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BRIEF COMMUNICATION

Chlorophyll fluorescence and anthocyanin content in chilled maize plants after return to a non-chilling temperature under various irradiances

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Abstract

The effect of irradiance on the ratio of variable to maximum fluorescence (F_v/F_m) and on the anthocyanin content of chilled (0.5 °C) young maize plants was investigated after returning the plants to a non-chilling temperature (25 °C). Compared to control plants grown throughout at 25 °C in the light, the F_v/F_m hardly changed during chilling or when returned to a non-chilling temperature in the dark, but there was a decrease in this parameter if the plants were shifted to the light after the cold treatment. Similarly, compared to the control plants there was no change in the anthocyanin content either at low temperature or after transfer to 25 °C in the dark. However, there was a sudden increase in the anthocyanin level after returning the plants from dark cold conditions to a non-chilling temperature in the light.

Additional key words: post-chilling symptoms, *Zea mays*.

Maize (*Zea mays* L.) plants, which are sensitive to chilling, may suffer severe damage at low temperature. The rate of damage depends not only on the temperature, but also on the irradiance (Long 1983). The exposure of plants to an irradiance higher than that which can be utilized in the photosynthetic processes may damage (photoinhibit) the photosynthetic apparatus, especially the photosystem 2 (PS2) (Kyle 1987). Chlorophyll (Chl) fluorescence induction parameters are much less affected by low temperature when the cold treatment is carried out at low irradiance or in complete darkness (Janda *et al.* 1994). Although low temperature may cause

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severe damage, some damage symptoms can only be seen after returning the plants to higher temperatures (Hetherington and Öquist 1988, Szalai *et al.* 1996). Chilling stress may also increase the expression of genes related to the phenylpropanoid metabolism (Hahlbrock and Scheel 1989). This process leads to the appearance of anthocyanin pigmentation in maize leaves. The effect of irradiance on certain post-chilling symptoms was investigated in the present study using the Chl fluorescence induction method and anthocyanin content determination.

Two-week-old maize (*Zea mays* L. hybrid Furio) plants grown at day/night temperatures of 25/23 °C, a 16 h photoperiod (200 μmol m^{-2} s^{-1} of photosynthetically active radiation) and 75 % relative humidity were exposed to an extremely low temperature (0.5 °C) in the dark in a climatized chamber (*Conviron PGV-36*). The chlorophyll fluorescence induction parameters in the third leaves were determined at room temperature using a *PAM-2000* fluorometer (*Walz,* Effeltrich, Germany) after 30 min dark adaptation. The method described by Kho *et al.* (1977) was used for the anthocyanin content determination with a slight modification: 0.5 g leaf tissue was homogenized in 4 cm^3 of methanol containing 1.0 M HCl and was kept at 4 °C for 4 h. After centrifugation at 10 000 *g* for 30 min the absorbance of the supernatant was measured at 530 nm.

The rate of photoinhibition can be followed by measuring the ratio between variable and maximum fluorescence (F_v/F_m) that determines the maximum photochemical efficiency of PS2 (Krause 1988). Compared to the control this parameter hardly changed after 2 d in the dark at low temperature (0.5 °C) or after a further 2 d at 25 °C following the cold treatment; however, there was a decrease after a shift back to the growth temperature (25 °C) in the light (Table 1). Thus, photoinhibition may also affect the post-chilling symptoms.

Table 1. Changes in F_v/F_m and anthocyanin content (characterized by absorbance at 530 nm) in 2-week-old maize plants during cold treatment at 0.5 °C in the dark and after returning them to a normal temperature (25 °C) in the dark (D) or light (L) (200 μmol m^{-2} s^{-1}) following 2 d of cold treatment in D. The results are means of at least 6 replications.

Treatment	F_v/F_m	Anthocyanin (A_{530})
Control	0.798 ± 0.010	0.08 ± 0.03
2 d D 0.5 °C	0.784 ± 0.009	0.11 ± 0.03
2 d D 0.5 °C + 2 d D 25 °C	0.771 ± 0.015	0.09 ± 0.03
2 d D 0.5 °C + 1 d L 25 °C	0.747 ± 0.016	0.32 ± 0.03
2 d D 0.5 °C + 2 d L 25 °C	0.705 ± 0.020	1.61 ± 0.19
2 d D 0.5 °C + 1 d D 25 °C + 1 d L 25 °C	0.719 ± 0.014	0.41 ± 0.16

Chilling may lead to an increase in the anthocyanin level of injured leaves (Dixon and Paiva 1995). Similarly to F_v/F_m, no change was detected in the anthocyanin level at 0.5 °C even after 4 d of dark cold treatment (data not shown) or at 25 °C in the dark following a 2-d cold treatment in the dark (Table 1). However, a dramatic increase in the pigment content was found in maize seedlings cold treated for 2 d after shifting them to 25 °C in the light if the low temperature treatment was carried

out in complete darkness (Table 1). The pigment content also increased when maize plants cold treated in the dark for 2 d were first kept at 25 °C in the dark for 1 d, during which time no change in the anthocyanin content occurred, and were then transferred to 25 °C in the light (Table 1).

In seedlings exposed to 5 °C and shifted to 25 °C, Christie *et al.* (1994) found only a slight change in the anthocyanin content, while chilling stress carried out at 10 °C caused a dramatic increase in transcript abundances for anthocyanin regulatory and structural genes, but only a slight increase in the anthocyanin content. After returning the plants to 25 °C, pigmentation increased several times, and hence a 10 °C stress enhanced anthocyanin gene transcription or transcript stabilities, but impaired the post-transcriptional processes important for anthocyanin biosynthesis, which were restored at 25 °C. Anthocyanin accumulation was found in all tested lines that were genotypically capable of anthocyanin production (Christie *et al.* 1994).

Our results suggest that the lack of the appearance of anthocyanin at low temperature, *i.e.* the impairment of the transcriptional and translational processes important for anthocyanin biosynthesis, is due not only to chilling, but also to light stress.

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