How do changes in temperature during growth affect leaf pigment composition and photosynthesis in Zea mays genotypes differing in sensitivity to low temperature?

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Abstract

The changes in photosynthetic activity and composition of pigments induced by changes in temperature were examined in the third leaf of three chilling-tolerant and three chilling-sensitive genotypes of maize (Zea mays L.). The plants were grown under a controlled environment at a photon flux density of 550 μmol m⁻² s⁻¹, a 12 h photoperiod and at a suboptimal temperature of 14/12 °C (day/night) until the full expansion of the third leaf. After this treatment, the chilling-tolerant genotypes, when compared with the sensitive ones, displayed a higher photosynthetic activity, a higher content of chlorophyll (Chl) a+b, a higher Chl a/b ratio, a larger total carotenoid pool size as well as a different carotenoid composition. When temperature was subsequently increased to 24/22 °C for 3 d the composition of the pigments changed, but the chilling-sensitive genotypes, while adjusting their lower Chl a/b ratio and their different carotenoid composition, were unable to adjust their lower content of chlorophyll, their smaller total carotenoid pool size or their lower photosynthetic performance. Moreover, while the chilling-tolerant genotypes converted the most part of zeaxanthin to violaxanthin in the xanthophyll cycle, the chilling-sensitive genotypes retained high amounts of zeaxanthin. The changes in pigment composition that occurred over the 3 d at 24/22 °C were largely conserved when the plants were returned to 14/12 °C, but photosynthetic activity decreased and zeaxanthin accumulated again. The results suggest that the capability of the chilling-tolerant genotypes, when compared with the sensitive ones, to retain high amounts of pigments and to form a competent photosynthetic apparatus at low temperature is the basis for their more vigorous growth in cool climates.

Key words: Carotenoids, chlorophylls, low temperature, photosynthesis, Zea mays.

Introduction

It is well established that when maize (Zea mays L.), a chilling-susceptible crop with a tropical/subtropical origin, is exposed to low temperature during growth the plants grow slowly and develop leaves with a reduced area and that have a pale green chlorotic appearance (Miedema, 1982; Stamp, 1984; Stamp et al., 1983; Baker and Nie, 1994). The phenomenon of low temperature-induced chlorosis in maize is not yet fully understood, but it has been suggested that chlorosis develops under conditions of low temperature and high irradiance because chlorophyll is photo-oxidized prior to its integration in pigment–protein complexes of the thylakoid membranes where the pigments are less prone to photo-oxidative damage (MacWilliam and Naylor, 1967). It has been demonstrated that chilling-sensitive plant species, when compared with chilling-resistant ones, are highly susceptible to low temperature-induced photooxidations (Wise and Naylor, 1987; Wise, 1995). It is also well known that the carotenoids in general and those of the xanthophyll cycle in particular play an essential role in protecting the photosynthetic apparatus against photo-oxidative damage (Pfündel and Bilger, 1994; Demmig-Adams and Adams, 1996; Demmig-Adams et al., 1996; Horton et al., 1996; Yamamoto and Bassi, 1996; Gilmore, 1997).
In temperate regions low temperature-induced chlorosis in maize is generally most pronounced in the early growing season. Leaves grown at low temperature, compared to those grown under normal conditions, are characterized by a very low photosynthetic performance (Nie and Baker, 1991; Stirling et al., 1991; Nie et al., 1992; Fryer et al., 1995; Haldimann et al., 1996), changes in the content and the composition of the pigments (Stamp et al., 1983; Nie and Baker, 1991; Nie et al., 1992; Haldimann et al., 1995, 1996; Haldimann, 1996, 1998), changes in the activities of several enzymes of photosynthetic carbon assimilation (Stamp, 1987) and oxygen scavenging system (Massacci et al., 1995; Hull et al., 1997; Fryer et al., 1998), reduced activities of photosystem I and photosystem II associated with modifications in the composition of thylakoid membranes (Nie and Baker, 1991; Bredenkamp and Baker, 1994; Robertson et al., 1993), and changes in the contents of the endogenous antioxidants ascorbate, glutathione and \( \gamma \)-tocopherol (Leipner et al., 1997; Fryer et al., 1998). Leaf anatomy and the number and general structure of the chloroplasts have been shown not to be significantly different from those of leaves continually grown at normal growth temperature (Robertson et al., 1993). Perhaps even more important is the fact that low growth temperature-dependent depressions in the photosynthetic activity do not recover easily when the environmental conditions become favourable for growth. In fact, maize leaves thoroughly developed at low temperature exhibit a sustained low photosynthetic performance even after several days of growth in an optimal growth environment (Fryer et al., 1995; Nie et al., 1995; Haldimann, 1996). It is important to note that the effects of long-term moderate chilling are less dramatic and recovery from chilling-induced reductions in photosynthesis faster and more complete when the low temperature stress is imposed on maize leaves that were initially thoroughly developed under normal conditions (Haldimann, 1997). It appears therefore that the low growth temperature-dependent inhibition of the development of a competent photosynthetic apparatus is likely to be the most important factor responsible for the poor photosynthetic productivity of maize in cool climates (Baker, 1994; Baker et al., 1994).

It has been demonstrated that within the general susceptibility of maize to low temperature chilling-tolerant genotypes exist which, compared to more sensitive ones, have the capability to form a more competent photosynthetic apparatus when exposed to low temperature during growth (Stamp, 1984; Haldimann, 1998). Despite the numerous studies on the effects of chilling on the physiology of maize, the genotypic variability in the capacity to recover from low temperature-induced changes to the structure and the function of the photosynthetic apparatus has been largely neglected. This work, therefore, examines the changes in photosynthetic activity and composition of the pigments that occur in maize genotypes with different sensitivities to low temperature when, after an initial period of growth at low temperature, the plants experience a period with favourable growth conditions before being again exposed to low temperature.

### Materials and methods

**Plant material and growth conditions**

Six *Zea mays* L. inbred lines were used in the experiments: chilling-tolerant Z7 (Breeding Company Zelder, Ottersum, The Netherlands), Z15 (Breeding Company Zelder, Ottersum, The Netherlands) and KW1074 (Kleinwanzlebener Saatzucht, Einbeck, Germany) with a European origin and chilling-sensitive MO17 (Experimental Station, University of Missouri, Columbia, Miss., USA), CM109 and Penjalinan (Suwan Farm, Kasetsart University, Bangkok, Thailand) with a tropical/subtropical origin (Stamp et al., 1983; Stamp, 1984; Kocsy et al., 1996). Seeds were first germinated between moistened filter paper at 24 °C for 3 d. Seedlings were then planted into 1 dm \(^3\) pots (2 plants per pot) containing a soil:peat mixture (5:1, v/v) and grown in a growth chamber (Conviron, model PGW36, Winnipeg, Canada) at a relative humidity of 60/70% (day/night), a 12 h photoperiod, and an irradiance of 550 μmol photons m \(^{-2}\) s \(^{-1}\) (PAR). Light was provided by fluorescent tubes (Sylvania cool white, VHO) and incandescent bulbs (100 W, Sylvania). The plants were first grown at a suboptimal temperature of 14/12 °C (day/night) until the full development of the third leaf (45 d). Temperature was then increased from 14/12 °C to 24/22 °C for 3 d and subsequently returned to 14/12 °C for 5 d. Temperature changes took place during the night at a rate of about 0.85 °C h \(^{-1}\). Photosynthetically active radiation (PAR) was measured with a quantum sensor (Li-185, Li-Cor, Lincoln, NE, USA). Canopy temperature in the growth chamber was measured with a psychrometer (type WVU) connected to a DL2 Delta Logger (Delta T Devices Ltd, Cambridge, UK).

**Measurement of photosynthesis**

The net rate of photosynthetic CO \(_2\) assimilation (\( A_{\text{net}} \)) was measured 6 h after the beginning of the light period using the portable photosynthesis system Li-Cor 6200 (Li-Cor, Lincoln, NE, USA) under environmental conditions equivalent to those which predominate during growth.

**Pigment analysis**

For pigment analysis two leaf segments (1.5 cm \(^2\)) were cut from the central part of the third leaf, frozen immediately in liquid nitrogen, and subsequently stored in a freezer at −80 °C until analysis. Plant material was collected 6 h after the beginning of the light period. Pigment extraction and high performance liquid chromatography (HPLC) analyses of chlorophylls and carotenoids were carried out essentially according to Thayer and Björkman (1990) as described elsewhere (Haldimann et al., 1995).

**Statistics**

All the measurements were made on six replicates. The significance (\( P<0.05 \)) of the differences in photosynthetic activity and contents of pigments observed among the genotypes was tested with analysis of variance using the STATGRAPHICS statistical system (STSC Inc., MA, USA).
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Fig. 1. Effects of changes in temperature on the net rate of photosynthetic CO₂ assimilation in the third leaf of three chilling-tolerant (Z7, Z15 and KW1074) and three chilling-sensitive (CM109, MO17 and Penjalinan) genotypes of Zea mays. The plants were initially grown at a suboptimal temperature of 14/12 °C until the full expansion of the third leaf. Values are means of six replicates. Bars indicate SD. Within a given day of measurement values carrying different letters are significantly different at *P* < 0.05.

Results

Photosynthetic activity

The chilling-tolerant genotypes (Z7, Z15 and KW1074) showed a much higher net rate of CO₂ assimilation (Aₙ) than the chilling-sensitive ones (CM109, MO17 and Penjalinan) when the third leaf of the plants had achieved full expansion after having been continually exposed to 14/12 °C (day/night) during growth (Fig. 1). The Aₙ increased in all the genotypes when the temperature was increased to 24/22 °C, but the chilling-sensitive genotypes were not able to take full advantage of the warmer conditions to increase their inferior photosynthetic activity although the Aₙ in MO17 approached that of the chilling-tolerant genotypes on the third day at 24/22 °C. When the plants were returned to 14/12 °C a reduction in the Aₙ could be observed in most of the genotypes during the first 3 d after the temperature change, but thereafter photosynthetic activity stabilized at a low level (Fig. 1). During this second period at 14/12 °C the chilling-tolerant genotypes still exhibited a higher Aₙ than the chilling-sensitive ones, but the differences between MO17 and Z7 or KW1074 were not any more statistically significant (Fig. 1).

Content of chlorophyll and total carotenoid pool size

At the third leaf stage, before the temperature was increased to 24/22 °C, the chilling-tolerant genotypes exhibited a higher content of chlorophyll (Fig. 2A) and a higher Chl a/b ratio (Fig. 2B) than the chilling-sensitive ones. Likewise, the total carotenoid pool size was generally larger in the chilling-tolerant genotypes than in the sensitive ones when expressed on a unit leaf area basis (Fig. 3A). No clear difference appeared between the two categories of genotypes when the total carotenoid content was expressed relative to the content of chlorophyll (Fig. 3B). The content of chlorophyll increased when the temperature was increased to 24/22 °C, but the chlorophyll content of the chilling-sensitive genotypes stayed significantly below that of the chilling-tolerant ones (Fig. 2A). At warmer conditions there was also an increase in the Chl a/b ratio and on the third day at 24/22 °C this ratio was similar in most of the genotypes (Fig. 2B). Since the content of total carotenoid (Fig. 3A) was affected by the temperature change to a much lesser extent than the content of chlorophyll (Fig. 2A), the ratio of total carotenoid to Chl a+b decreased markedly during the treatment at 24/22 °C (except in Z15 and KW1074 where the changes were less pronounced) and on the third day at 24/22 °C this ratio became similar in all the genotypes (Fig. 3B). During the second period at 14/12 °C the chilling-tolerant genotypes still showed a higher content of chlorophyll (Fig. 2A) and a larger total carotenoid pool size (Fig. 3A) than the chilling-sensitive ones, but the two categories of genotypes showed no clear difference in terms of their Chl a/b ratio (Fig. 2B) or their ratio of total carotenoid to Chl a+b (Fig. 3B).

Components of the carotenoid pool

When the plants had been continually exposed to low temperature during growth a genotypic variability...
appeared in the contents of lutein, neoxanthin, β-carotene, and xanthophyll cycle carotenoids (violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z)) regardless of whether the content of pigment was expressed relative to the content of chlorophyll (Fig. 4A–D) or the total carotenoid pool size (Fig. 5A–D). However, concerning the ratio of neoxanthin to Chl _a+b_ none of the differences that appeared among the genotypes was statistically significant (Fig. 4B). The chilling-tolerant genotypes, when compared with the sensitive ones, displayed a higher relative content of β-carotene (Fig. 5C) and a lower ratio of V+A+Z to β-carotene (Fig. 6), but the differences between Z7 and MO17 were not statistically significant. Increasing the temperature changed the carotenoid composition. In particular, there was a decrease in the ratios of lutein and V+A+Z to Chl _a+b_ (Fig. 4A, D), a decrease in the relative content of V+A+Z (Fig. 5D), an increase in the relative content of β-carotene (Fig. 5C), and a decrease in the ratio of V+A+Z to β-carotene (Fig. 6). All these changes were less pronounced in Z15 and KW1074 than in the other genotypes. On the third day at 24/22 °C, the two categories of genotypes no longer showed a clear difference in terms of their relative content of β-carotene (Fig. 5C) or their ratio of V+A+Z to β-carotene (Fig. 6), but the chilling-tolerant genotypes displayed a higher ratio of β-carotene to Chl _a+b_ than the chilling-sensitive ones (Fig. 4C). More generally, at warmer conditions, the amplitude of the genotypic variability in the contents of the different components of the carotenoid pool was reduced regardless of whether the content of pigment was expressed relative to the chlorophyll content (Fig. 4A–D) or the total carotenoid pool size (Fig. 5A–D). The changes in the contents of the different components of the carotenoid pool that occurred over the 3 d at warmer conditions were largely conserved over the following 5 d at 14/12 °C (Figs 4, 5). Thus, the amplitude of the genotypic variability remained reduced and the two categories of genotypes showed no clear difference in terms of their carotenoid composition (Figs 4, 5, 6).

**Xanthophyll cycle carotenoids**

The analysis of the effects of changes in temperature on the composition of the xanthophyll cycle pool (V+A+Z) revealed that prior to the increase of the temperature to...
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24/22 °C zeaxanthin accounted for about 80–90% and 40–60% of the pool in the chilling-sensitive genotypes and the chilling-tolerant ones, respectively (Fig. 7C). The relative content of antheraxanthin was significantly higher in the chilling-tolerant genotypes (18–21%) than in the chilling-sensitive ones (6–10%) (Fig. 7B). On the first day at 24/22 °C, zeaxanthin was largely converted to violaxanthin in the chilling-tolerant genotypes, whereas in the chilling-sensitive ones much less conversion took place with the result that zeaxanthin accounted for about 15–20% of the V+A+Z pool in the former genotypes while this portion was still around 60% (CM109 and MO17) or 80% (Penjalinan) in the latter genotypes (Fig. 7A-C). Even on the third day at 24/22 °C, zeaxanthin still accounted for 30% (MO17) or 40% (CM109 and Penjalinan) of the V+A+Z pool in the chilling-sensitive genotypes while the portion of zeaxanthin had fallen below 12% in the chilling-tolerant ones (Fig. 7C).

When the plants were subsequently returned to 14/12 °C violaxanthin was de-epoxidized to zeaxanthin, but the relative content of zeaxanthin was always significantly higher in the chilling-sensitive genotypes than in the tolerant ones (Fig. 7A, C).

**Discussion**

Photosynthetic activity increased in all the maize genotypes when the temperature was increased from 14/12 °C to 24/22 °C (Fig. 1). However, even on the third day at warmer conditions, the rate of CO$_2$ assimilation stayed much below that measured in the same genotypes when the plants were continually developed at 24/22 °C (about 25 μmol CO$_2$ m$^{-2}$ s$^{-1}$; see Haldimann, 1998). Moreover, the treatment at 24/22 °C did not permit the chilling-sensitive genotypes to adjust their inferior photosynthetic performance (Fig. 1). It has been demonstrated that...
Fig. 6. Effects of changes in temperature on the ratio of total xanthophyll cycle carotenoids (V + A + Z) to β-carotene in the third leaf of three chilling-tolerant (Z7, Z15 and KW1074) and three chilling-sensitive (CM109, MO17 and Penjalinan) genotypes of *Zea mays*. V, A, and Z denote the contents of violaxanthin, antheraxanthin and zeaxanthin, respectively. See Fig. 2 for more details.

young leaf tissue, in which the photosynthetic apparatus is still under development, is better able to recover from low growth temperature-induced depressions in photosynthetic activity than mature leaf tissue (Haldimann, 1996). Thus, it is possible that the comparatively large increase in photosynthetic activity observed in MO17 at warmer conditions is related to the fact that the third leaf of this genotype was not yet fully mature when the temperature was increased as this genotype was growing very slowly at low temperature. The chilling-sensitive genotypes, starting from a lower content of chlorophyll (Fig. 2A) and a smaller total carotenoid pool size (Fig. 3A) than the chilling-tolerant genotypes, were not able to compensate for these deficiencies during the treatment at 24/22°C. This is likely to be an important factor responsible for the permanent weak photosynthetic performance observed in the chilling-sensitive genotypes (Fig. 1).

As discussed in more detail elsewhere (Haldimann, 1998), the reduced Chl a/b ratio (Fig. 2B) together with the reduced content of β-carotene (Figs 4C, 5C) observed in maize leaves grown at low temperature is likely to be related to the fact that such leaves display reduced proportions of reaction centre core complexes to light-harvesting antenna complexes in photosystem I (PSI) and photosystem II (PSII) (Nie and Baker, 1991). This feature is associated with reduced electron transport activities in both photosystems (Nie and Baker, 1991; Nie et al., 1995). As the activities of both photosystems have been shown to increase when the plants are exposed to warmer conditions (Nie et al., 1995), the increase in the Chl a/b ratio (Fig. 2B) and the increase in the content of β-carotene (Figs 4C, 5C) together with the less marked changes in the contents of lutein (Fig. 5A) and neoxanthin (Fig. 5B) that occurred on exposure of the plants to warmer conditions have been thought indicative for a preferential resynthesis of PSI and PSII reaction centre core complexes relative to light-harvesting antenna complexes (Haldimann, 1996). Maize has two types of chloroplasts, mesophyll and bundle sheath, with different PSII/PSI compositions. Thus, since it has been demonstrated that large amounts of β-carotene are associated with PSI and that PSI displays a high Chl a/b ratio (Thayer and Björkman, 1992), it is also possible that changes in the composition of the pigments reflect differential changes in these two populations of chloroplasts.

The finding that at low temperature the chilling-sensitive genotypes generally displayed a lower Chl a/b ratio (Fig. 2B) and a lower content of β-carotene (Figs 4C, 5C) than the chilling-tolerant genotypes might be indicative of a higher proportion of damaged reaction centres in the former genotypes than in the latter ones. However, since the chilling-sensitive genotypes had the capability to adjust their lower Chl a/b ratio (Fig. 2B) and their lower content of β-carotene (Figs 4C, 5C) at warmer conditions, it appears that efficient mechanisms of repair were operating in these genotypes, but that they did not permit these genotypes to adjust their inferior photosynthetic competence (Fig. 1). That the chilling-sensitive genotypes displayed a higher ratio of V + A + Z to β-carotene than the chilling-tolerant ones when the plants had been continually exposed to low temperature during growth (Fig. 6) may reflect some differences in the structure of PSII and PSI. Indeed, β-carotene is mainly localized in the reaction centre core complexes of PSII.
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(Figs 4, 5, 6) and, more generally, of the composition of the thylakoid membranes (Nie et al., 1995) maize leaves grown at low temperature are incapable of achieving full photosynthetic competence when exposed to warmer conditions remains an open question, but the organization and stability of proteins in the membranes may play a central role (Nie et al., 1995).

The plants responded to the low temperature stress by reducing their chlorophyll content (Fig. 2A), increasing their ratio of total carotenoid to chlorophyll (Fig. 3B) and by the accumulation of large amounts of zeaxanthin and antheraxanthin (Fig. 7B, C). This leads to a reduction in the absorption of excitation energy on one hand and to an increase in the capacity for non-radiative energy dissipation on the other hand. Differences in the ratio of total carotenoid to chlorophyll (Fig. 3B) and in the accumulation of zeaxanthin (Fig. 7C) that appeared among the genotypes prior to the treatment at 24/22 °C, the content of zeaxanthin being higher in the chilling-sensitive genotypes than in the tolerant ones, may reflect differences in the need to dissipate excitation energy that cannot be dissipated through the electron transport chain. As expected, the ratio of total carotenoid to chlorophyll (Fig. 3B) and the content of zeaxanthin (Fig. 7C) decreased as photosynthetic activity increased (Fig. 1) at warmer conditions. However, even at warmer conditions the chilling-sensitive genotypes retained higher amounts of zeaxanthin than the chilling-tolerant ones (Fig. 7C).

Zeaxanthin accumulated again when the plants were returned to 14/12 °C, but here again the content of zeaxanthin was higher in the chilling-sensitive genotypes than in the tolerant ones (Fig. 7C). Collectively these results suggest that the requirement of excitation energy dissipation was generally higher in the chilling-sensitive genotypes than in the tolerant ones.

In conclusion, none of the maize genotypes tested in this study had the capacity to recover fully from low temperature-induced depressions in photosynthetic activity when the plants were exposed to warmer conditions. Nevertheless, the chilling-tolerant genotypes, when compared with the chilling-sensitive ones, had the capability to accumulate higher amounts of pigments and to form a more competent photosynthetic apparatus at low temperature. Warmer conditions permitted the chilling-sensitive genotypes to adjust their carotenoid composition, but neither their lower content of pigment nor their inferior photosynthetic performance. Thus, the capacity of the chilling-tolerant genotypes to form a more competent photosynthetic apparatus at low temperature is likely to be an important factor responsible for their more vigorous growth in cool climates.

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