

# Photosynthetic performance of two maize genotypes as affected by chilling stress

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## ABSTRACT

The effect of chilling on light dependence of photosynthetic and chlorophyll *a* fluorescence characteristics in two maize genotypes CE 704 and CE 810 grown in a glasshouse during spring and autumn was studied. In spring, the net photosynthetic rate ( $P_N$ ) of CE 704 plants was not affected by chilling under moderate irradiance but it was strongly affected under a saturating one. This indicates that efficiency of photosynthetic apparatus was not affected by chilling but its capacity was decreased. Contrary to CE 704, CE 810 plants were not affected by chilling under saturating irradiance. In autumn, CE 704 plants adapted to chilling and no statistically significant differences in  $P_N$  and Fv/Fm between chilled and control plants in the whole range of irradiance were found. Enhanced activity of non-photochemical quenching (NPQ) in chilled CE 704 plants under saturating irradiance corresponded with an increased level of xanthophyll cycle pigments and an increased deepoxidation state of these pigments.

**Keywords:** maize (*Zea mays* L.); genotype; chilling; light dependence of photosynthetic characteristics; net photosynthetic rate ( $P_N$ ); chlorophyll *a* fluorescence; photosynthetic pigments; growth

Despite its tropical origin, maize (*Zea mays* L.) has become a widely cultivated crop in temperate regions. Low spring temperatures can seriously damage growth and ontogeny of young maize plants due to the inhibition of their photosynthesis (Nie and Baker 1991, Nie et al. 1992, Dolstra et al. 1994, Massacci et al. 1995, Haldimann et al. 1996). Maize as a biological species includes a large number of genotypes which differ in their tolerance to low temperatures (chilling or cold) (Dolstra et al. 1994, Massacci et al. 1995, Fracheboud et al. 1999). Differences in photosynthetic characteristics are particularly important because the yield of the photosynthetic processes is used for growth during plant ontogeny.

A great deal of work has been done in investigating key photosynthetic characteristics which are responsible for chilling tolerance in young maize plants, but little is still known about the impact of chilling on photosynthetic apparatus in the whole range of irradiance. Fluorescence measurements are widely used as noninvasive indicators of state of PSII (e.g. Krause and Weis 1991).

The aim of this work was to investigate how chilling affects light dependence of  $P_N$  and chlorophyll *a*

fluorescence and what the observed changes under the whole range of irradiance can tell us about the impact of chilling on the photosynthetic apparatus, particularly on PSII. Our intention was to study the impact of chilling in two maize genotypes with different photosynthetic performance. The other main idea was to compare the influence of chilling during two different seasons – spring and autumn where low but positive temperatures could occur. The impact of chilling on composition of photosynthetic pigments was also investigated.

## MATERIAL AND METHODS

### Plant material

Seeds of two maize inbred lines CE 704 and CE 810 (Breeding station CEZEA, Čejč, Czech Republic) were sown into pots filled with garden soil. Plants were grown in tempered greenhouse (mean daily temperatures 20–25°C; mean daily RH 60–70%) for 10 days and after this period one half of plants was transferred into non-tempered greenhouse (mean daily temperatures 10–15°C; large differences

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during spring day: from 0 a.m. to 6 a.m. mean temperatures 5–7°C while at noon mean temperatures 20–25°C; smaller differences during the autumn day: from 0 a.m. to 6 a.m. mean temperatures 8–10°C while at noon mean temperatures 15–17°C; mean daily RH 70–80%; Kosova 2004). No additional fertiliser was applied during growth period. After three weeks leaf discs from matur, fully expanded leaves (3<sup>rd</sup> leaf in control plants, 2<sup>nd</sup> leaf in chilled ones) were cut and used for simultaneous measurement of  $P_N$  and chlorophyll *a* fluorescence parameters, and probes for the determination of photosynthetic pigments were harvested.

### Growth characteristics

Length of internodes, number of leaves per plant and length of leaf used for measurements of photosynthetic and fluorescence characteristics, were determined during the experiments. After measuring  $P_N$ , dry matter of leaf discs was determined after a period of drying at 80°C for 24 hours.

### Net photosynthetic rate

Net photosynthetic rate ( $P_N$ ) was measured by a Clark type leaf disc oxygen electrode (LD2/2, Hansatech, King's Lynn, UK). Actinic illumination was provided by a halogen lamp and its intensity was changed by neutral optical filters. Irradiance (PAR, 400–700 nm) was measured by quantum radiometer Li-Cor (Li-Cor Instruments, Lincoln, NE, USA).

### Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured with a PAM fluorometer (PAM 101-103, Walz, Effeltrich,

Germany) which was connected with a source of light saturation pulses (KL 1500 electronic, Schott, Germany). Ten leaf discs (each of an area of 0.5 cm<sup>2</sup>) were used for one measurement. At least 30 min of dark adaptation was applied before initial fluorescence  $F_0$  was measured. After determining of  $F_0$ , a light saturation pulse was applied and maximum fluorescence  $F_m$  was obtained. Variable fluorescence  $F_v$  was calculated as the difference between  $F_m$  and  $F_0$ . Under actinic illumination steady-state fluorescence  $F_s$  was measured and  $F_m'$  was determined under simultaneous application of actinic illumination and a saturation pulse. Fluorescence parameters characterising either dark-adapted state or light-adapted state were calculated according to the following formulae: maximum quantum yield of PSII,  $\Phi_{PSII} = F_v/F_m = (F_m - F_0)/F_m$ ; actual rate of photochemical reduction of RC PSII,  $Q_r/Q_t = (F_s - F_0)/(F_m' - F_0)$  and Stern-Volmer definition of non-photochemical quenching,  $NPQ = (F_m - F_m')/F_m'$  according to Rohacek (2002). For more details see, e.g., Ticha et al. (1998).

### Pigment analysis

Samples of leaf tissue used for quantitative pigment analysis were immediately frozen in liquid nitrogen, lyophilized and then stored at –80°C. Photosynthetic pigments were extracted in acetone with BHT (butylhydroxytoluene) and MgCO<sub>3</sub> and separated using HPLC (Spectra-Physics, San Jose, USA) on reversed-phase column (Sepharon SGX C18, Tessek, Praha). The solvent system was a linear gradient of acetonitrile/methanol/water (80:12:6) followed by 100% methanol. More details can be found in Haisel et al. 1999. DEPS, deepoxidation state of xanthophyll cycle pigments violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) was calculated as  $(1/2A + Z)/(V + A + Z)$ .

Table 1. Growth characteristics of plants used for photosynthetic and fluorescence measurements; data represent means ± standard errors; means have been calculated from at least 25 plants

Genotype	Experimental season	Length of leaf used for measurements (mm)	Number of leaves per plant	Average dry mass of leaf disc per area (mg/cm <sup>2</sup> )
704C	spring	167 ± 4.28	5.62 ± 0.12	2.06 ± 0.40
704S		87 ± 2.15	4.07 ± 0.08	2.13 ± 0.36
810C	spring	190 ± 7.32	4.72 ± 0.15	2.13 ± 0.54
810S		102 ± 5.10	3.88 ± 0.11	2.13 ± 0.29
704C	autumn	176 ± 7.15	4.3 ± 0.07	1.57 ± 0.26
704S		85 ± 4.82	3.3 ± 0.08	1.71 ± 0.29

C – unchilled plants, S – plants affected by chilling stress

## Statistical analysis

Differences between chilled and control plants were evaluated using one-way ANOVA.  $P$ -values lower than 0.05 were considered statistically significant,  $P$ -values lower than 0.01 were regarded as highly significant.

## RESULTS AND DISCUSSION

### Growth characteristics

Decrease in growth rate was found in both chilled genotypes. After three weeks of chilling treatment chilled plants had fewer leaves and internodes in comparison with non-chilled ones (Table 1, Figure 1A, B). Decrease in growth rate, but no effects on plant ontogeny, were observed by Nie and Baker (1991), Nie et al. (1992), Sowinski et al. (2003). Sowinski et al. (2003) also found thicker leaf blades in chilled maize plants.

### Net photosynthetic rate $P_N$

In spring, no significant differences in  $P_N$  in chilled CE 704 plants and the non-chilled ones under moderate irradiance (in the linear part of photosynthetic light response) were found, but  $P_N$  in chilled plants significantly decreased under

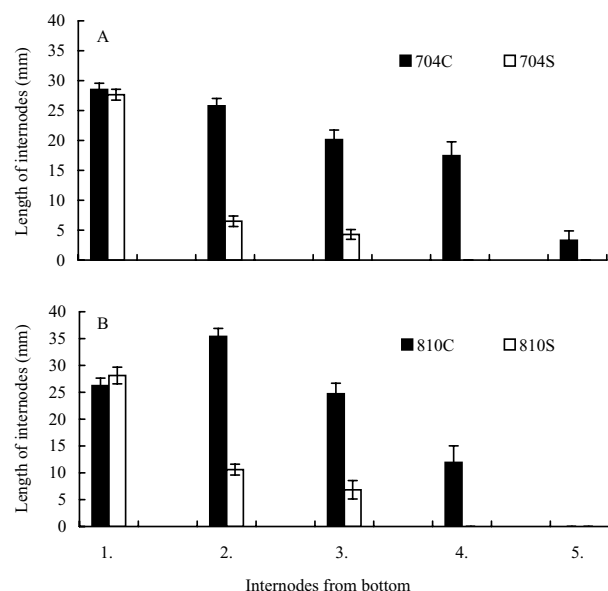


Figure 1. Length of internodes from the bottom of the plant. Each value represents the mean  $\pm$  standard error from at least 25 maize plants. A = CE 704 genotype, B = CE 810 genotype, C = unchilled control plants, S = plants affected by chilling stress

satürating irradiance (Figure 2A). This observation is in accordance with results of Massacci et al. (1995), Kingston-Smith et al. (1997), Fryer et al. (1998) who had measured photosynthetic light curves in chill-treated maize leaves. Contrary to CE 704 plants, values of  $P_N$  under saturating irradiance in chilled CE 810 plants were slightly higher than those measured in non-chilled ones (Figure 2B). Similar results were found also by other authors who had done experiments with genotypes which had contrasting ability to adapt to chilling (Massacci

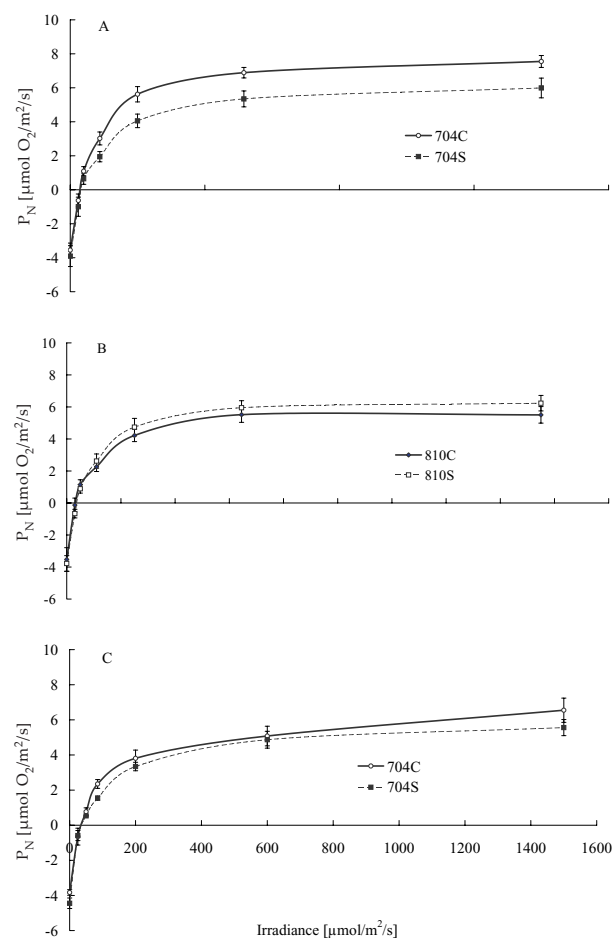


Figure 2. Comparison of light response of  $P_N$  expressed as  $\mu\text{mol O}_2/\text{m}^2/\text{s}$  (photosynthetic light curves). A represents data from spring experiments measured on CE 704 genotype, B represents data from spring experiments measured on CE 810 genotype, C represents data from autumn experiments measured on CE 704 genotype. C = unchilled plants, S = plants affected by chilling stress. Data represent means from 8 independent measurements  $\pm$  standard errors. In A, differences in  $P_N$  between control and chilled plants are statistically significant at irradiance higher than  $110 \mu\text{mol}/\text{m}^2/\text{s}$  while in B and C no statistically significant differences between control and chilled plants were found in the whole range of irradiance

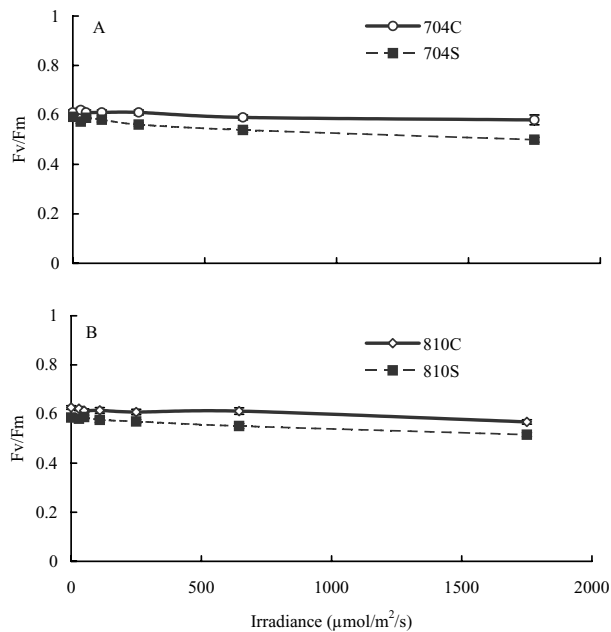


Figure 3. Light dependence of maximum quantum yield of RC PSII  $F_v/F_m$  measured in spring experiments on CE 704 genotype (A) and CE 810 genotype (B). C = unchilled plants, S = plants affected by chilling stress. Data represent means of 8 independent measurements  $\pm$  standard errors. In A and B, statistically significant differences between control and chilled plants were found in the whole range of irradiance

et al. 1995) and can be supported by Körnerová (2000) who had found enhanced PSII activity in isolated chloroplasts of CE 810 plants. However, during autumn chilled CE 704 plants managed to acclimate to low temperature and to maintain  $P_N$  sufficiently high under saturating irradiances, too (Figure 2C).

### Fluorescence characteristics

Under chilling, the ratio  $F_v/F_m$  in both genotypes was decreased (Figure 3A, B). This indicates a decreased capacity of PSII to capture solar energy under stress conditions and the development of a slow-relaxing photoinhibitory component of non-photochemical quenching  $q_I$  (Krause and Weis 1991). Similar results were observed by, e.g., Massacci et al. (1995), Haldimann et al. (1996), Aguilera et al. (1999), Fracheboud et al. (1999), Leipner et al. (1999). Low temperatures also led to a decrease in  $Q_r/Q_t$  under saturating irradiance, but only in CE 704 plants (Figure 4A). This parameter is the ratio of reduced (closed) RC PSII  $Q_r$  to the total amount of RC PSII  $Q_t$  and indicates the fraction of RC PSII, which is used in photochemical reactions. These results are in agreement with

Hurry et al. (1995) who observed a similar effect of chilling on young plants of spring and winter wheat cultivars. In spring (Figure 5A), there was no difference between chilled CE 704 plants and the non-chilled ones in the non-photochemical quenching calculated according to Stern-Volmer equation, whereas in autumn (Figure 5B), chilling increased NPQ especially under saturating irradiance. These results implicate development of xanthophyll cycle activity in chilled plants because thermal dissipation mediated by xanthophyll cycle activity is considered one of the major compounds of NPQ under high irradiances. These observations are in accordance with hypotheses of many other authors who have studied the relationship between mechanisms of non-photochemical quenching, thermal dissipation and xanthophyll cycle activity (Demmig-Adams and Adams 1996, Gilmore 1997, Havaux and Kloppstech 2001).

### Pigment analysis

Results obtained from quantitative pigment analysis in CE 704 maize plants in autumn showed an

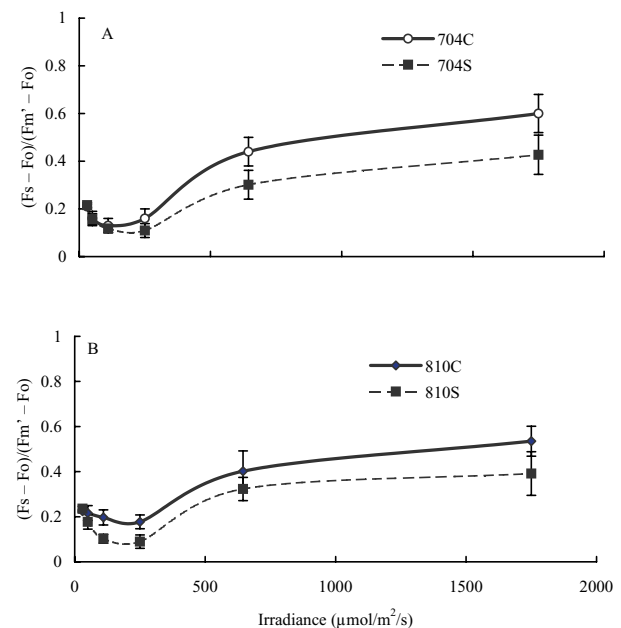


Figure 4. Comparison of light response of actual rate of photosynthetic reduction of RC PSII in spring experiments measured on CE 704 genotype (A) and CE 810 genotype (B). C = unchilled control plants, S = plants affected by chilling stress. Data represent means of 8 independent measurements  $\pm$  standard errors. In A, a decrease of  $P$ -values with increasing irradiance was observed, but, however, in both A and B no statistically significant differences between unchilled and chilled plants in the whole range of irradiance were found

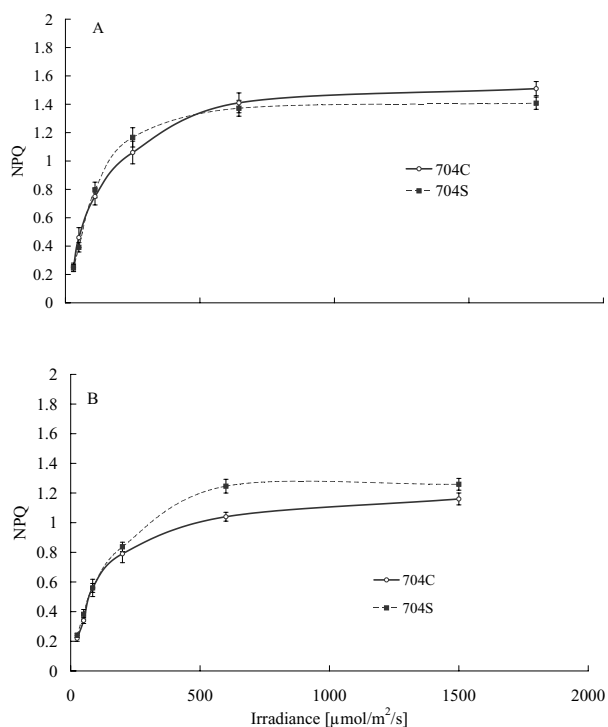


Figure 5. Light response of non-photochemical quenching NPQ measured with CE 704 plants in spring (A) and autumn (B). C = unchilled plants, S = plants affected by chilling stress. Data represent means of 8 independent measurements  $\pm$  standard errors. In A, no statistically significant differences between unchilled and chilled plants were found in the whole range of irradiance while in B, statistically significant differences between control and chilled plants were found at irradiance higher than 600  $\mu\text{mol}/\text{m}^2/\text{s}$

increase in the content of xanthophyll cycle pigments after chilling (Figure 6A). Especially, there was an increase in the deepoxidation state of xanthophyll cycle pigments which is the parameter expressing the activity of thermal dissipation processes (Figure 6B). These results are in accordance with the observations of, e.g., Haldimann (1996), Haldimann et al. (1996) and Venema et al. (1999). Surprisingly, under chilling conditions no decrease of total chlorophyll and chlorophyll *a* content was found. There was a slight (but statistically significant) increase in both parameters (Figure 6C) which is in contrast with the results of Massacci et al. 1995, Haldimann (1996), Haldimann et al. (1996) and Tichá et al. (2002). These results could explain the relatively high  $P_N$  in chilled CE 704 plants under saturating irradiance in autumn experiments. Holá et al. (2003) observed a similar effect of moderate chilling temperatures during autumn on chlorophyll *a* content and values of PSII activity in plants of the same genotypes grown under similar experimental conditions.

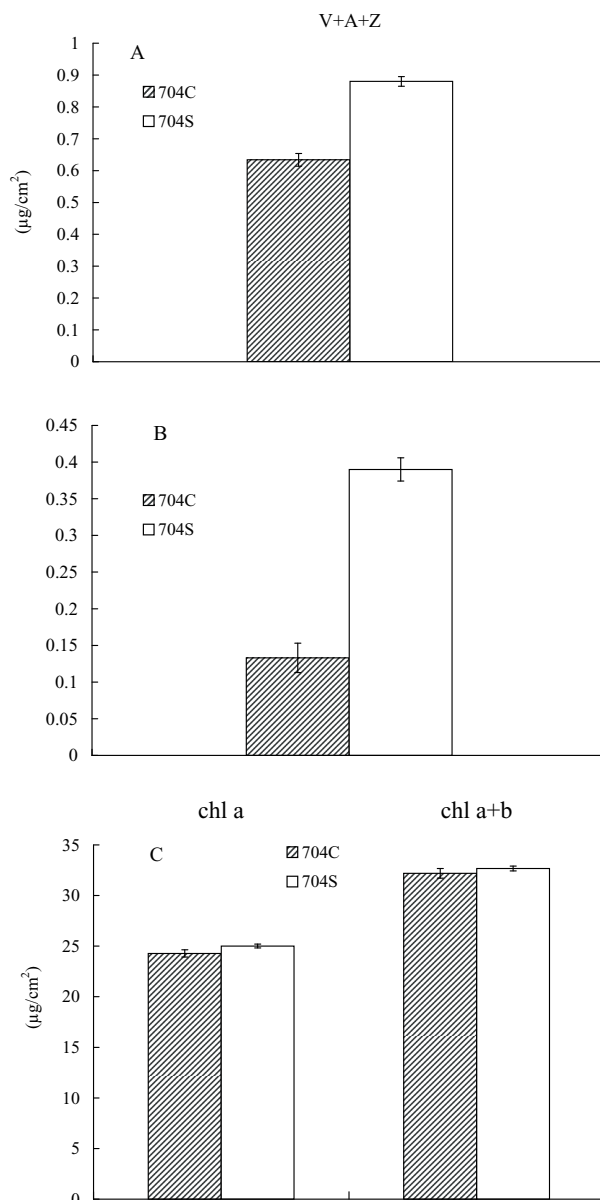


Figure 6. Total content of xanthophyll cycle pigments (A), deepoxidation state of xanthophyll cycle pigments (B) and chlorophyll *a* content and total chlorophyll content (C) in CE 704 plants measured in autumn experiments. C = unchilled plants, S = plants affected by chilling stress. Data represent means from 10 independent measurements  $\pm$  standard errors. Statistically significant differences were found in all characteristics shown and, with exception of C, the differences between unchilled and chilled plants were highly significant

It could be concluded that the chilling of CE 704 plants during **spring** did not affect the efficiency of the photosynthetic apparatus but decreased its capacity. The data obtained from chlorophyll fluorescence measurements suggest that chilling did not damage the photosynthetic apparatus directly.

The plants managed to adapt to a decreased rate of carbon assimilation processes. Thus, the capacity of primary photochemical processes in RC PSII was lowered as well. Decreased activity of PSII after chilling treatment was already found in our previous paper (Tichá et al. 2002). Contrary to CE 704 plants, CE 810 ones could probably better adapt to chilling, and stressed plants had nearly the same  $P_N$  as the unchilled ones in the whole range of irradiance. CE 810 plants can be considered more tolerant to chilling than CE 704 ones. Chilling tolerance itself is very important for the further development of maize plants in temperate climates but it is not the only factor determining the final yield.

In **autumn**, chilling probably affected photosynthetic apparatus of CE 704 plants more moderately than during spring. The plants were able to adapt to chilling stress and maintain the rate of  $P_N$  and chlorophyll *a* fluorescence parameters comparable to the control ones. This idea is also supported by the results obtained from analysis of photosynthetic pigments: an increased level of xanthophyll cycle pigments (antheraxanthin and zeaxanthin) and increased deepoxidation state of these pigments indicate an enhanced rate of thermal dissipation processes. The increased content of chlorophyll *a* which is in contrast with data found in the literature may be the reason for relatively high  $P_N$  under saturating irradiance.

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## ABSTRAKT

### Fotosyntetické charakteristiky dvou genotypů kukuřice ovlivněných chladovým stresem

Sledovali jsme vliv chladu na světelné křivky fotosyntetických a fluorescenčních charakteristik u dvou genotypů kukuřice (*Zea mays* L., CE 704 a CE 810) pěstovaných ve skleníku v jarním a v podzimním období. Rychlost čisté fotosyntézy ( $P_N$ ) jsme měřili pomocí Clarkovy kyslíkové elektrody, fluorescenci chlorofylu *a* pomocí PAM fluorometru a obsah fotosyntetických pigmentů pomocí HPLC. Na jaře nebyly rostliny genotypu CE 704 ovlivněny chladem při nízké ozáření, byly však silně ovlivněny při saturační ozáření. Chlad neměl vliv na účinnost fotosyntetického aparátu, ale snížil jeho kapacitu. Na rozdíl od rostlin CE 704 nebyly rostliny CE 810 ovlivněny chladem ani při saturační ozáření. Na podzim se rostliny genotypu CE 704 aklimovaly na chlad, takže jsme v celém rozmezí ozáření nezjistili žádné statisticky významné rozdíly v  $P_N$  a Fv/Fm mezi kontrolními rostlinami a rostlinami ovlivněnými chladem. Zvýšená aktivita nefotochemického zhášení NPQ u rostlin CE 704 vystavených chladu při saturační ozáření souvisela se zvýšeným obsahem pigmentů xanthofylového cyklu a se zvýšeným stupněm deepoxidace pigmentů xanthofylového cyklu.

**Klíčová slova:** kukuřice (*Zea mays* L.); genotyp; chlad (chilling); světelné křivky fotosyntetických charakteristik; rychlost čisté fotosyntézy ( $P_N$ ); fluorescence chlorofylu *a*; fotosyntetické pigmenty; růst

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