor structural changes but photosynthetic function was considerably impaired, and thus functional losses (photoinhibition) in chilled maize leaves can occur without significant structural damage.

A gradient of chloroplast modifications across the maize leaf was observed at the initial stages of treatment *i.e*. 4 h from the onset of the stress (4.5 °C and PPFD 950 µmol m^{-2} s^{-1}). Upper mesophyll chloroplasts, which were directly exposed to the light showed a contraction of intrathylakoid space relative to that of unchilled controls.

Murakami and Packer (1970a,b) gave evidence that the lumen contraction in thylakoids of *Porphyra thalli*, which are transferred from dark to light, was the result of an acidity-induced loss of organic acids and the subsequent osmotic collapse. Kramer *et al*. (1999) reported that even though a moderate trans-thylakoid ΔpH initiates down-regulatory processes (formation of zeaxanthin), an excessively low lumen pH (below 5), which can be generated only under stress conditions, contributes possibly to photoinhibitory damage. It would seem therefore that the first alterations observed in chilled maize upper mesophyll chloroplasts could be the result of excess light causing an abnormally low pH in the lumen of the thylakoids and contributing to photoinhibitory damage. This lumen contraction remained till the recovery phase when less destructive conditions (chilled at 9 ºC) were applied. It should be reported that after 72 h of chilling at 9 °C with PPFD 950 ± 150 μmol m^{-2} s^{-1} (10-h photoperiod) the apparent maximum quantum yield of CO_2 assimilation decreased by 61 % (of control level) in Batan and by 74 % in Bastion seedlings (Saropulos and Drennan 2002).

Another ultrastructural modification, which appeared during the first treatment day, was the development of multiple layers of peripheral reticulum in bundle sheath chloroplasts from cv. Batan 8686 seedlings. The formation of peripheral reticulum has also been detected in chloroplasts of chilled bean, cotton (Wise *et al*. 1983) and soybean (Musser *et al*. 1984) leaves, even though a peripheral reticulum surrounding the stroma is characteristic of C_4 species (Laetsch 1974). Kratsch and Wise (2000) suggested that these peripheral vesicles can facilitate the metabolite transport across the inner membrane of the chloroplast envelope, and thus help the chloroplast to regulate its osmotic potential and avoid the excessive swelling which is associated with chilling. The fact that bundle sheath chloroplasts of cv. Batan 8686 were not swollen and showed less damage than bundle sheath chloroplasts of cv. Bastion after 50 h of chilling, clearly supports the hypothesis of a protective role of peripheral reticulum. In this context, the formation of an extensive peripheral reticulum during the delayed response of maize plants to chilling at 9 ºC should not be related to a possible programmed cell death (Kratsch and Wise 2000).

The higher photosynthetic capacity of cv. Batan 8686 compared to that of cv. Bastion, when chilled at 9 ºC or chilled at 4.5 ºC for less than 50 h (Table 1), was correlated with the enhanced formation of peripheral reticulum and the preservation of shape and structure of bundle sheath chloroplasts in cv. Batan. Thus, there is genotypic variability to the chilling-induced ultrastructural damage and photosynthetic impairment in maize populations. Recently, Kutík *et al*. (2004) reported that chilling-stressed hybrid maize plants showed positive heterosis in ultrastructural alterations of mesophyll chloroplasts, while, Jatimliansky *et al.* (2004) gave evidence that hybrid (*Zea mays* × *Tripsacum dactyloides*) plants have higher capacity to recover from chilling injury than their parents. This genotypic variability, which can also be observed in other chilling-sensitive species such as rice (Huang and Guo 2005), should be identified and exploited in breeding programs.

References

Baker, N.R., Long, S.P., Ort, D.R.: Photosynthesis and temperature, with particular reference to effects on quantum yield. - In: Long, S.P., Woodward, F.I. (ed.): Plants and Temperature. Pp. 347-375. The Company of Biologists Limited, Cambridge 1988.

Baker, N.R., Nie, G.Y.: Chilling sensitivity of photosynthesis in maize. - In: Bajaj, Y.P.S. (ed.): Biotechnology in Agriculture and Forestry: Maize. Vol. 9. Pp. 465-481. Springer-Verlag, Berlin 1994.

Bonner, L.J.: Pollination Biology of *Lycopersicon esculentum* Mill. in Summer and Winter. - PhD Thesis. The University of Reading, Reading 1988.

Doulis, A.G., Debian, N., Kingston-Smith, A.H., Foyer, C.H.: Differential localization of antioxidants in maize leaves. - Plant Physiol. **114**: 1031-1037, 1997.

Fryer, M.J., Andrews, J.R., Oxborough, K., Blowers, D.A., Baker, N.R.: Relationship between CO_2 assimilation, photosynthetic electron transport and active O_2 metabolism in leaves of maize in the field during periods of low temperature. - Plant Physiol. **116**: 571-580, 1998.

Giles, K.L., Beardsell, M.F., Cohen, D.: Cellular and ultrastructural changes in mesophyll and bundle sheath cells of maize in response to water stress. - Plant Physiol. **54**: 208-212, 1974.

Gomez, L.D., Vanacker, H., Buchner, P., Noctor, G., Foyer, C.H.: Intercellular distribution of glutathione synthesis in maize leaves and its response to short-term chilling. - Plant Physiol. **134**: 1662-1671, 2004.

Haldimann, P.: How do changes in temperature during growth affect leaf pigment composition and photosynthesis in *Zea mays* genotypes differing in sensitivity to low temperature? - J. exp. Bot. **50**: 543-550, 1999.

Halliwell, B. (ed.): Chloroplast Metabolism: The Structure and Function of Chloroplasts in Green Leaf Cells. - Clarendon Press, Oxford 1984.

Hatch, M.D.: C_4 pathway photosynthesis: mechanism and physiological function. - Trends Biochem. Sci. **2**: 199-201, 1977.

Huang, M., Guo, Z.: Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity. - Biol. Plant. **49**: 81-84, 2005.

Jatimliansky, J.R., García, M.D., Molina, M.C.: Response to chilling of *Zea mays*, *Tripsacum dactyloides* and their hybrid. - Biol. Plant. **48**: 561-567, 2004.

Kingston-Smith, A.H., Foyer, C.H.: Bundle sheath proteins are more sensitive to oxidative damage than those of the mesophyll in maize leaves exposed to paraquat or low temperatures. - J. exp. Bot. **51**: 123-130, 2000.

Kramer, D.M., Sacksteder, C.A., Cruz, J.A.: How acidic is the lumen? - Photosynth. Res. **60**: 151-163, 1999.

Kratsch, H.A., Wise, R.R.: The ultrastructure of chilling stress. - Plant Cell Environ. **23**: 337-350, 2000.

Kutík, J., Holá, D., Kočová, M., Rothová, O., Haisel, D., Wilhelmová, N., Tichá, I.: The ultrastructure and dimensions of chloroplasts in leaves of three maize (*Zea mays* L.) inbred lines and their F_1 hybrids grown under moderate chilling stress. - Photosynthetica **42**: 447-455, 2004.

Laetsch, W.M.: The C_4 syndrome: A structural analysis. - Annu. Rev. Plant Physiol. **25**: 27-52, 1974.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. - Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 633-662, 1994.

Ma, S., Lin, C., Chen, Y.: Comparative studies of chilling stress on alterations of chloroplast ultrastructure and protein synthesis in the leaves of chilling-sensitive (mungbean) and -insensitive (pea) seedlings. - Bot. Bull. Acad. sin. **31**: 263-272, 1990.

Murakami, S., Packer, L.: Light-induced changes in the conformation and configuration of the thylakoid membrane of *Ulva* and *Porphyra* chloroplasts *in vivo*. - Plant Physiol. **45**: 289-299, 1970a.

Murakami, S., Packer, L.: Protonation and chloroplast membrane structure. - J. Cell Biol. **47**: 332-351, 1970b.

Murphy, C., Wilson, J.M.: Ultrastructural features of chilling-injury in *Episcia reptans*. - Plant Cell Environ. **4**: 261-265, 1981.

Musser, R.L., Thomas, S.A., Wise, R.R., Peeler, T.C., Naylor, A.W.: Chloroplast ultrastructure, chlorophyll fluorescence and pigment composition in chilling-stressed soybeans. - Plant Physiol. **74**: 749-754, 1984.

Pastori, G., Foyer, C.H., Mullineaux, P.: Low temperature-induced changes in the distribution of H_2O_2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. - J. exp. Bot. **51**: 107-113, 2000.

Pinhero, R.G., Paliyath, G., Yada, R.Y., Murr, D.P.: Chloroplast membrane organization in chilling-tolerant and chilling-sensitive maize seedlings. - J. Plant Physiol. **155**: 691-698, 1999.

Saropulos, A.S.: Chilling-induced Physiological Dysfunction in Leaves of *Zea mays* L. and *Capsicum annuum* L. Seedlings. - PhD Thesis. The University of Reading, Reading 1995.

Saropulos, A.S., Drennan, D.S.H: Leaf photosynthetic parameters of two maize (*Zea mays* L.) cultivars in response to various patterns of chilling temperatures and photon flux densities. - Ann. appl. Biol. **141**: 237-245, 2002.

Slack, C.R., Roughan, P.G., Bassett, H.C.M.: Selective inhibition of mesophyll chloroplast development in some C_4-pathway species by low night temperature. - Planta **118**: 57-73, 1974.

Sopher, C.R., Krol, M., Huner, N.P.A., Moore, A.E., Fletcher, R.A.: Chloroplastic changes associated with paclobutrazol-induced stress protection in maize seedlings. - Can. J. Bot. **77**: 279-290, 1999.

Taylor, A.O., Craig, A.S.: Plants under climatic stress. II. Low temperature, high light effects on chloroplast ultrastructure. - Plant Physiol. **47**: 719-725, 1971.

Tevini, M., Steinmüller, D.: Composition and function of plastoglobuli. II. Lipid composition of leaves and plastoglobuli during beech leaf senescence. - Planta **163**: 91-96, 1985.

Von Caemmerer, S., Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-387, 1981.

Wise, R.R.: Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light. - Photosynth. Res. **45**: 79-97, 1995.

Wise, R.R., Cook, W.B.: Development of ultrastructural damage to chloroplasts in a plastoquinone-deficient mutant of maize. - Environ. exp. Bot. **40**: 221-228, 1998.

Wise, R.R., Naylor, A.W.: Chilling-enhanced photooxidation. The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. - Plant Physiol. **83**: 272-277, 1987.

Wise, R.R., McWilliam, J.R., Naylor, A.W.: A comparative study of low-temperature-induced ultrastructural alterations of three species with differing chilling sensitivities. - Plant Cell Environ. **6**: 525-535, 1983.