PHOTOSYNTHETICA 43 (1): 125-134, 2005

Increase in photosynthesis of maize hybrids (*Zea mays* L.) at suboptimal temperature (15 ºC) by selection of parental lines on the basis of chlorophyll *a* fluorescence measurements

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Abstract

We tested the usefulness of chlorophyll *a* fluorescence quenching analysis for the selection of maize parental inbred lines able to produce F_1 hybrids with a high CO_2 assimilation rate during growth at suboptimal temperature. Fifty inbred lines, grown at 15 °C, showed at 6 °C a broad genetic variability regarding the quantum yield of photosynthetic electron transport (Φ_{PS2}). A decrease of Φ_{PS2} in sensitive lines was caused more by reduction of the efficiency of excitation energy capture by open photosystem 2 (PS2) reaction centres (F_v'/F_m') than by a drop in photochemical quenching (q_P). Selected inbred lines with the highest (H) and the lowest (L) values of Φ_{PS2} were used for separate crossings in a diallelic arrangement. Twenty-one of H×H hybrids and 21 of the L×L hybrids were grown at 15 °C. The H×H hybrids showed at suboptimal temperature a significantly higher transport of photosynthetic electrons than the L×L hybrids at lower (400) as well as at higher [800 μmol(photon) m^{-2} s^{-1}] irradiance. The mean net photosynthetic rate (P_N) in H×H and L×L hybrids amounted to 8.4 and 5.8 (second leaf) and 8.5 and 7.6 μmol(CO_2) m^{-2} s^{-1}(third leaf), respectively. Among the best 20 hybrids with regard to P_N (values larger than the average) of second leaves, as many as 15 were derived from H lines (75 % of hybrids), whereas among the best 21 hybrids with regard to P_N of the third leaves, 16 were derived from H lines (76 % of hybrids). The intensive P_N of H×H hybrids was most often accompanied by less water lost *via* transpiration in relation to photosynthesis than in the hybrids of L lines. Hence an analysis of chlorophyll *a* fluorescence quenching enables the selection of inbred lines, which can produce hybrids with improved CO_2 fixation and with efficient water management during growth at suboptimal temperature.

Additional key words: breeding; chilling tolerance; intercellular CO_2 concentration; net photosynthetic rate; photosynthetic efficiency; stomatal conductance; temperature stress; transpiration rate.

Introduction

The susceptibility to chilling of the photosynthetic apparatus of maize (Baker *et al.* 1994) is the most important factor decreasing its productivity on the northern hemisphere (Torsell *et al.* 1959). Temperatures below 15 °C during springtime cause injuries as well as developmental and functional disturbances of the photosynthetic apparatus in maize (Stirling *et al.* 1993). A reduction of CO_2 assimilation during the chilling periods (Stirling *et al.* 1993) does not only decrease the current biomass production of seedlings (Pietrini *et al.* 1999), but it also reduces, as an after-effect, the final yield of maize (Kościelniak and Biesaga-Kościelniak 2000).

At low temperature developmental defects in maize chloroplasts occur (Robertson *et al.* 1993) and the chlorophyll (Chl) content decreases (Haldimann 1997). Extensive disorder and damage of the photosynthetic apparatus can be caused particularly by the simultaneous influence of chilling temperature and strong irradiance (Farage and Long 1987, Baker *et al.* 1994, Krause 1994, Massacci *et al.* 1995). Reduction of carbon metabolism at

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Received 2 January 2004, *accepted* 9 June 2004.

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Abbreviations: C_i = intercellular CO_2 concentration; Chl = chlorophyll; F_v'/F_m' = efficiency of excitation energy capture by open PS2 reaction centres; H, L = maize inbred lines with high or low Φ_{PS2} at 15 °C, respectively; LSD = least significant difference; P_N = net photosynthetic rate; PS2 = photosystem 2; q_P = photochemical fluorescence quenching; SE = standard error of the mean; Φ_{PS2I} = quantum efficiency of PS2 electron transport.

Acknowledgements: The work was supported by the Committee for Scientific Research (KBN), grant no. 5 P06A 028 21. We thank Prof. Karl Dörffling for his careful reading of the manuscript and for his valuable comments.

low temperature in light induces an over-energization of the photosynthetic apparatus, which may cause the formation of reactive oxygen species, which, in turn, induce diverse damages (Prasad *et al.* 1994a,b). All of these undesirable effects are sometimes accompanied by a drastic decrease in the efficiency of photosystem (PS) 1 and 2 (Baker *et al.* 1994, Baker and Nie 1994, Long *et al.* 1994). Carotenoids fulfil a special protective function against the photoinhibition of photosynthesis. They function by increasing the dissipation of a part of the absorbed radial energy as heat in the antenna system (Pfündel and Bilger 1994, Demmig-Adams *et al.* 1995). The content of protective carotenoids in maize leaves can significantly increase at suboptimal temperature (Leipner *et al.* 1997, 1999). This results in easily detectable changes in quenching of Chl fluorescence (Leipner *et al.* 1997, 1999, Haldimann 1997, Fracheboud *et al.* 1999).

The increase of acreage of maize cultivation in cool regions requires the selection of genotypes with a chilling-tolerant photosynthetic apparatus. Since the fundamental paper on quenching of Chl fluorescence (Kautsky and Hirsch 1931) was published, this non-destructive technique to acquire fast and reliable information about plant responses to environmental stresses has been progressively developed. The use of Chl fluorescence measurements as screening methods for chill tolerance has already been investigated in experiments in which maize genotypes were compared (Hetherington *et al.* 1983, Havaux 1987, Janda *et al.* 1998, Schapendonk *et al.* 1989, Dolstra *et al.* 1994). However, Fracheboud *et al.* (1999) were the first to show that quenching analysis of Chl *a* fluorescence can be a useful tool for selection in breeding programmes aimed at increasing CO_2 assimilation in new maize genotypes at suboptimal temperature. Using maize genotypes extremely differing in chilling tolerance, they characterised the conditions of seedling growth and fluorescence measurements, which are most suitable for selection of genotypes tolerant to low temperature stress with regard to photosynthesis. They proved the effectiveness of the fluorescence quenching analysis for maize breeding by selection of families (F_3) from a population and created hybrids with increased CO_2 assimilation at suboptimal temperature.

The aim of present work was to test the usefulness of the quenching analysis method of Chl *a* fluorescence for selection of inbred lines, which would be able to produce single hybrids with high photosynthesis at suboptimal temperature. The basis for the evaluation of the effectiveness of this selection method was the estimation of the possibility to increase the tolerance of the photosynthetic apparatus of single hybrids derived from lines with high combining abilities and currently used in breeding programmes in Poland.

Materials and methods

Plants: The study was performed with 50 inbred lines of maize (*Zea mays* L., Table 1) as well as with 42 single F_1 hybrids produced from lines selected in this study. Used inbred lines are components of the registered and cultivated maize cultivars in Poland. The seed material came from the Crop Breeding Station *Nasiona Kobierzyc Ltd*, Poland.

Growth: Plants of inbred lines or hybrids were grown in growth rooms in pots (5 000 cm^3) filled with a mixture of clay, peat, and sand (3 : 2 : 1, v/v/v) at a 16-h photoperiod, an irradiance of 350 $\mu mol(photon)\,m^{-2}\,s^{-1}$ (provided by high pressure sodium lamps, *Philips SON-T AGRO*, 400 W), and at 50–60 % air humidity. During germination (5 d) a constant temperature of 25 °C was kept, after emergence during the next 8 d the temperature was 25/17 °C (day/night) and then 15/13 °C (10 d). Afterwards the seedlings were imposed to chilling at 6 °C (inbred lines) or the fluorescence and the gas exchange measurements were done (hybrids). These measurements were carried out on the fully developed 2^{nd} and 3^{rd} leaves. Seedlings were watered and fertilised with half-strength Hoagland nutrient solution as required.

Table 1. Symbols and ID numbers of maize inbred lines used in the study. The inbred lines are in actual breeding programs of the Crop Breeding Station *Nasiona Kobierzyc Ltd.*, Poland.

Symbol	ID	Symbol	ID	Symbol	ID	Symbol	ID	Symbol	ID
K208	1	K186	11	K184	21	K376	31	K85	41
K130	2	K230	12	K137	22	K215	32	K152	42
K2812	3	K310	13	K324	23	K242	33	K212	43
K383	4	K180	14	K96	24	K202	34	K216	44
K261	5	K296	15	K312	25	K117	35	K192	45
K259	6	K182	16	K194	26	K354	36	K203	46
K255	7	K154	17	K167	27	K278	37	K185	47
K243	8	K387	18	K153	28	K258	38	K217	48
K373	9	K218	19	K112	29	K155	39	K256	49
K257	10	K231	20	K372	30	K244	40	K150	50

Measurements of fluorescence parameters: Chl *a* fluorescence was measured by means of a pulse amplitude modulation fluorometer (*FMS2*, *Hansatech*, UK). The efficiency of excitation energy capture by open photosystem 2 (PS2) reaction centres (F_v'/F_m'), the photochemical quenching (q_P), and the quantum yield of electron transport at PS2 (Φ_{PS2}) were determined (Genty *et al.* 1989). A clip with optical fibre was fastened on the central part of an attached leaf. The leaf was lighted by constant "actinic light" for a period longer than 10 min until stabilisation of fluorescence (F_s). The saturating pulse (F_m') had an intensity of about 5 800 µmol(photon) $m^{-2}\cdot s^{-1}$ and lasted 0.9 s. F_0' was measured after turning off the "actinic light" by immediately irradiating the leaf for 3 s with a far red emitting diode with about 15 W m^{-2}. Measurements on inbred lines were performed on the third leaf after 1 and 24 h of chilling at 6 °C at two actinic irradiances: 150 and 250 µmol(photon) m^{-2} s^{-1}. Studies on hybrids were carried out at a temperature of 15 °C at 400 and 800 µmol(photon) m^{-2} s^{-1} (2nd leaf) or at 400 µmol(photon) m^{-2} s^{-1} (3rd leaf). The leaf temperature was kept constant by a thermostat system.

Crossing of inbred lines with high and low Φ_{PS2} at low temperature: In the next vegetation period nine inbred lines with high Φ_{PS2} and nine with low Φ_{PS2} were crossed separately for each group, in a diallelic and one-sided arrangement (altogether 72 hybrids). The crossing programme was performed on the experimental field of the Crop Breeding Station *Nasiona Kobierzyc Ltd*. For each line 20 kernels were sown in two rows with 20 cm spacing between the plants and 70 cm between the rows. To ensure full synchronisation of stigmation and pollination of lines differing in the flowering time (different earliness), sowing of each line was repeated at three different dates. Stigmas of lines, which were selected as a maternal component, were isolated against an uncontrolled pollination. Unfortunately, not all plants produced enough kernels to proceed with further measurements. In some cases, lack of grains was caused by damages of plants as a result of strong wind, in other cases plants did not produce sufficient amounts of kernels. To avoid decrease in frequency of representation of some lines in hybrid populations, two separate sets of hybrids were chosen for further investigations, in which each line occurred 7 times (altogether 42 hybrids).

Measurements of gas exchange: Net photosynthetic (P_N) and transpiration (E) rates, stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of hybrids were measured by an infrared gas analyser (*Ciras-1*, *Hansatech*, UK) with a Parkinson leaf chamber (*PLC6*) automatically controlling the measurement conditions. The irradiation system consisted of halogen lamps. The leaf chamber was fastened on the middle part of the second and third leaves. The flow rate of air with constant CO_2 concentration [360 µmol(CO_2) mol^{-1}(air)] through the assimilation chamber was 300 cm^{-3} min^{-1}. The temperature of the leaves was 15 °C, the air humidity 45 %, and the irradiance 400 µmol(photon) m^{-2} s^{-1}.

Assessment of leaf necrotic injuries and seedling survival: After Chl fluorescence or gas exchange measurements (18 d after emergence), seedlings of inbred lines and hybrids were transferred to 6 °C for five days and afterwards for three days to 25/17 °C (day/night) for recovery. During the recovery period a photoperiod of 15 h [350 µmol(photon) m^{-2} s^{-1}] and a relative air humidity 50–60 % were kept. After the recovery period, an assessment of necrotic injuries of the third leaves according to an eight-gradual scale in percent of injured leaf area (0, 5, 15, 25, 35, 50, 75, 100 %) and an evaluation of seedling survival were performed. All measurements were made in nine replicates.

Statistical analyses: A fully randomised experimental design was used. Presented data are the mean values of nine replicates for all measured traits. Least significant differences (LSD) were calculated ($p<0.05$).

Results

Selection of inbred lines on the basis of Φ_{PS2} at 6 °C: Φ_{PS2} of inbred lines grown at suboptimal temperature (15 °C) was measured under severe chilling conditions (6 °C). The differentiation of lines with regard to Φ_{PS2} after two chilling periods (1 and 24 h) as well as at two irradiances [150 and 250 µmol(photon) m^{-2} s^{-1}] was examined. The ranking of inbred lines as regards Φ_{PS2} was similar at both irradiances (Fig. 1*A,B*). After the longer chilling period (24 h), values of Φ_{PS2} at both irradiances correlated higher ($r^2 = 0.94$) than those after 1 h ($r^2 = 0.82$). However, differences among investigated lines were greater at 150 than 250 µmol(photon) m^{-2} s^{-1}. This is shown by a greater slope of regression lines of Φ_{PS2} for all inbred lines at weaker "actinic light". When prolonged to 24 h, chilling at 150 µmol(photon) m^{-2} s^{-1} did not cause the same decrease of Φ_{PS2} in all lines (Fig. 2). In spite of this, Φ_{PS2} values after both chilling periods correlated highly ($r^2 = 0.84$) and enabled the selection of two groups of lines: nine with high (H) and nine with low (L) Φ_{PS2}. The mean values of Φ_{PS2} (after 24 h chilling) for H and L lines at 150 µmol(photon) m^{-2} s^{-1} amounted to 0.247 and 0.070, respectively. The extension of chilling at 6 °C reduced the photosynthetic electron transport more in L than in H lines (Table 2). The reason for this phenomenon was a greater drop in F_v'/F_m' than in photochemical quenching (q_P).

Φ_{PS2} and gas exchange of hybrids at 15 °C: Φ_{PS2} in hybrids produced from H or L lines showed significant differentiation at lower (400) as well as at higher [800 μmol(photon) m^{-2} s^{-1}] irradiance (Table 3). At both irradiances, values of Φ_{PS2} of hybrids produced from L lines were lower by 23 and 27 % in comparison to H×H hybrids, respectively. Similar to inbred lines, the decrease in Φ_{PS2} of hybrids derived from L lines was connected more with disturbances of light-harvesting antennae (decrease of F_v'/F_m') than with a decrease in q_P.

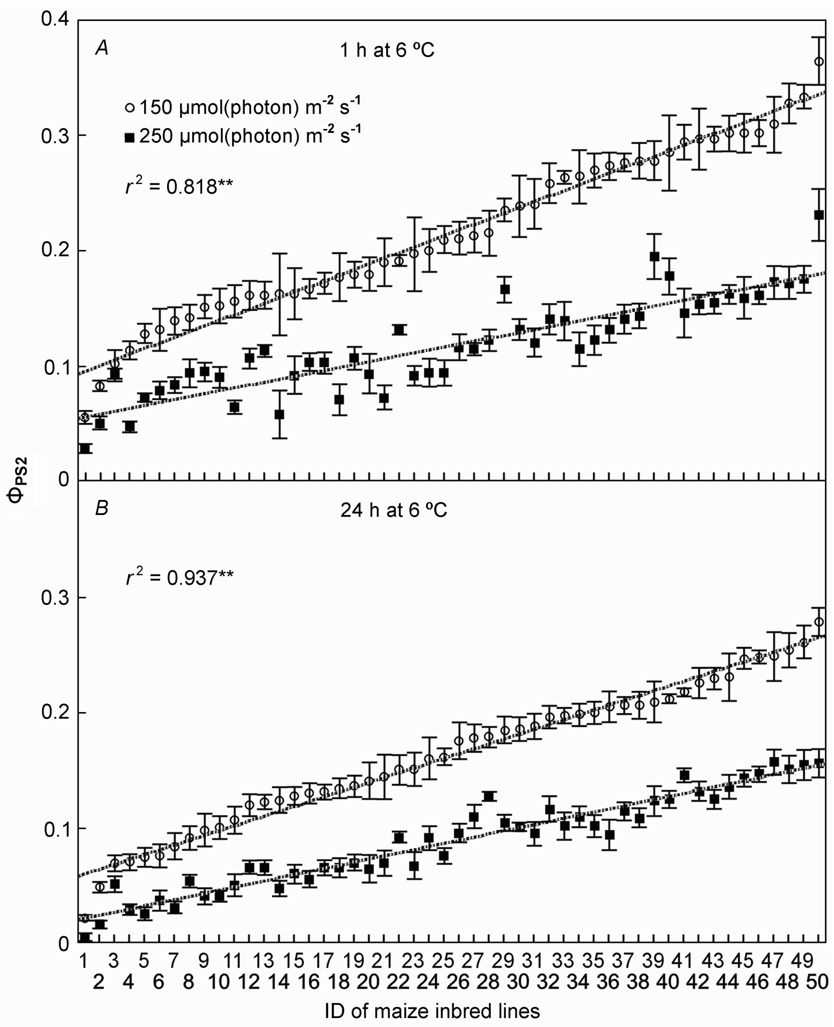

Fig. 1. Quantum yield of electron transport (Φ_{PS2}) of the third seedling leaves of 50 maize inbred lines after 1 h (*A*) and 24 h (B) chilling at 6 °C depending on different irradiances. Before chilling the seedlings were grown for 10 d at 15/13 °C (day/night). The inbred lines are ordered according to the increasing Φ_{PS2} at 150 μmol(photon) m^{-2} s^{-1}. Means (±SE) of nine measurements, $^{**}r^2$ significant at $p<0.001$. Symbols of inbred lines are in Table 1.

Table 2. Chlorophyll fluorescence parameters of the third leaves in H and L maize inbred lines (compare Fig. 2) after 1 h and 24 h at 6 °C and an irradiance of 150 μmol(photon) m^{-2} s^{-1}. Means (±SE) of 9 inbred lines (*n* = 9).

		Φ_{PS2}	F_v'/F_m'	u_p
1 h	H	0.305±0.008 (100.0 %)	0.405±0.008 (100.0 %)	0.747±0.006 (100.0 %)
	L	0.117±0.005 (38.4 %)	0.199±0.006 (49.1 %)	0.575±0.001 (76.9 %)
24 h	H	0.247±0.005 (100.0%)	0.355±0.005 (100.0 %)	0.694±0.007 (100.0 %)
	L	0.070±0.004 (28.3 %)	0.144±0.006 (40.6 %)	0.476±0.012 (68.6 %)
24 h [% of 1 h]	H	81.0	87.7	92.9
	L	59.8	72.4	82.8

P_N of hybrids was clearly dependent on chilling tolerance of the photosynthetic apparatus of the parental lines (Fig. 3*A,B*). The mean P_N of H×H and L×L hybrids amounted to 8.4 and 5.8 (second leaf) and 8.5 and 7.6 µmol(CO_2) m^{-2} s^{-1} (third leaf), respectively. The ranking of hybrids with regard to P_N in both investigated leaves was similar ($r^2 = 0.88$).

Some of L×L hybrids at suboptimal temperature showed lower g_s than the H×H hybrids (Fig. 4*A*). In spite of this, their photosynthesis often proceeded at higher intracellular CO_2 concentration, C_i (Fig. 4*B*). The intensive CO_2 assimilation of H×H hybrids was most often accompanied by smaller lost water *via* transpiration in relation to photosynthesis than the hybrids of L lines (Fig. 5). Similar results were obtained for second leaves (values not presented).

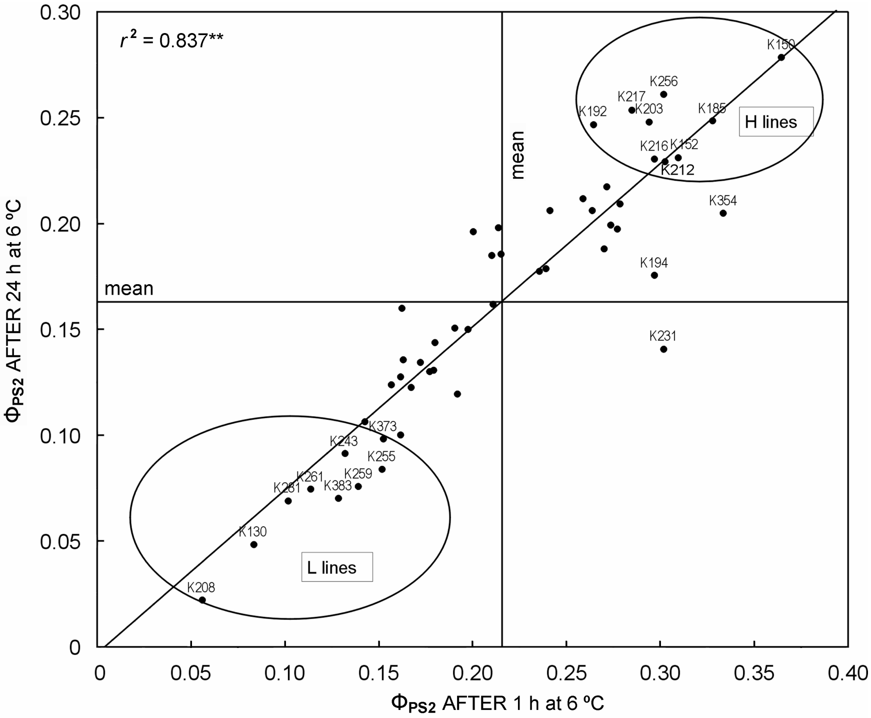

Fig. 2. Relationship between Φ_{PS2} measured on the third leaves of 50 maize inbred lines after 1 and 24 h chilling at 6 °C and at 150 µmol(photon) m^{-2} s^{-1}. Before chilling the seedlings were grown for 10 d at 15/13 °C (day/night). The inbred lines with high (H) and low (L) Φ_{PS2} at 6 °C, selected for production of hybrids, are labelled within the ellipses. $^{**}r^2$ significant at $p<0.001$.

Table 3. Chlorophyll fluorescence parameters in the second leaves of maize hybrids created by crossing either H or L inbred lines (compare Fig. 3), measured at 15 °C and two irradiances [µmol(photon) m^{-2} s^{-1}]. Means (±SE) of 21 hybrids (*n* = 9).

Irradiance	Hubrids	Φ_{PS2}	F_v'/F_m'	u_p
400	H×H	0.298±0.006 (100.0 %)	0.463±0.005 (100.0 %)	0.635±0.006 (100.0 %)
	L×L	0.231±0.006 (77.5 %)	0.375±0.007 (81.0 %)	0.600±0.006 (94.5 %)
800	H×H	0.170±0.004 (100.0 %)	0.391±0.005 (100.0 %)	0.425±0.007 (100.0 %)
	L×L	0.125±0.004 (73.5 %)	0.319±0.006 (81.6 %)	0.379±0.006 (89.2 %)

Chl fluorescence parameters of inbred lines measured after 24 h chilling at 6 °C and at 150 µmol(photon) m^{-2} s^{-1} correlated moderately but significantly with the extent of necrotic injury assessed 3 d after long-term chilling (6 d at 6 °C). The correlation coefficients between necrotic injury and Φ_{PS2}, q_P, and F_v'/F_m' amounted to –0.606, –0.755, and –0.605, respectively. Mean necrotic damages of H and L lines assessed after the recovery period covered 14 and 45 % of the third leaf area, respectively. However, Chl fluorescence parameters and differentiation in P_N of hybrids at suboptimal temperature were not linked with the resistance to leaf damage and plant survival during long exposure of the seedlings (5 d) to severe chilling (6 °C). Mean necrotic damages of L×L and H×H hybrids assessed after the recovery period covered in both cases 62 % of the third leaf area. Seedling survival of both hybrid groups also did not differ significantly and amounted to 33.5 % (L×L hybrids) and 28.0 % (H×H hybrids).

Discussion

The observed great differentiation in Φ_{PS2} of inbred lines grown at 15 °C indicates the large resource of broad genetic variability and adaptability of the photosynthetic apparatus to low temperature in *Z. mays* (Fig. 1*A,B*). The genotypic differences in Φ_{PS2} at 6 °C were largest at low irradiance. The differentiation in activity of photosynthetic electron transport in maize at lowered temperature and low irradiance is in accordance with results of Fracheboud *et al.* (1999). They showed that the genetically determined variability of Φ_{PS2} in seedlings grown at suboptimal temperature disappeared completely under high irradiance [more than 400 µmol(photon) m^{-2} s^{-1}] and temperature of 15 °C. Our measurements are only partly consistent with these results. The H×H and L×L hybrids differed with regard to Φ_{PS2} at 15 °C not only at an irradiance of 400 µmol(photon) m^{-2} s^{-1} but also at 800 µmol(photon) m^{-2} s^{-1}, although the difference was relatively smaller than among parental H and L lines (Tables 2 and 3). Because the growth conditions of seedlings and the method of Φ_{PS2} measurement were in our experiments similar to those of Fracheboud *et al.* (1999), it may be supposed that the main reason for this discrepancy was the greater ability of genotypes investigated in this study to adapt their photosynthetic apparatus to low temperature during growth at 15 °C and during chilling at 6 °C. This may also explain the finding of genotypic differentiation of q_P in reaction to low temperature in leaves of maize grown even at 25 °C (Havaux 1987).

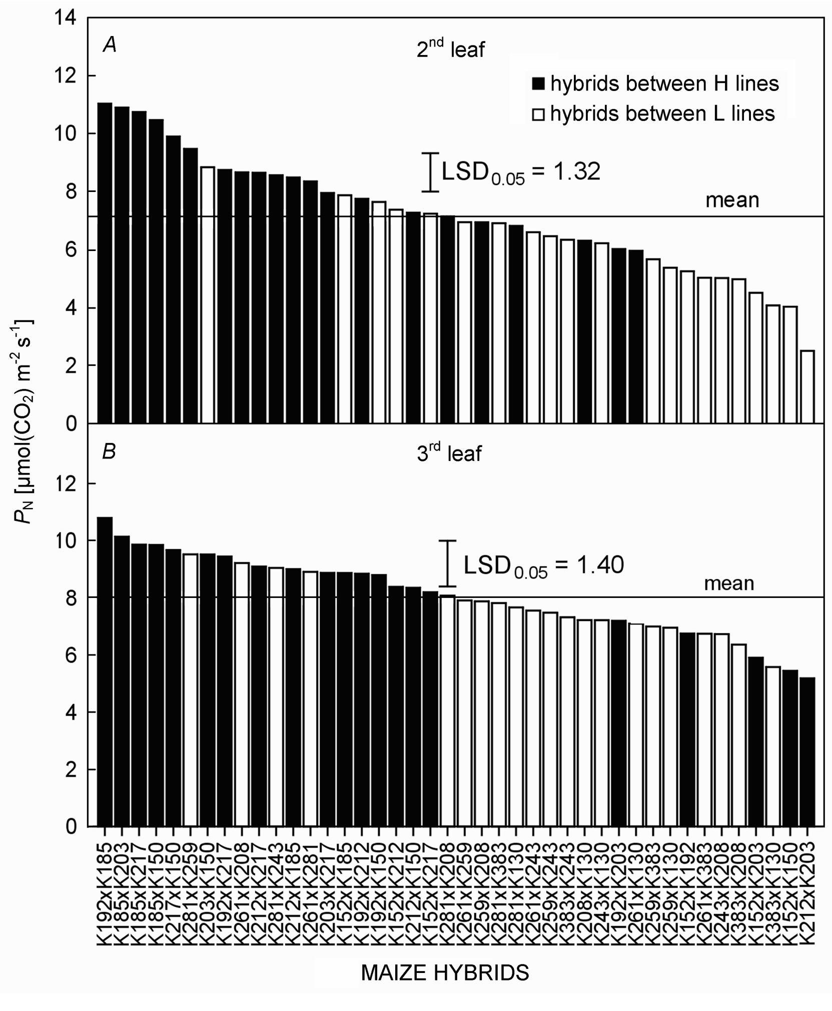

Fig. 3. Net photosynthetic rate (P_N) at 15 °C (growth temperature) in the second (*A*) and the third (*B*) leaves of maize hybrids produced from H and L inbred lines (compare Fig. 2). Measurements were performed at an irradiance of 400 µmol(photon) m^{-2} s^{-1}. The hybrids are ordered according to the decreasing P_N.

In the presented experiments the ranking of inbred lines with regard to Φ_{PS2} was similar after 1 and 24 h of chilling at 6 °C (Fig. 2). The drop in leaf temperature from 15 to 6 °C probably did not cause large structural changes of the photosynthetic apparatus during a 1 h treatment. The high correlation between Φ_{PS2} of lines after 1 and 24 h shows that processes of damage and adaptation to chilling of the photosynthetic apparatus in most studied genotypes proceeded similarly during this time. However, the prolongation of chilling to 24 h disclosed that some inbred lines decreased Φ_{PS2} earlier than others, *e.g.* the lines K354, K194, and K231. Moreover, Φ_{PS2} of L lines decreased at 6 °C faster than Φ_{PS2} of the H ones (Table 2). Thus prolonging the chilling period at 6 °C to 24 h is advisable during screening, as it permits the disclosure of adaptation processes to severe chilling and by that the achievement of larger differentiation of H and L lines.

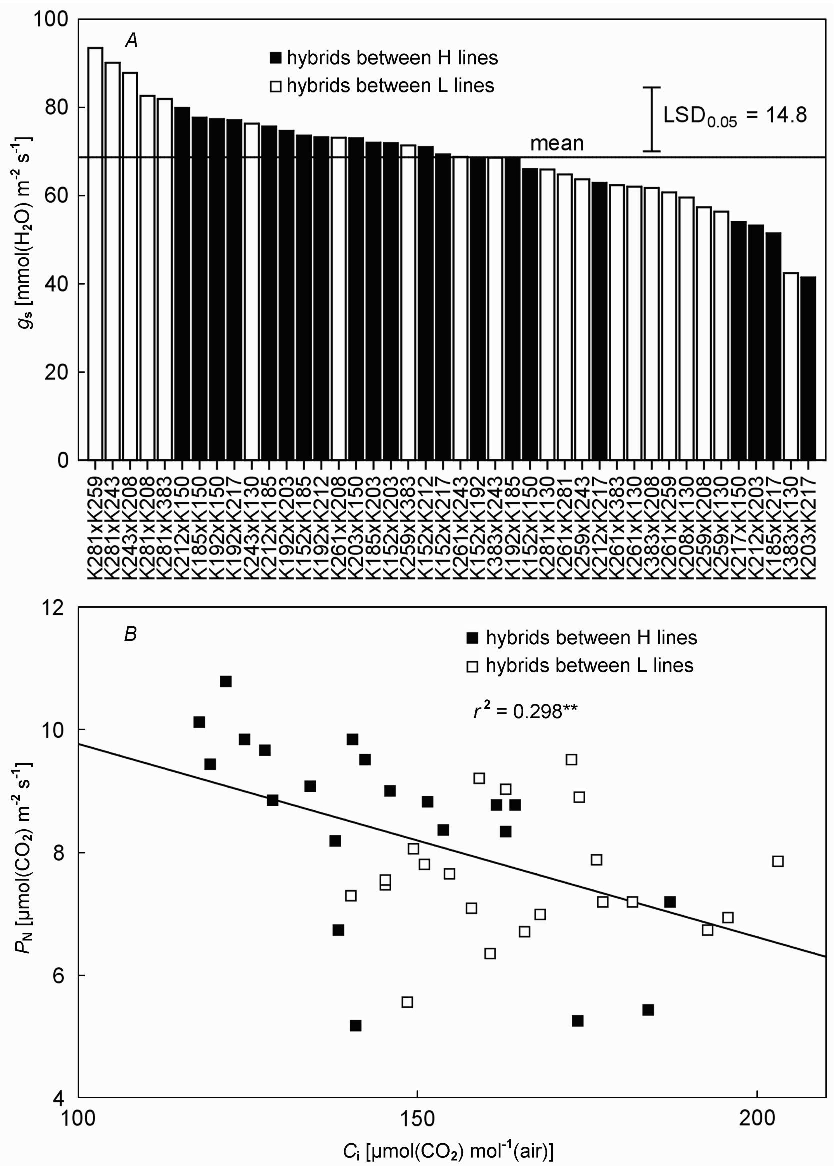

Fig. 4. Stomatal conductance, g_s (*A*) and relationship between net photosynthetic rate, P_N and intercellular CO_2 concentration, C_i (*B*) at 15 °C in the third leaves of maize hybrids produced from H and L inbred lines (compare Fig. 2). Measurements were performed at an irradiance of 400 μmol(photon) m^{-2} s^{-1}. $^{**}r^2$ statistically significant at $p<0.001$.

The reasons for genetic differentiation of Φ_{PS2} in maize at suboptimal temperature are complex. They can result from different speeds of forming non-active reaction centres (Krause 1994), from the de-epoxidation of violaxanthin to zeaxanthin in the xanthophyll cycle (Demmig-Adams *et al.* 1995, Haldimann 1997), or finally from the conformation changes in the light-harvesting complexes of PS2 (Horton *et al.* 1994). The concentration of zeaxanthin increases in leaves of maize plants developing at a low temperature (Leipner *et al.* 1997). The zeaxanthin content in leaves of maize plants growing at suboptimal temperature is higher in sensitive genotypes than in the tolerant ones (Haldimann 1997). This increases the ability to dissipate the excessive absorbed energy in PS2 antennae in the form of heat (Demmig-Adams *et al.* 1995). It also causes a decrease of energy utilisation in photochemical processes, but it can contribute to lesser damages to the photosynthetic apparatus. The speed of de-epoxidation of violaxanthin is low in maize leaves at 6 °C (Haldimann 1997). Even so, maize leaves are able to accumulate large quantities of carotenoids during their first hours under these unfavourable conditions. Moreover, a growth temperature of 15 °C inhibits the down regulation of zeaxanthin in the form of after-effects even at 5 °C through a period of 24 h (Leipner *et al.* 1997).

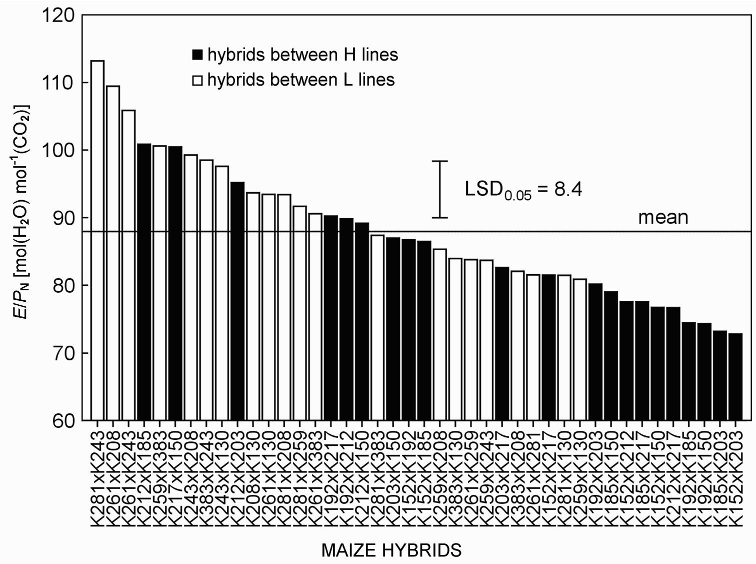

Fig. 5. Proportions of transpiration and photosynthesis rates, E/P_N at 15 °C in the third leaves of maize hybrids produced from H and L inbred lines (compare Fig. 2). Measurements were performed at an irradiance of 400 μmol(photon) m^{-2} s^{-1}.

The photosynthetic apparatus of maize plant, especially PS2, suffers from photo-damages under chilling stress (Lidon *et al.* 2001, Holá *et al.* 2003). Furthermore, a severe low temperature increased susceptibility to photoinhibition in a cold sensitive maize hybrid (Szalai *et al.* 1996). Additionally, under these conditions degradation of lipids and increase in content of free fatty acids in the chloroplast membrane take place as a result of increased galactolipase activity. Comparison of various plant species showed a higher galactolipase activity in the chilling-sensitive plants than in the resistant ones (Kaniuga 1997) Thus, the observed differences in Φ_{PS2} and the gas exchange rate at low temperatures among H and L inbred lines and their hybrids can be caused by varying the extent of photoinhibition (Janda *et al.* 1998). Furthermore, the differences could be additionally affected by their different antioxidant capacities, since chilling-sensitive maize hybrids have lower antioxidant capacities than the tolerant ones (Hodges *et al.* 1997).

The decrease in F_v'/F_m' can be attributed to an increasing level of energy dissipation and a decreasing utilisation of the absorbed energy in photochemical processes (Demmig-Adams *et al.* 1995). In accordance with this, L lines as well as their hybrids showed a strong increase in the energy dissipation in antennae (Tables 2 and 3). A similar reaction at suboptimal temperature was observed in maize by Fracheboud *et al.* (1999). An experimentally well-founded reason for the differences is the existence of a mechanism preventing excessive reduction of electron acceptors of PS2 in maize under chilling and high irradiance. This is manifested in the form of keeping high q_P values even at strongly lowered temperatures (Fracheboud *et al.* 1999). The same effect was observed in the present study in H lines as well as in their hybrids both at 6 and 15 °C (Tables 2 and 3).

The greater ability of H than L lines to maintain a high rate of electron transport in PS2 at suboptimal temperature does not guarantee a high quantum yield of carbon fixation. A linear relationship between Φ_{PS2} and the quantum yield of carbon fixation (Massacci *et al.* 1995) can be lost in leaves developing at low temperature in the field (Fryer *et al.* 1995). We found that P_N in the second and third leaves of H×H hybrids was significantly higher than that of L×L hybrids (Fig. 3). Among the best 20 hybrids with regard to P_N (P_N larger than the average) of the second leaves, as many as 15 were derived from H lines (75 % of hybrids), whereas among the best 21 hybrids with regard to P_N of the third leaves, 16 were derived from H lines (76 % of hybrids).

Exposure of young maize plants to low temperatures decreased g_s. A relationship between stomatal closure at low temperatures and cold sensitivity in maize was demonstrated (Vígh *et al.* 1981), but this relationship has been not always observed (Janda *et al.* 1998). We found that part of the hybrids created from L lines had lower g_s than hybrids of the H lines (Fig. 4*A*). Therefore, the susceptibility of L×L hybrids to stomatal closure during chilling might be the main cause of low P_N, and in consequence, of decrease in the efficiency of photosynthetic electron transport. The analysis of relationships between C_i and P_N of leaves did not confirm this presumption (Fig. 4*B*). In spite of reduced g_s, hybrids of L lines photosynthesised usually under higher C_i than the H×H hybrids. This result is consistent with observations about the lack of stomatal limitation of photosynthesis in chilled maize (Irigoyen *et al.* 1996) and it points to metabolic limitations of photosynthetic efficiency in L×L hybrids under low temperature. Additionally, in our study the H×H hybrids showing a high P_N at suboptimal temperature did not demonstrate any breakdown of the relation between water loss and P_N (Fig. 5).

Our results show the usefulness of Chl fluorescence quenching analysis in breeding programmes aimed at increasing CO_2 assimilation efficiency of maize seedlings at suboptimal temperature. Relatively large differences in the tolerance of the photosynthetic apparatus to low temperature among inbred lines, revealed in our study, in addition enlarge the chance of hybrid improvement with the proposed selection method. On the basis of measurements of Φ_{PS2} at 6 °C and low irradiance it is possible to select inbred lines, which most likely will produce hybrids with high CO_2 assimilation as well as efficient and effective in water management.

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