Cold Tolerance of the Photosynthetic Apparatus

Pleiotropic Relationship between Photosynthetic Performance and Specific Leaf Area of Maize Seedlings

Molecular Breeding (2005) 16: 321–331
DOI 10.1007/s11032-005-1642-7

Cold tolerance of the photosynthetic apparatus: pleiotropic relationship between photosynthetic performance and specific leaf area of maize seedlings

Andreas Hund[1,*], Elisabetta Frascaroli[2], Jörg Leipner[1], Choosak Jompuk[3], Peter Stamp[1] and Yvan Fracheboud[1]
[1]*Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), Universitätstrasse 2, 8092 Zurich, Switzerland;* [2]*Dip. Scienze e Tecnologie Agroambientali (DiSTA), Università di Bologna, Bologna, Italy;* [3]*Department of Agronomy, Kasetsart University, Phaholyothin Road, Bangkok 10903, Thailand; *Author for correspondence (e-mail: hundan@ipw.agrl.ethz.ch; phone: +41-1- 6323829; fax.: +41-1-6321143)*

Received 1 April 2005; accepted in revised form 1 August 2005

Key words: Chilling stress, Chlorophyll fluorescence, Quantitative trait locus (QTL), Specific leaf area, *Zea mays*

Abstract

The objective of this study was to elucidate the genetic relationship between the specific leaf area (SLA) and the photosynthetic performance of maize (*Zea mays* L.) as dependent on growth temperature. Three sets of genotypes: (i) 19 S_5 inbred lines, divergently selected for high or low operating efficiency of photosystem II (Φ_{PSII}) at low temperature, (ii) a population of 226 $F_{2:3}$ families from the cross of ETH-DL3 × ETH-DH7, and (iii) a population of 168 $F_{2:4}$ families from the cross of Lo964 × Lo1016 were tested at low (15/13 °C day/night) or at optimal (25/22 °C day/night) temperature. The latter cross was originally developed to study QTLs for root traits. At 15/13 °C the groups of S_5 inbred lines selected for high or low Φ_{PSII} differed significantly for all the measured traits, while at optimal temperature the groups differed only with regard to leaf greenness (SPAD). At low temperature, the SLA of these inbred lines was negatively correlated with Φ_{PSII} ($r = -0.56$, $p < 0.05$) and SPAD ($r = -0.80$, $p < 0.001$). This negative relationship was confirmed by mapping quantitative trait loci (QTL) in the two mapping populations. A co-location of three QTLs for SLA with QTLs for photosynthesis-related traits was detected in both populations at 15/13 °C, while co-location was not detected at 25/22 °C. The co-selection of SLA and Φ_{PSII} in the inbred lines and the co-location of QTL for SLA, SPAD, and Φ_{PSII} at 15/13 °C in the QTL populations strongly supports pleiotropy. There was no evidence that selecting for high Φ_{PSII} at low temperature leads to a constitutively altered SLA.

Abbreviations: CER – carbon exchange rate; Φ_{PSII} – operating efficiency of photosystem II; PPFD – photosynthetic photon flux density; QTL – quantitative trait locus; SLA – specific leaf area; SPAD – soil plant analysis development, leaf greenness

Introduction

In temperate regions, the maize crop is often exposed to long-term low temperature during early phases of development, resulting in poor photosynthetic performance (Stirling et al. 1991) and the maintenance of a high carbon exchange rate (CER) is among the most limiting factors for cold

tolerance (Lee et al. 2002). At optimal temperatures, when CER varies little among genotypes, other factors, like the rate of development might be of greater importance, leading to an earlier canopy closure and thus to an increased light interception (Richards 2000). The necessary increase in leaf area can be achieved by a change in partitioning of carbohydrates between or within organs. For example, a high specific leaf area (SLA, ratio of leaf area to leaf weight) is one way by means of which barley achieves its early growth advantage over wheat (Lopez-Castaneda et al. 1995). However, there is evidence that the SLA is negatively correlated with the CER at various irradiance and concentrations of atmospheric CO_2 (Evans 1998; Poorter and Van der Werf 1998). Fichtner et al. (1993) reported an increase in the SLA in tobacco plants transformed with antisense *rbcS* (Rubisco small subunit), which were characterized by a lower CER and chlorophyll content due to the lower level of Rubisco. In maize, a negative relationship between SLA and CER was found for three tropical and three temperate inbred lines early sown in the field (Verheul et al. 1996). The above studies indicate that SLA is negatively correlated with CER whenever the variation in the CER, due to environmental or genetic influences, is large. In contrast to the negative relationship between SLA and photosynthetic performance, in general a high SLA and, thus, a high rate of CO_2 assimilation per unit leaf mass seems to be most important for achieving fast growth. For example, a high SLA is considered to be an important trait of crops like sugarcane (Terauchi and Matsuoka 2000) and temperate cereals (Richards 2000), leading to a higher light interception during early development. Furthermore, slow-growing alpine species have a lower SLA than fast-growing lowland species (Atkin and Lambers 1998). In a comprehensive review based on 57 studies, Poorter and Van der Werf (1998) concluded that the leaf area ratio (LAR, ratio of leaf area to plant weight), and more specifically SLA, are the most important factors in the variation of the relative growth rate. The introduction of exotic maize germplasm, showing temperature-stable, strong photosynthetic performance, in European breeding programs is considerably limited by its linkage to a small SLA (Leipner et al. 1999; Soldati et al. 1999; Stehli et al. 1999). It has been shown that an improvement in the CER at low temperature can be achieved indirectly by selecting for a high operating quantum efficiency of photosystem II (Φ_{PSII}) at low temperature (Fracheboud et al. 1999). The chlorophyll fluorescence parameter Φ_{PSII} measures the proportion of the light absorbed by chlorophyll associated with PSII that is used for photochemistry (Genty et al. 1989). At constant light intensity there is a strong linear correlation between Φ_{PSII} and CER as it was shown, for example, in maize seedlings grown at suboptimal temperature (Fracheboud et al. 2004). Furthermore Φ_{PSII} measurements are much faster compared to CER measurements taking only few seconds in comparison to several minutes. This makes Φ_{PSII} a useful tool for the routine screening of the photosynthetic performance of maize under suboptimal growth temperatures. However, since maize is not constantly exposed to suboptimal temperatures during its life cycle, a constitutive high photosynthetic capacity, if achieved in combination with a small SLA, is an undesirable attribute (Leipner et al. 1999).

The research herein presented was conduced in maize in order to: (i) determine whether a divergent selection for Φ_{PSII} at low temperature led to a co-selection of SLA and greenness, (ii) evaluate whether this selection led to a constitutive expression of low SLA, and (iii) verify whether QTLs for SLA were co-located with QLTs for other traits in two mapping populations.

Material and methods

Plant material

The plant material consisted of a set of 19 experimental inbred lines and two mapping populations of maize. The inbred lines had been divergently selected for the operating quantum efficiency of photosystem II (Φ_{PSII}), measured at 6 °C and 60 μmol m^{-2} s^{-1} in seedlings grown at suboptimal temperature (15/13 °C, day/night). The selection was from three breeding populations (i.e. one dent, one flint, and one exotic population with a 25% flint and 75% Mexican highland background; Fracheboud et al. 1999). After four cycles of inbreeding selection, the families were further selfed to obtain S_5 inbred lines. For this study, 11 of the lines selected for high photosynthetic efficiency (H) and eight selected for low photosynthetic

efficiency (L) were available: seven dent lines (D), four exotic lines (E), and eight flint lines (F): ETH-DH1 (a), ETH-DH2 (b), ETH-DH5 (c), ETH-DH7 (d), ETH-DL3 (e), ETH-DL4 (f), ETH-DL7 (g), ETH-EH2 (h), ETH-EH3 (i), ETH-EH5 (k), ETH-EL2 (l), ETH-FH1 (m), ETH-FH6 (n), ETH-FH7 (o), ETH-FH8 (p), ETH-FL1 (q), ETH-FL3 (r), ETH-FL5 (s), ETH-FL8 (t). The letters in brackets identify the inbred lines in Figure 1.

From the cross of ETH-DL3×ETH-DH7 a QTL population of 226 $F_{2:3}$ families was developed (Fracheboud et al. 2004), referred to as ETH-population. The second population, referred to as Lo-population, consisted of 168 $F_{2:4}$ families derived from the Lo964×Lo1016 cross (Tuberosa et al. 2002), two dent lines contrasting in root morphology (Sanguineti et al. 1998) and the cold tolerance at germination (Frascaroli et al. unpublished data). The parents and the $F_{2:3}$ families of the Lo-population were provided by Dr. M. Motto (Experimental Institute for Cereal Crops, Bergamo, Italy). The $F_{2:4}$ seeds were produced at the Department of Agro-environmental Science and Technology (DiSTA, Bologna, Italy).

Growth conditions

The set of 19 experimental inbred lines and the ETH-population were grown in growth chambers (PGW36, Conviron, Winnipeg, Canada) in 1-l pots containing a commercial mixture of soil, peat, and compost (Torf und Pikiererde 140, Ricoter, Aarberg, Switzerland). Two treatments were applied, i.e. optimal and suboptimal temperatures. The control plants grew for 13 days at 25/22 °C (optimal temperature) with a photoperiod of 12 h at 400μmol m^{-2} s^{-1} and a relative humidity of 60/70% (day/night). Chilling-treated (suboptimal temperature) plants were first grown for 6 days as control plants and then for 14 days at 15/13 °C; the other conditions were the same as those for the control plants. In both temperature regimes the plants were measured and harvested at the 3-leaf (V3) stage, i.e. when the whole collar of the third leaf was visible (Ritchie and Hanway 1984).

For the Lo-population, pre-germinated seeds were placed in growth columns (7 cm diameter, 25 cm height) in a mixture of quartz sand (particle size 0.08–0.2 mm) and 5% (w/w) vermiculite powder (Vermex Pulver E, Vermica AG, Bözen, Switzerland). The sand substrate was used to ease the evaluation of root traits after harvest, the results of which are described elsewhere (Hund et al. 2004). After coleoptile emergence, the plants were grown under the same conditions as the plants at suboptimal temperature treatment described above. The plants of the Lo-population were harvested after 21 days when most had reached the 1-leaf (V1) stage.

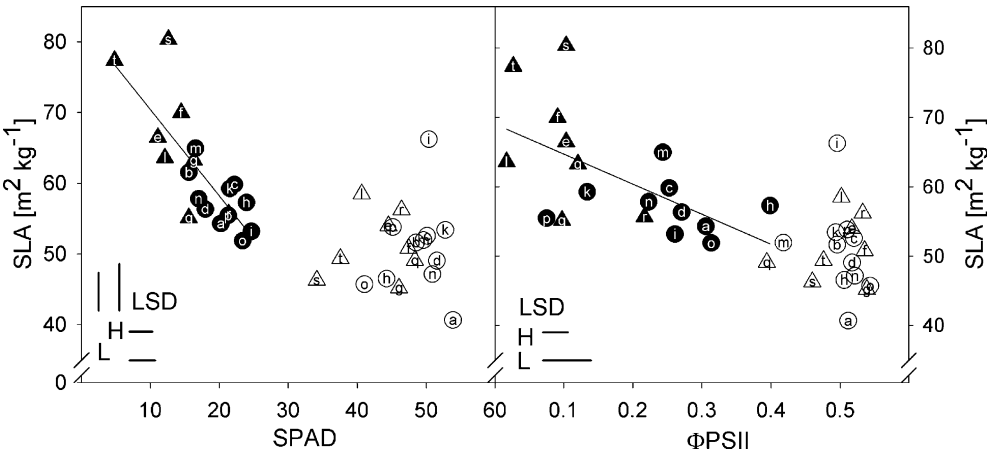

Figure 1. Relationship between chlorophyll content (SPAD) and SLA and operating quantum efficiency of PSII (Φ_{PSII}) and SLA of inbred lines selected for high (circles) and low (triangles) Φ_{PSII} at low temperature. Lines were grown at 15/13 °C (closed symbols) or 25/22 °C (open symbols). See Material and methods with regard to letters, which indicate the line. Bars (left hand corner) indicate Fishers's least significant difference (LSD) for the comparison of trait values at optimal (H) and suboptimal (L) growth temperature.

Determination of morpho-physiological traits

All the measurements on the ETH material (S5 lines and the ETH-population) were performed on fully expanded third leaves after 14 (25/22 °C) and 20 (15/13 °C) days. The operating quantum efficiency of photosystem II (Φ_{PSII}) and the CER were measured as described by Fracheboud et al. (2004) with a LI-6400 instrument equipped with an LI-6400-40 pulse-amplitude modulation fluorometer (LI-COR, Lincoln, NE, USA). The greenness of the third leaf was recorded with a chlorophyll meter (SPAD-502, Minolta Corporations, Ramsey, NJ, USA). The area of the third leaf was measured with a leaf-area meter (LI-COR 3100, Lincoln, NE, USA); its dry weight was recorded after drying at 65 °C for 42 h. The SLA was calculated as SLA = leaf area/leaf dry weight ($m^2\ kg^{-1}$).

For the Lo-population, all the morpho-physiological measurements were performed on the second leaf after 19 and 20 days (blocks 1 and 2, respectively) after imbibition. The Φ_{PSII} was measured at $400\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density (PPFD) with a pulse-amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany) as described by Hund et al. (2004). The SPAD values of the second leaves and the leaf area of the fully developed first leaf and the successive leaves were measured as described above. SLA was calculated as the overall leaf area per overall leaf dry matter.

Experimental design and statistics

For the evaluation of the experimental inbred lines, a randomized complete-block design combined over temperature treatments was used. Each temperature treatment consisted of one growth chamber run containing three replications and each experimental unit consisted of one pot containing two plants.

The plants of the ETH-population were sown and analyzed at intervals of 24 h in incomplete blocks of 22 families. Six to nine plants of each $F_{2:3}$ family and six plants of each parental line were analyzed at each growth temperature (for further details see Fracheboud et al. 2004).

The plants of the Lo-population, including the two parental inbred lines and the 168 $F_{2:4}$ families, were arranged in a randomized complete-block design with two growth chamber runs and two replications per run. For technical reasons different growth chambers were used, slightly differing from each other ($50\ \Phi mol\ m^{-2}\ s^{-1}$ PPFD and about 0.5 °C) and were thus considered as different environments. Experimental units (PVC columns), each containing three plants, were arranged as a central and a border block, according to the light intensity in the growth chamber.

All the data were analyzed using the general linear model procedure (PROC GLM) of SAS 8.02 (SAS Institute Inc., 1999–2001, Cary, NC, USA). Skewness was calculated with the e1071 package in R (Ihaka and Gentleman 1996).

QTL analyses

QTLs were identified by composite interval mapping using QTL Cartographer (Jansen and Stam 1994; Zeng 1994) with different model thresholds, depending on the population and the experimental design. The QTL analyses of the ETH-population were performed with a linkage map with 118 SSR markers (Fracheboud et al. 2004). The QTLs were identified using model 6 of QTL Cartographer, with a blocking window size of 30 cM. The co-factors were selected by forward and backward regressions with the 'in' and 'out' thresholds at a *p*-value of 0.01. The presence of a QTL was considered to be significant when the likelihood of odds (LOD) value was higher than 3.5. This value corresponds to an experiment wise type I error rate (α') of 0.021 for a single trait analysis in an F_2 population with three degrees of freedom (df), assuming that all the chromosome arms segregate independently.

The QTL analyses of the Lo-population were performed with a linkage map with 135 markers (Tuberosa et al. 2002). The co-factors were selected as described by Hund et al. (2004). A QTL was declared significant when a critical LOD threshold of 3.5 was exceeded. For a joint analysis of an F_2 population in two environments (i.e. growth chambers) this threshold is equivalent to α' of 0.11. A joint analysis of the phenotypic data of the two growth chamber runs enabled the evaluation of the QTL-by-environment (Q×E) interaction (Jiang and Zeng 1995). The factor

'environment' accounts for the variability in time and space. A LOD threshold of 1.3 for a significant Q×E interaction was based on the type-I error rate of 0.05 for a single locus for an F_2 with two df. Multiple regressions were used to evaluate the actual additive effects of the QTL and the total percentage of phenotypic variation accounted for by the identified QTL. Considering that the phenotypic evaluation was made on the $F_{2:4}$, only the additive effects of the QTLs are reported.

Results

For the experimental inbred lines, the analysis of variance revealed significant differences between temperature treatments, genotypes and their interaction ($p < 0.001$) for all evaluated traits. A separate analysis of the optimal and suboptimal temperature treatments showed highly significant differences among the genotypes ($p < 0.001$) for all traits at both temperatures (data not shown). At suboptimal temperature significant differences were detected between the group of genotypes selected for high Φ_{PSII} (H) and the group selected for low Φ_{PSII} (L) for all the measured traits (Table 1). At optimal temperature, the only significant difference between the two groups was in the SPAD values.

At suboptimal temperature, there were modest to very strong correlations among the measured traits (Table 2). The SLA correlated negatively with all the other traits; the strongest correlation was with SPAD values. At suboptimal temperature, a very strong correlation was found between CER and Φ_{PSII}. At optimal temperatures, there were no significant correlations among the traits, with the exception of a strong correlation between CER and Φ_{PSII}. The significant correlations between values of SLA and SPAD and of SLA and Φ_{PSII} for inbred lines selected for high and low Φ_{PSII} at low temperature are given in Figure 1. The two groups of inbred lines are displayed with different symbols in order to underline that for these materials, the high correlation is not only due to differences among groups, but also within groups.

With respect to the ETH-population, the growing temperature had a significant effect on all traits. The values of SLA, SPAD, Φ_{PSII}, CER, and shoot dry weight at 25 °C where 52.5 $m^2\ kg^{-1}$, 37.2, 0.52, 15.0μmol $m^{-2}\ s^{-1}$, and 218 mg and where significantly reduced at low temperature by 13.5, 45.2, 74.6, 81.7, and, 25.2% respectively. The variation among $F_{2:3}$ families was significant at both temperatures. The correlations between SPAD values or Φ_{PSII} with SLA at low temperatures were not as close as expected from the results obtained from the S5 inbred lines. The phenotypic correlation coefficients between SLA and the other above traits ranged from −0.23 (CER) to −0.38 (shoot dry weight) for plants grown at 15 °C and from −0.15 (shoot dry weight) to −0.28 (SPAD) for plants grown at 25 °C (data not shown). The QTL analysis of the ETH-population grown at 25 °C revealed two significant QTLs for SLA, whereas at 15 °C a total of four QTLs for SLA were detected (Table 3). There were no common QTLs for the 15 °C-treatment and the 25 °C-treatment. In a comparison with previous results (Fracheboud et al. 2004) loci for SLA were co-located with loci for photosynthesis-related traits (Figure 2), including two major loci on chromosomes 2 and 6 as well as a locus on chromosome 1. At the locus on chromosome 6, SLA was co-located with CER, Φ_{PSII} and shoot dry weight; at the locus on

Table 1. Mean values of SLA ($m^2\ kg^{-1}$), leaf greenness (SPAD), quantum yield of electron transport at photosystem II (Φ_{PSII}), and CER (μmol $CO_2\ m^{-2}\ s^{-1}$) of 19 S_5 inbred lines selected for high (H) and low (L) Φ_{PSII} at low temperature.

Temperature	Selection	SLA	SPAD	Φ_{PSII}	CER
15/13 °C	H	57.6	20.3	0.25	8.1
	L	66.5	13.5	0.10	3.0
	Significance	***	***	***	***
25/22 °C	H	50.8	48.9	0.50	16.8
	L	51.2	42.9	0.49	17.4
	Significance	ns	***	ns	ns

***Significance for the contrast between the 'H' and 'L' group at $p \leq 0.001$; ns: not significant.

Table 2. Pearson correlation coefficients between mean values of photosynthesis-related traits of 19 S_5 inbred lines divergently selected for Φ_{PSII} at low temperatures.

15 °C	CER	Φ_{PSII}	SPAD	SLA
25 °C				
CER		0.98^{***}	0.78^{***}	-0.63^{**}
Φ_{PSII}	0.83^{***}		0.73^{**}	$\mathbf{-0.58}^{*}$
SPAD	−0.03	0.07		$\mathbf{-0.80}^{***}$
SLA	−0.27	**−0.04**	**0.07**	

Correlations at 15/13 °C (day/night) (above the diagonal), and 25/22 °C (below the diagonal). Significance of the regressions at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ is indicated by *, **, and ***, respectively. The scatter plots corresponding to the correlation coefficients (bold) are shown in Figure 1.

chromosome 2, SLA was co-located with CER and leaf greenness (SPAD) and at the locus on chromosome 1, SLA was co-located with Φ_{PSII}. At all three loci the additive effects of SLA were negative, indicating that the alleles increasing the trait values were contributed by the parent selected for low photosynthetic efficiency (i.e. ETH-DL3), while the signs of the additive effects for the photosynthesis-related traits were positive, indicating that the alleles that increased the trait values were contributed by the parent selected for high photosynthetic efficiency (i.e. ETH-DH7). At optimal growth temperature, no common QTLs for SLA or photosynthetic-related traits or shoot dry weight were detected (Figure 2).

A second QTL population, namely the Lo-population, whose parental inbred lines were not selected for photosynthetic traits in the cold, was used to test the association between Φ_{PSII}, SPAD, and SLA in an independent set of genotypes. According to the analysis of variance the two growth chamber runs were significantly different for all three traits (data not shown). The parents differed significantly ($p = 0.001$) in Φ_{PSII} and SPAD. All frequencies were more or less normally distributed, but the direction of the asymmetric tail (skewness) depended on the growth chamber run. The skewness of the distribution of Φ_{PSII} and SPAD was negative in replication 1 (−0.52 and −0.53, respectively) and positive in replication 2 (0.57 and 0.10, respectively).

For the $F_{2:4}$ families of the Lo-population there was a significant genotype-by-environment (growth chambers) interaction for Φ_{PSII} and SPAD but not for SLA. The correlations between Φ_{PSII}, SPAD, and SLA were closer in the second growth chamber run, in which the variability of all trait values was higher (Figure 3). The QTL analysis revealed three QTLs for SLA, which were located on chromosomes 1, 5, and 10 (Table 4). In

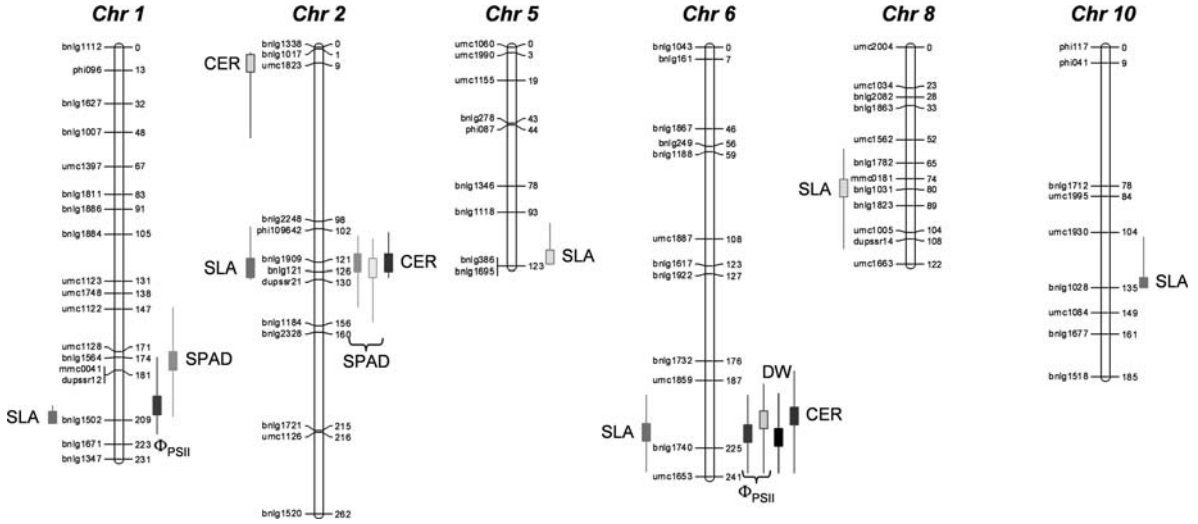

Figure 2. QTLs for SLA, leaf greenness (SPAD), shoot dry weight (DW), CER, and operating quantum efficiency of PSII (Φ_{PSII}) above an LOD threshold of 3.5 in the ETH-population. Hatched bars indicate QTLs of plants grown at optimal temperature (25 °C), filled bars indicate QTLs of plants developed at suboptimal temperature (15 °C). Bars to the left of the chromosome represent QTLs with negative additivity, bars to the right of the chromosome QTLs with positive additivity. Only, chromosomes with QTLs for SLA are shown. The map and the QTLs for Φ_{PSII}, CER, SPAD, and shoot dry weight were taken from Fracheboud et al. (2004).

Table 3. Putative QTLs for the SLA (m^2 kg^{-1}) (LOD > 3.5) of 226 $F_{2:3}$ families of the ETH-population (ETH-DL3×ETH-DH7) grown at suboptimal (15/13 °C) or optimal (25/22 °C) temperature.

Temp	Chr	cM	Nearest marker	LOD	R^2	Add[a]	Dom
15 °C	1	209	bnlg1502	4.1	0.06	−0.97	0.56
	2	108	phi109642	5.1	0.09	−1.18	0.11
	6	221	bnlg1740	6.6	0.12	−1.13	−1.84
	10	132	bnlg1028	4.7	0.08	1.05	1.26
25 °C	5	123	bnlg386	5.6	0.09	0.85	−0.64
	8	78	bnlg1031	5.6	0.09	−0.95	0.55

Temp, growth temperature; Chr, chromosome number; cM, position of the peak of the QTL in centimorgan; R^2, phenotypic variance explained by genotype class at LOD peak; Add, additivity; Dom, dominance.
[a]Additive and dominant effects represent the substitution of an ETH-DL3 allele with an ETH-DH7 allele.

replication 1, the locus on chromosome 10 explained the highest portion of variability for SLA, while in replication 2 this was the case for the loci on chromosomes 1 and 5. By comparing with previous results (Hund et al. 2004), for all the SLA QTLs, a co-location with QTLs for leaf greenness was observed (Figure 4). Moreover, a QTL for Φ_{PSII} (chromosome 1) and QTLs for Φ_{PSII} and shoot dry weight (chromosome 10) were located at the same positions as the QTLs for SLA. The additivity of the QTLs for SLA always had the opposite sign to those for the other traits. There was no overlap of the QTLs for SLA between the Lo and the ETH populations.

Discussion

Improving photosynthesis at low temperature is one of the most relevant breeding goals for the adaptation of maize to low temperature during early growth in a temperate climate. In the present work, we used inbred lines, which were exclusively selected for high or low Φ_{PSII} at low temperature (Fracheboud et al. 1999) to study the co-selection of other traits. Divergent selection was successful and led to an increase in the CER, of up to 31%, in hybrids derived from inbred lines selected upwards (Fracheboud 1999). In contrast to studies with unrelated, contrasting genotypes, the material used

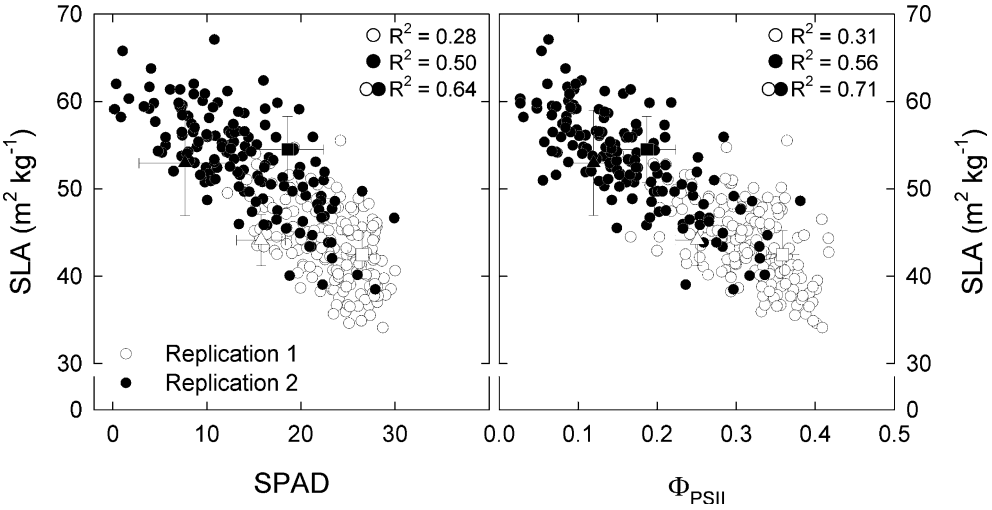

Figure 3. Relationship between chlorophyll content (SPAD values) and SLA and between the operating quantum efficiency of photosystem II (Φ_{PSII}) and SLA in the Lo-Population. The lines were grown at low 15/13 °C temperatures in two growth chamber runs (open and closed symbols). Circles: $F_{2:4}$ families; triangles: Lo1016; squares: Lo964. Error bars show 95 % confidence intervals.

Table 4. The putative QTLs for SLA (LOD > 3.5) of 168 $F_{2:4}$ families of the Lo-population (Lo964×Lo1016) grown at 15 °C.

Chr	cM	Nearest marker	LOD		R^2		Add[a]
			Joint	Q×E	Repl. 1	Repl. 2	
1	0	PGAMCTA310	5.0	0.6	0.07	0.09	−1.88[a]
5	118	PGAMCGT165	4.4	2.8	0.03	0.08	−1.31
10	59	PGAMCTT250	4.0	0.8	0.13	0.02	1.95

Chr, chromosome number; cM, position of the peak of the QTL in centimorgan; Joint, joint analysis of the two experiments; Q×E, QTL-by-environment interaction; Repl., replication; R^2, phenotypic variance explained by genotype class at LOD peak; Add, additivity.

[a]Additive effects represent the substitution of a Lo964 allele with a Lo1016 allele.

has the advantage that the association between traits is not biased by selection pressure on traits other than Φ_{PSII}. Grown at suboptimal temperature, the group of lines selected for high Φ_{PSII} differed significantly from the group selected for low Φ_{PSII} for all the traits. This supports the hypothesis that pleiotropic effects are the cause of the close phenotypic correlations among these traits at suboptimal temperature. At optimal temperature, significant effects between the two groups were found only for leaf greenness. Thus, the selection did not constitutively change the leaf

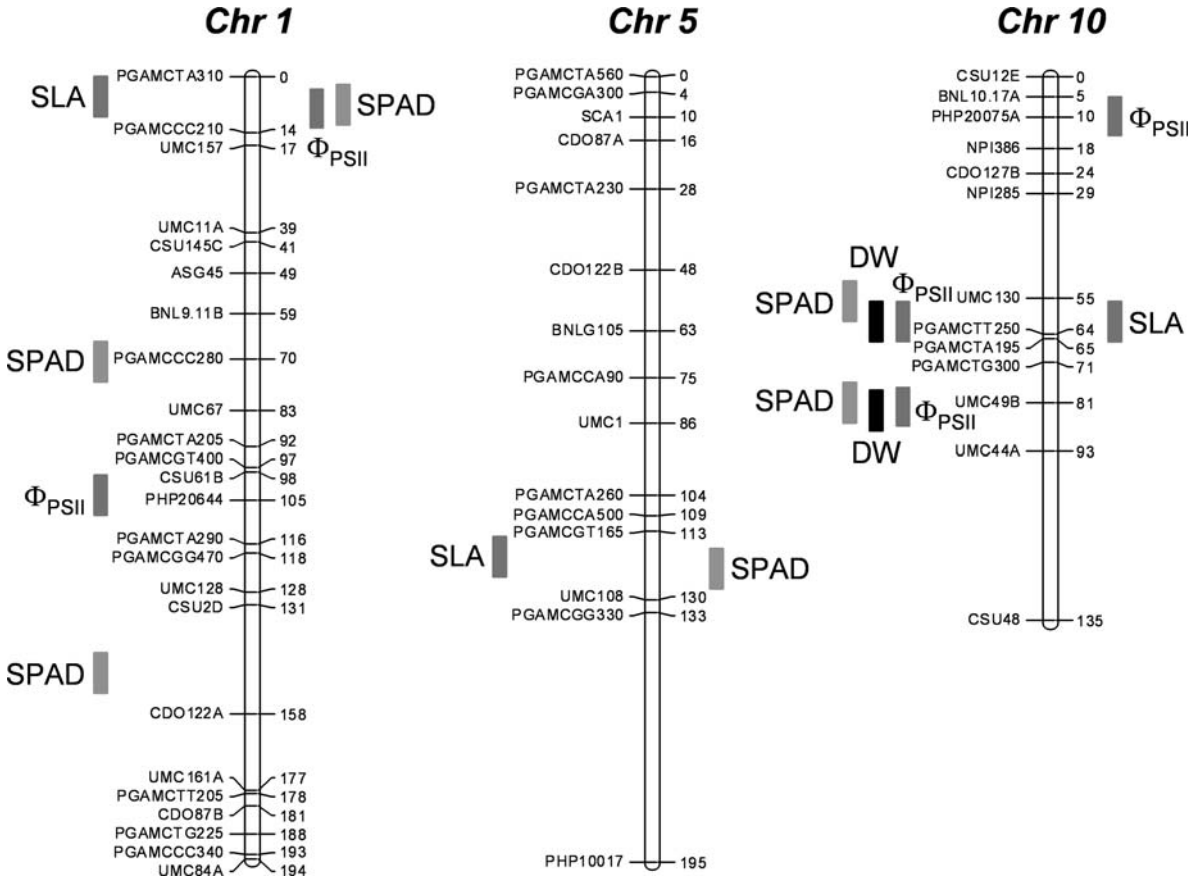

Figure 4. QTLs for SLA, leaf greenness (SPAD), shoot dry weight (DW), and operating quantum efficiency of PSII (Φ_{PSII}) above an LOD threshold of 3.5 in the Lo-population. Bars to the left of the chromosome represent QTLs with negative additivity, bars right of the chromosome with positive additivity. Only chromosomes with QTLs for SLA are shown. The QTLs for Φ_{PSII}, SPAD, and shoot dry weight were taken from Hund et al. (2004).

physiology and morphology. This is important since an unfavorable linkage between temperature-stable high Φ_{PSII} and low SLA might lead to a decrease in productivity at optimal temperature (Leipner et al. 1999; Poorter and Van der Werf 1998). The association between photosynthetic performance and SLA was observed in a previous study in maize (Verheul et al. 1996). However, unrelated genotypes had been used, which were pre-selected for high and low vigor, as defined by a large leaf area and high greenness. Thus, the relationship between the traits may have been due to a co-selection of independent loci or to random genetic drift.

Despite the low correlation between SLA and photosynthesis-related traits in the ETH-population, the QTL analysis of plants grown at suboptimal temperature yielded three of four QTLs where SLA was co-located with photosynthesis-related traits. The opposite sign of the additive effects matches with the phenotypic observations. In contrast, the QTLs for SLA from seedlings developed at optimal temperature were not associated with QTLs for SLA at suboptimal temperature as well as with QTLs for other measured traits. Furthermore, a high SLA at optimal temperature had no positive effect on dry matter accumulation but was negatively correlated with the shoot dry weight at suboptimal temperature. This shows clearly that SLA was under different genetic control at suboptimal and at optimal temperature. The characteristics of leaves developed at suboptimal temperature compared to leaves grown at optimal temperature with respect to SLA, leaf greenness, and photosynthetic performance are very similar to the characteristics of leaves grown at low and high light intensity (see for example Evans and Poorter 2001). However, while in plants acclimated to low or to high light intensity the SLA was positively correlated with the assimilation rate per leaf weight (calculated from the data provided by Evans and Poorter 2001), this was the case only when the ETH-population was grown at optimal but not at suboptimal temperature (data not shown). Therefore, it seems unlikely that the increase in the SLA of the chilling sensitive genotypes at suboptimal temperature is an optimization reaction. Rather, it seems that a high SLA at suboptimal temperature is the consequence of a reduced availability of assimilates.

The close (negative) relationship between SLA on the one hand and photosynthesis-related traits and shoot dry matter accumulation on the other was verified in the Lo-population grown at suboptimal temperature. The two different growth chamber runs were designed as replications in time. However, there was a significant difference between the two replications, which cannot be attributed to one distinct factor. The small difference of 50μmol m^{-2} s^{-1} PPFD and of about 0.5 °C between the two growth chamber runs seem to cause the weaker performance of the plants in the second replication. However, the opposite skewness of the frequency distributions of the SPAD values and Φ_{PSII} in the two replications shows that the environmental conditions were in a range where the genotypes differed most. The significant genotype-by-environment interaction for Φ_{PSII} and SPAD was reflected by the significant QTL-by-environment interaction for many of the loci (see Hund et al. 2004). This indicates that, at the critical temperature threshold of 15 °C, small changes in the environmental conditions resulted not only in a shift in photosynthetic performance, but also in a shift in the set of genes responsible for its regulation. Despite these interactions, the association between Φ_{PSII}, leaf greenness, and SLA was detected at the phenotypic as well as at the genetic level in both growth chamber runs.

By searching the maize genetics and genomics database, interesting candidate genes were identified for the major QTLs. For the ETH-population, *ssu2* (coding for Rubisco small subunit 2) and *agp2* (coding for ADP glucose pyrophosphorylase small subunit) were found to be promising candidate genes for the QTLs on chromosomes 2 and 6, respectively (Fracheboud et al. 2004). The major QTL of the Lo-population, which was localized at chromosome 1, is close to the gene *cat2* when the Lo964 × Lo1016 map is aligned with the BNL2002 map (Maize Genetics and Genomics Database). The *cat2* encodes catalase 2, which is expressed in photosynthetic tissue of maize (Scandalios et al. 1984). Catalase, an antioxidative enzyme that detoxifies hydrogen peroxide, was shown to be involved in the induced acclimation of maize seedlings to chilling stress (Prasad 1997). The significance of catalase for photosynthesis was demonstrated in antisense tobacco plants lacking catalase; antisense suppression of catalase resulted

in a strong decrease in *rbcS* (Rubisco small subunit) expression and in a reduced photosynthetic capacity (Rizhsky et al. 2002). It is clear that the detected QTLs span large chromosome regions with possibly thousands of genes. Therefore getting the underlying gene out of the database would be highly unlikely. However, all the detected candidate genes (*ssu2*, *agp2*, and *cat2*) are indirectly associated with the functioning of the photosynthetic apparatus through CO_2 fixation, storage, and oxidative stress response, respectively. This matches observations of transgenic tobacco with reduced Rubisco levels and, thus, weaker photosynthesis and a higher SLA (Quick 1998; Stitt and Schulze 1994).

In conclusion, we can state that the finding of a relationship among Φ_{PSII}, leaf greenness, and SLA at suboptimal temperature in two independent populations strongly supports pleiotropy more than linkage disequilibrium as the genetic basis of the relationship among traits. However, the question remains as to whether this increase in SLA, observed at low temperature with the photosynthesis decline, is due to a shortage of assimilates and the formation of leaf structure or to a compensation reaction. Our results did not provide evidence that selection for good photosynthetic performance at low temperature leads to a co-selection of a constitutive low SLA, which is not a desirable attribute because it can cause a low productivity. We therefore, consider Φ_{PSII} to be a valuable trait for selecting genotypes with improved cold tolerance, especially since the trait measurements are reasonably fast, as required for plant breeding applications. However, the actual merit of a selection for Φ_{PSII} and its effects on SLA must be confirmed by testing plants under field conditions.

Acknowledgements

The authors thank Susan Blumer for laboratory measurements, Ernst Merz for managing the ETH lines in the field nursery, Dr M. Motto for supplying Lo964 and Lo1016 and Dr S. Salvi for supplying the genetic map of their cross. The authors would further like to thank the anonymous reviewers for their valuable comments in improving the manuscript.

References

Atkin O.K. and Lambers H. 1998. Slow-growing alpine and fast-growing lowland species: a case study of factors associated with variation in growth rate among herbaceous higher plants under natural and controlled conditions. In: Lambers H., Poorter H. and Van Vuuren M.M.I. (eds), Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences. Backhuys Publishers, Leiden.

Evans J.R. 1998. Photosynthetic characteristics of fast- and slow-growing species. In: Lambers H., Poorter H. and Van Vuuren M.M.I. (eds), Inherent Variation in Plant Growth Physiological Mechanisms and Ecological Consequences. Backhuys Publishers, Leiden, pp. 101–119.

Evans J.R. and Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ. 24: 755–767.

Fichtner K., Quick W.P., Schulze E.-D., Mooney H.A., Rodermel S.R., Bogorad L. and Stitt M. 1993. Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' rbcS. V. Relationship between photosynthetic rate, storage strategy, biomass allocation and vegetative plant growth at three different nitrogen supplies. Planta 190: 1–9.

Fracheboud Y. 1999. Cold adaptation of the photosynthetic apparatus of maize by growth at suboptimal temperature. In: Sanches-Diaz M., Irigoyen J.J., Aguirreola J. and Pithan K. (eds), Crop Developement for the Cool and Wet Climate of Europe. Office for the Official Publications of the European Communities, Brussels, pp. 88–89.

Fracheboud Y., Haldimann P., Leipner J. and Stamp P. 1999. Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). J. Exp. Bot. 50: 1533–1540.

Fracheboud Y., Jompuk C., Ribaut J.-M., Stamp P. and Leipner J. 2004. Genetic analysis of cold-tolerance of the photosynthesis in maize. Plant Mol. Biol. 56: 241–253.

Genty B., Briantais J.-M. and Baker N.R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990: 87–92.

Hund A., Frachboud Y., Soldati A., Frascaroli E., Salvi S. and Stamp P. 2004. QTL controlling root and shoot traits of maize seedlings under cold stress. Theor. Appl. Genet. 109: 618–629.

Ihaka R. and Gentleman R. 1996. R: a language for data analysis and graphics. J. Comput. Graph. Stat. 5: 299–314.

Jansen R.C. and Stam P. 1994. High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136: 1447–1455.

Jiang C. and Zeng Z.B. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140: 111–127.

Lee E.A., Staebler M.A. and Tollenaar M. 2002. Genetic variation and physiological discrimination for cold tolerance in maize (*Zea Mays* L.) during an early autotrophic phase of development. Crop Sci. 42: 1919–1929.

Leipner J., Stehli A. and Soldati A. 1999. Photosynthetic performance of exotic maize (*Zea mays* L.) germplasm from tropical highlands at low and high temperature. J. Appl. Bot. 73: 20–24.

Lopez-Castaneda C., Richards R.A. and Farquhar G.D. 1995. Variation in early vigour between wheat and barley. Crop Sci. 35: 472–479.

Poorter H. and Van der Werf A. 1998. Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance* A review of herbaceous species. In: Lambers H., Poorter H. and Van Vuuren M.M.I. (eds), Inherent Variation in Plant Growth Physiological Mechanisms and Ecological Consequences. Backhuys Publishers, Leiden, pp. 309–332.

Prasad T.K. 1997. Role of catalase in lnducing chilling tolerance in pre-emergent maize seedlings. Plant Physiol. 114: 1369–1376.

Quick W.P. 1998. Transgenic plants as a tool to analyse the mechanistic basis for variation in plant growth. In: Lambers H., Poorter H. and Van Vuuren M.M.I. (eds), Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences. Backhuys Publishers, Leiden, pp. 199–219.

Richards R.A. 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. J. Exp. Bot. 51: 447–458.

Ritchie S.W. and Hanway J.J. 1984. How a corn plant develops. Special Report No. 48. Cooperative Extension Service, Ames. http://maize.agron.iastate.edu/corngrows.html.

Rizhsky L., Hallak-Herr E., Van Breusegem F., Rachmilevitch S., Barr J.E., Rodermel S., Inzé D. and Mittler R. 2002. Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J. 32: 329–342.

Sanguineti M.C., Giuliani M.M., Govi G., Tuberosa R. and Landi P. 1998. Root and shoot traits of maize inbred lines grown in the field and in hydroponic culture and their relationship with root lodging. Maydica 43: 211–216.

Scandalios J.G., Tsaftaris A.S., Chandlee J.M. and Skadsen R.W. 1984. Expression of the developmentally regulated catalase (cat) genes in maize. Dev. Genet. 4: 281–293.

Soldati A., Stehli A. and Stamp P. 1999. Temperature adaptation of tropical highland maize (*Zea mays* L.) during early growth and in controlled conditions. Europ. J. Agron. 10: 111–117.

Stehli A., Soldati A. and Stamp P. 1999. Vegetative performance of tropical highland maize (*Zea mays* L.) in the field. J. Agron. Crop Sci. 183: 193–198.

Stirling C.M., Nie G.Y., Aguilera C., Nugawela A., Long S.P. and Baker N.R. 1991. Photosynthetic productivity of an immature maize crop: changes in quantum yield of CO_2 assimilation, conversion efficiency and thylakoid proteins. Plant Cell Environ. 14: 3905–3914.

Stitt M. and Schulze D. 1994. Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology Plant Cell Environ. 17: 465–487.

Terauchi T. and Matsuoka M. 2000. Ideal characteristics for the early growth of sugarcane. Jpn. J. Crop. Sci. 69: 286–292.

Tuberosa R., Sanguineti M.C., Landi P., Giuliani M.M., Salvi S. and Conti S. 2002. Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Mol. Biol. 48: 697–712.

Verheul M.J., Picatto C. and Stamp P. 1996. Growth and development of maize seedlings under chilling conditions in the field. Europ. J. Agron. 5: 31–43.

Zeng Z.B. 1994. Precision mapping of quantitative trait loci. Genetics 136: 1457–1468.