Journal of Experimental Botany, Vol. 53, No. 376, pp. 1967–1977, September 2002
DOI: 10.1093/jxb/erf040

Journal of Experimental Botany

Identification of quantitative trait loci for cold-tolerance of photosynthesis in maize (*Zea mays* L.)

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Received 20 February 2002; Accepted 13 May 2002

Abstract

The effects of low growth temperature (15 °C) on the photosynthetic apparatus of maize were investigated in a set of 233 recombinant inbred lines by means of chlorophyll fluorescence, gas exchange measurements and analysis of photosynthetic pigments. A quantitative trait loci (QTL) analysis of five traits related to the functioning of the photosynthetic apparatus revealed a total of eight genomic regions that were significantly involved in the expression of the target traits. Four of these QTLs, located on chromosomes 1 (around 146 cM), 2 (around 138 cM), 3 (around 70 cM), and 9 (around 62 cM), were identified across several traits and the phenotypic correlation observed among those traits confirmed at the genetic level. The two QTLs on chromosomes 1 and 9 were also expressed in leaves developed at near-optimal temperature (25 °C) whilst the two QTLs on chromosomes 2 and 3 were specific to leaves developed at sub-optimal temperature. A QTL analysis conducted on traits related to the pigment composition of the leaves developed at 15 °C detected the QTL on chromosome 3 around 70 cM in 7 of the 11 traits analysed. This QTL accounted for up to 28% of the phenotypic variance of the quantum yield of electron transport at PSII in the fourth leaf after about 3 weeks at a sub-optimal temperature. The results presented here suggest that key gene(s) involved in the development of functional chloroplasts of maize at low temperature should be located on chromosome 3, close to the centromere.

Key words: Chlorophyll fluorescence, cold-tolerance, maize, photosynthesis, pigment composition, QTL.

Introduction

Despite its subtropical origin, maize is now cultivated extensively in temperate areas. During the last 50 years it has become a major crop in northern regions with a cool climate which often impairs the early development of the canopy and the photosynthetic productivity of the seedlings (Stirling *et al*., 1991; Fryer *et al*., 1998; Leipner *et al*., 1999). Maize leaves that develop at low temperature are characterized by a change in the composition of leaf pigments (Haldimann *et al*., 1995), changes in anti-oxidative defences (Leipner *et al*., 1997; Fryer *et al*., 1998; Kingston-Smith *et al*., 1999) and modifications of the protein composition of the thylakoid membrane (Nie and Baker, 1991; Robertson *et al*., 1993). These changes probably reflect adaptation processes, which might influence the tolerance of the photosynthetic apparatus to low temperature. Comparisons of genotypes indicate that there is wide genetic variation in the *Zea mays* species for cold tolerance of the photosynthetic apparatus (Fracheboud *et al*., 1999; Haldimann, 1999).

Over the past ten years, molecular markers have been extensively used to identify quantitative trait loci (QTLs) involved in the expression of traits of agronomic importance (for a review see Lee, 1995). This includes the genetic dissection of yield components (Kraja and Dudley, 2000; Austin *et al*., 2000), resistance to diseases (Moon *et al*., 1999; Welz and Geiger, 2000) and, to a lesser extent, tolerance to abiotic stress, especially crop production under water-limited environments (for a review see Ribaut and Poland, 2000).

Because the genetic basis of cold tolerance in maize has not yet been investigated using molecular markers, the objective of this study was to conduct a QTL analysis on a set of 233 recombinant lines (RILs) to understand further

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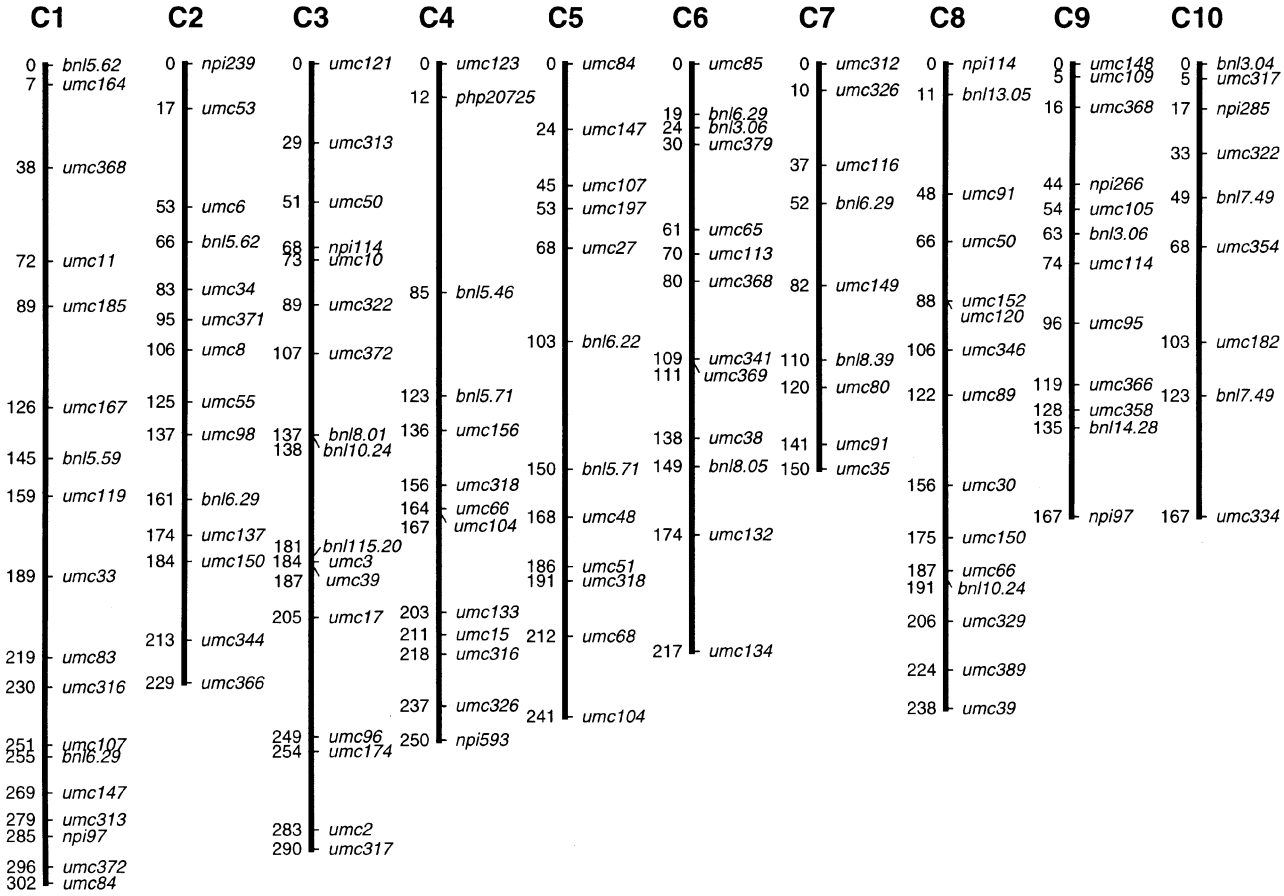

Fig. 1. RFLP linkage map based on the cross Ac7643×Ac7729 and the allelic segregation of 132 loci in 233 S5 recombinant inbred lines. Loci names are on the right and cumulative distances in centimorgans on the left.

the cold tolerance of photosynthesis at the genetic level. Information about the number and the characteristics of the genomic regions responsible for cold-tolerance will be useful for future germplasm improvement. QTL analysis also allows the investigation, to some extent, of the causal relationships between different traits (Lebreton *et al*., 1995). This could provide useful information about the physiological responses of maize to low temperature.

Materials and methods

Plant material and growth conditions

By crossing a drought-tolerant with a drought-susceptible line (Ac7643 and Ac7729/TZSRW) two segregating populations for drought tolerance were developed at the second and sixth inbreeding levels at CIMMYT. Genetic dissection for drought tolerance has already been conducted using phenotypic data obtained from the F_3 families (Ribaut *et al*., 1996, 1997) and the recombinant inbred lines (RILs) (Fracheboud *et al*., unpublished data) derived by single seed descent from F_2 plants. First measurements on the two parental lines indicated that the families derived from this specific cross were also segregating for cold tolerance of photosynthesis. Therefore, an analysis for cold tolerance was performed on the 233 RILs. One seedling of each line was grown in a growth chamber (PGW36, Conviron, Winnipeg, Canada) in 10×10×10 cm pots containing a commercial mixture of soil, peat and compost (Topf und Pikiererde 140, Ricoter, Aarberg, Switzerland). The plants were first developed for 6 d at 25/22 °C (day/night) under a photoperiod of 12 h at 450 µmol quanta m^{-2} s^{-1} at a relative humidity of 60/70% (day/night). The temperature was then switched to 15/13 °C (day/night). If not otherwise specified, all measurements were performed on fully expanded third leaves after 18–21 d culture at 15 °C. Six plants of each parent line were grown together with the population. The whole experiment was performed twice. Samples were harvested for pigment analysis at the end of the first experiment (see below). In the second experiment, the quantum yield of electron transport at photosystem II (ΦPSII, see below) was monitored over time, from 2.5 h before exposure to low temperature on leaf 1, until 26 d at 15 °C on leaf 4.

In a control experiment, the RIL population and the two parents were grown under the same conditions, except that the temperature was kept at 25/22 °C (day/night) during the whole growth period. The control experiment was performed once, and the measurements of photosynthetic traits were made on fully expanded third leaves 13–15 d after sowing.

Photosynthesis and chlorophyll fluorescence

Photosynthesis and stomatal resistance were measured under growth conditions using a LI-6200 (Li-Cor, Lincoln, USA) instrument. Chlorophyll fluorescence was recorded with a pulse-amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany), equipped with a leaf clip holder 2030-B. The maximum quantum

efficiency of PSII primary photochemistry (F_v/F_m) was measured by applying a 1 s saturating flash (>8000 μmol m^{-2} s^{-1}) after about 1 h dark adaptation. The quantum yield of electron transport at PSII (ΦPSII) was measured under growing conditions according to Genty *et al*. (1989).

Pigments analysis

At the end of the first experiment (after 21 d at low temperature), leaf discs were punched from the third leaf, frozen in liquid N_2 and stored at –80 °C until analysis. Pigments were analysed by HPLC according to the method of Gilmore and Yamamoto (1991) and the modifications described in Leipner *et al*. (2000).

Linkage map and QTL identification

According to the protocols of Hoisington *et al*. (1994), DNA from a bulk of 10 leaf samples was extracted for each RIL. The DNA was quantified and then digested with the restriction enzymes EcoRI or Hind III; the fragments were separated according to size on agarose gel and the digested DNA was transferred to a nylon membrane by the Southern blotting process. Labelled RFLP probes (digoxigenin-dUTP) were then hybridized to the complementary sequences on the immobilized fragment DNA sample in the membranes to detect polymorphism. Using 132 RFLP probes, a linkage map of a total length of 2250 cM with an average density of 17 cM was constructed (Fig. 1) using Mapmaker 3.0 (Lander *et al*., 1987). The longest distance between two consecutive markers (72.2 cM) was located on chromosome 4, between 12.3 and 84.5 cM. Apart from this large gap, five other gaps of 40–50 cM were identified on chromosomes 3 (two of them), 5, 6 and 10.

The QTL identification was conducted using the composite interval mapping (CIM) software developed by Zeng (1994). This approach is based on mixture models and maximum likelihood techniques and enables an accurate evaluation of QTL characteristics by reducing QTL interaction when using markers as cofactors. For more details related to the practical procedure of CIM see Ribaut *et al*. (1997). The presence of a QTL was declared significant if the likelihood of odds (LOD) value was >2.5 for a single trait analysis or in the joint analysis of data from the two different experiments at low temperature. Additivity at each significant QTL was obtained directly from the output of the program. A joint analysis of phenotypic data from the two experiments enabled the evaluation of the effects of environmental interaction on QTL identification (Jiang and Zeng, 1995). Multiple regressions were used to evaluate the total percentage of phenotypic variation accounted for by the identified QTLs.

Results

Photosynthetic parameters

The rate of carbon fixation was well correlated with the quantum yield of electron transport at PSII both in leaves developed at 25 °C and 15 °C. (Fig. 2A). Compared with leaves developed at 25 °C leaves developed at 15 °C showed an average reduction of photosynthesis of 66% and a higher variation between lines, suggesting a large genetic variation in the cold-tolerance of the population. There was also a non-linear correlation between CO_2 fixation and the maximum quantum efficiency of PSII primary photochemistry, F_v/F_m in leaves developed at 15 °C but not in leaves developed at 25 °C (Fig. 2B). A decrease in F_v/F_m of 15 °C-grown leaves was clearly associated with a low rate of photosynthesis, but only when lower than 0.4–0.5. Above a threshold of 0.5, the maximum quantum efficiency of PSII primary photochemistry was sufficient to sustain photosynthesis; the variation in CO_2 fixation among lines was due to other factors. Although photosynthesis and stomatal resistance showed some correlation in both leaf types (Fig. 2C), in leaves developed at 15 °C, it was lower than the correlation between CO_2 fixation and ΦPSII (Fig. 2A), indicating that stomatal closure was not the main factor that controlled the rate of photosynthesis. In these leaves, the rate of CO_2 fixation was also inversely correlated with F_o, the fluorescence yield emitted by the leaf in the absence of actinic illumination used to calculate F_v/F_m, whilst F_o in leaves developed at 25 °C showed little variation and was not correlated with the rate of CO_2 fixation (Fig. 2D). The correlation between F_o and F_v/F_m of leaves developed at 15 °C was moderate (r^2=0.37, data not shown) while the correlation between F_m and F_v/F_m was not significant (r^2=0.05, data not shown), indicating that a low F_v/F_m was due to a high F_o rather than a low F_m. According to the value of the two parental lines for the different traits presented in Fig. 2, it appears that all the traits in leaves developed at 15 °C presented a transgressive segregation in this RIL population due to the presence of favourable alleles in the susceptible parental line.

The QTL analysis (Table 1) detected four QTLs for CO_2 fixation measured on the third leaf of the segregating families grown at 15 °C. Those QTLs were located on chromosomes 1 (146 cM), 2 (137 cM), 3 (70 cM), and 9 (62 cM). For three of these QTLs, located on chromosomes 1, 2 and 3, an increase in CO_2 fixation was due to the allelic contribution of the drought-tolerant parent (P1), as demonstrated by the positive value of the mean additivity at those QTLs. By contrast, for the QTL on chromosome 9, an increase in the CO_2 fixation was due to the allelic contribution of the drought-susceptible parent (P2). All four QTLs were also detected for the expression of ΦPSII, confirming at the genetic level the tight phenotypic correlation observed between the two traits in Fig. 2. The two QTLs on chromosomes 1 and 9 were also probably detected for CO_2 fixation and ΦPSII in leaves developed at 25 °C at, respectively, 157 and 73 cM, indicating that these QTLs are not specific to low temperature. By contrast the two QTLs on chromosomes 2 (137–138 cM) and 3 (70 cM), only detected in leaves developed at 15 °C for these traits, were specific to low growth temperature. From the QTL data presented in Table 1, several QTLs detected consistently across the different target traits can be identified in leaves developed at 15 °C. The most obvious is the QTL on chromosome 3 (70 cM) which was detected for all traits investigated between 67 and 80 cM. This QTL explained the highest proportion of the phenotypic variance for almost all the traits and resulted in the highest LOD score compared to the other QTLs. This QTL was perhaps also detected for F_o in leaves developed at 25 °C (at 83 cM). The QTL on chromosome 1 at 146 cM for CO_2

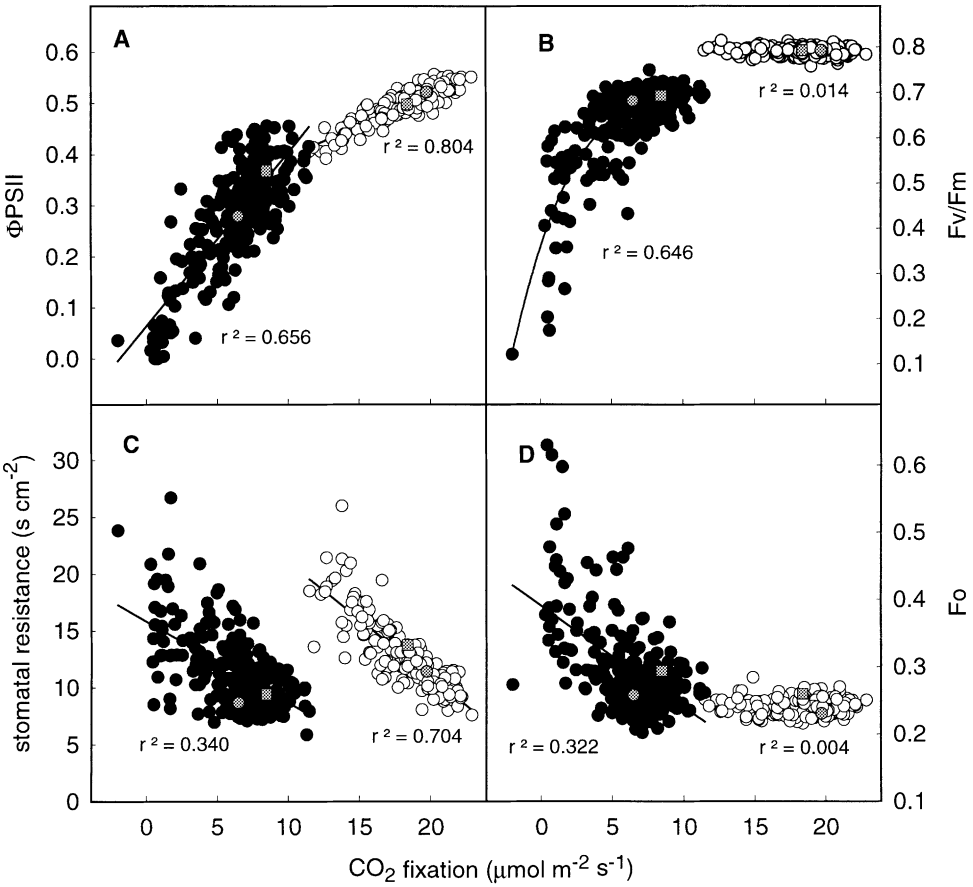

Fig. 2. Relationship between the rate of carbon fixation and the quantum yield of electron transport at PSII (A), the maximum quantum efficiency of PSII primary photochemistry (B), the stomatal resistance (C) and the 'dark' level of chlorophyll fluorescence (D) of the third leaf of maize seedlings grown at 25 °C or 15 °C. White circles: recombinant inbred lines grown at 25 °C (single values); black circles: recombinant inbred lines grown at 15 °C (average of two independent experiments); grey circles: parent P1 (average of six replicates); grey squares: parent P2 (average of six replicates). Lines represent linear regressions except for F_v/F_m of leaves developed at 15 °C, where line represents cubic regression.

fixation and ΦPSII was also probably detected for F_v/F_m (at 158 cM) in leaves developed at 15 °C. Besides the four QTLs involved in CO_2 fixation and ΦPSII, leaves grown at 15 °C showed additional QTLs for individual parameters on chromosomes 3 (289 cM) for ΦPSII, 6 (24 cM) for F_v/F_m, and 4 (202 and 239 cM) for stomatal resistance. Leaves developed at 25 °C showed additional QTLs on chromosomes 1 (106 cM) for ΦPSII, 2 (164 cM) for F_v/F_m and 8 (161 cM) for F_o. None of the QTLs identified for leaves developed at 15 °C presented a significant interaction with the environment (Q×E in Table 1, threshold at 3.84), meaning that their expression was quite stable across the two experiments. This result is important because it demonstrates the reliability of the single measurement per genotype.

The QTLs identified both for CO_2 fixation and ΦPSII of leaves developed at 15 °C on chromosomes 2, 3 and 9 are quite consistent because they were significant for both traits in both experiments. When considering the effects of the QTLs together, 24% and 31% of phenotypic variance are expressed for CO_2 fixation and ΦPSII in the second experiment at low temperature. In leaves developed at 25 °C, 16.1% and 19.3% of the phenotypic variance was explained by the sum of the detected QTLs for CO_2 fixation and ΦPSII, respectively. The QTL on chromosome 3 alone accounted for most of this phenotypic expression in leaves developed at 15 °C, whilst the QTL on chromosome 9 seemed the most important in leaves developed at 25 °C. Considering all the QTLs presented in Table 1, the QTL on chromosome 3 (70 cM) is considered to be a major QTL, and key gene(s) for the tolerance of photosynthesis to low temperature are assumed at this genomic location.

Leaf pigment composition

Figure 3 shows the relationship between CO_2 fixation and pigment composition in the third leaf developed at 15 °C in Exp.1. There was a weak, but significant, positive linear relationship between the rate of carbon fixation and the accumulation of chlorophyll on a leaf area basis (Fig. 3A).

Table 1. *Genetic characteristics of QTLs involved in photosynthetic traits of the third leaf of maize seedlings developed at 15 °C or 25 °C with a LOD score above a threshold of 2.5*

T°, growth temperature; Chr, chromosome number; cM, position of the peak of the QTL in centimorgans; Joint, LOD score in the joint analysis of experiment 1 (Exp. 1) and 2 (Exp. 2) for leaves developed at 15 °C; Q×E, LOD score value for QTL–environment interaction in the joint analysis of Exp. 1 and Exp. 2; V(%), % of phenotypic variance explained by the QTL; A, additivity expressed in the unit of the trait.

Trait	T°	Chr	cM	Nearest marker	LOD score				V (%)		A
					Exp. 1	Exp. 2	Joint	Q×E	Exp. 1	Exp. 2	
CO_2 fixation (μmol m^{-2} s^{-1})	15	1	146	*bnl5.59*	2.12	0.08	2.75	2.60	4.2	0.1	0.13
		2	137	*umc98*	3.35	1.81	3.91	0.70	7.9	5.7	0.68
		3	70	*umc10*	3.54	8.42	9.31	0.10	7.6	18.8	1.24
		9	62	*bnl3.06*	2.06	2.05	3.13	0.12	1.7	1.4	–0.66
									19.6	24.4	
	25	1	157	*umc119*	2.90				1.5		0.75
		9	73	*umc114*	4.96				14.1		–1.08
									16.1		
ΦPSII	15	1	146	*bnl5.59*	2.94	0.71	3.04	0.27	6.0	1.8	0.024
		2	138	*umc98*	2.83	1.57	3.35	0.01	6.5	5.8	0.028
		3	70	*umc10*	3.09	9.97	10.47	3.55	7.9	20.4	0.044
			289	*umc317*	0.06	3.01	3.66	3.29	0.1	3.8	–0.009
		9	62	*bnl3.06*	2.55	1.65	3.23	0.00	2.4	1.4	–0.028
									20.7	31.3	
	25	1	106	*umc185*	3.50				3.4		–0.013
		9	74	*umc114*	7.63				13.8		–0.015
									19.3		
F_v/F_m	15	1	158	*umc119*	2.87	0.09	3.01	1.93	6.2	0.2	0.014
		3	70	*umc10*	3.02	5.33	6.21	0.09	7.2	13.0	0.039
		6	24	*bnl3.06*	2.71	0.11	2.81	1.69	2.6	0.0	–0.015
									16.2	13.2	
	25	2	164	*bnl6.29*	6.93				17.0		0.004
F_o (rel.)	15	3	67	*npi114*	3.34	5.87	7.28	0.58	4.6	13.9	–0.028
	25	3	83	*csu30*	3.69				8.0		0.004
		8	161	*umc30*	3.55				7.4		–0.004
									15.3		
Stomatal resistance (s cm^{-2})	15	3	80	*umc10*	0.65	3.83	4.10	2.47	0.9	7.2	–0.55
		4	202	*umc133*	2.29	0.45	2.54	0.00	2.2	0.0	0.60
			239	*umc326*	0.73	2.84	3.24	1.66	0.4	4.4	–0.48
									4.6	12.4	
	25	9	74	*umc114*	4.42				11.1		–1.08

As mentioned above for stomatal resistance, the weakness of the correlation indicated that the rate of carbon fixation is only marginally influenced by the amount of chlorophyll in the leaf. Similarly, the rate of CO_2 fixation was also correlated linearly with the chlorophyll *a/b* ratio (Fig. 3B) and with the ratio between β-carotene and lutein (Fig. 3C). Furthermore, there was an inverse linear relationship between the rate of carbon fixation and the accumulation of the xanthophyll zeaxanthin on a chlorophyll basis (Fig. 3D).

As for photosynthetic traits, the QTL analysis of leaf pigment composition (Table 2) revealed the presence of a very consistent QTL around 68–80 cM on chromosome 3 because it was detected for 7 of the 11 traits investigated. The sign of the additivity parameter (A in Table 2) indicated that a positive allele at this QTL contributed to an

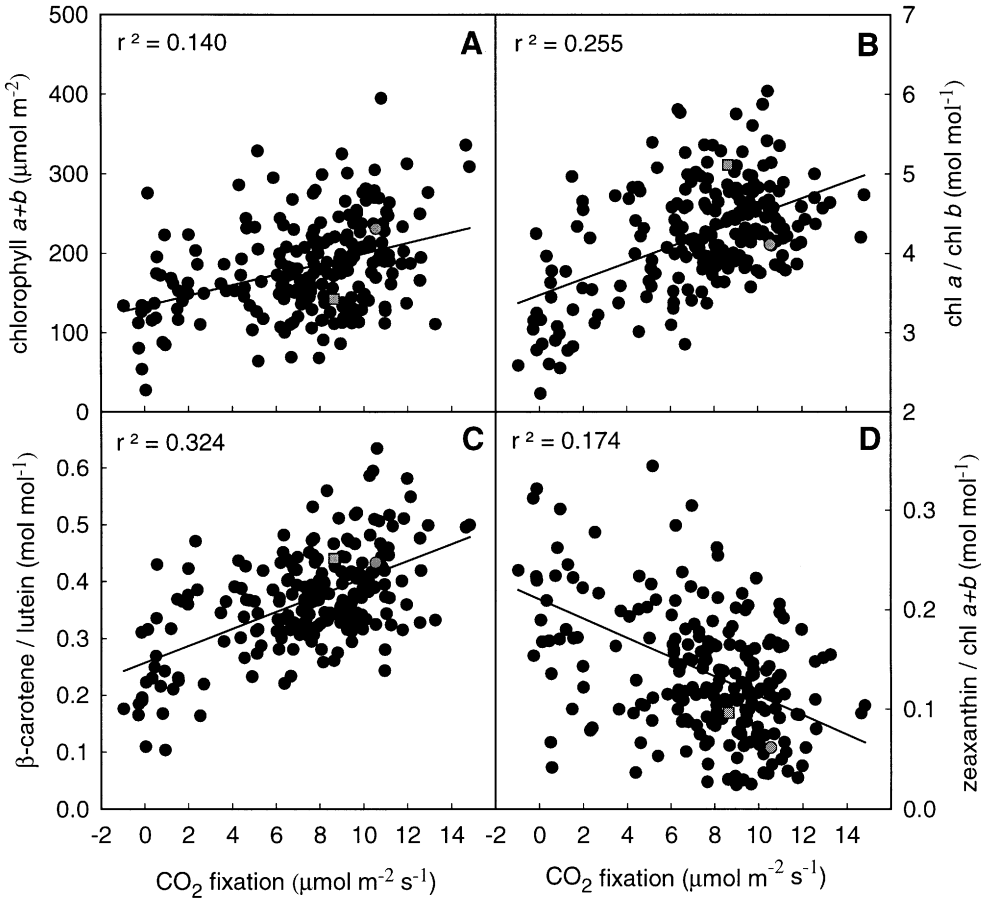

Fig. 3. Relationship between the rate of carbon fixation and the chlorophyll content (A), the ratio between chlorophyll ***a*** and ***b*** (B), the ratio between β-carotene and lutein (C) and the amount of zeaxanthin per chlorophyll (D) of the third leaf of maize seedlings grown at sub-optimal temperature (Exp. 1). Black circles: recombinant inbred lines (single values), grey circles: parent P1 (average of six replicates); grey squares: parent P2 (average of six replicates).

increase in the amount of chlorophyll per leaf area and to maintain a higher ratio of β-carotene/lutein. It also prevented the accumulation of lutein, xanthophyll cycle pigments pool (indicated by V+A+Z/chl *a*+*b*) and zeaxanthin (indicate by Z/chl *a*+*b* and Z/V+A+Z). These observations confirm the presence of a key gene at this genomic location. Besides this locus, the analysis revealed only two additional QTLs on chromosome 7 at 118 cM for the ratio β-carotene/lutein and on chromosome 3 (289 cM) for the accumulation of chlorophylls.

Time-course of ΦPSII

During the second experiment at low temperature, ΦPSII was monitored on different leaves, from leaf 1 *c*. 2.5 h before exposure to 15 °C until 26 d at 15 °C on leaf 4. Based on the results of the first experiment, special attention was paid to the kinetics of the genetic effects at the four loci detected for CO_2 fixation and ΦPSII. In the first leaf, which was fully developed prior to exposure to low temperature, no significant QTL were identified and the LOD score at the four selected loci was low (Table 3).

In the second leaf, which was partially developed before exposure to low temperature, the LOD score for the QTLs on chromosomes 1, 2 and 3 was higher compared to the values obtained on the first leaf, although none of them reached the LOD threshold of 2.5. In leaf 3, which developed entirely at low temperature, the QTL on chromosome 3 became highly significant and remained at a very significant level at all developmental stages of the leaf. By contrast, the QTL on chromosome 1 was most significant at early stages of the leaf development (days 8 and 12) and became less involved in the expression of ΦPSII thereafter. The QTL on chromosome 2 was not very significant in this experiment and seemed to be more important at the late stage of leaf 2 (day 12) and intermediate stages of leaf 3 (days 12 and 19). The QTL on chromosome 9 showed the lowest LOD values, because it was less important in this experiment than in the first one (Table 1). The importance of the QTLs on chromosomes 1, 2 and 9 of leaf 4 tended to decrease in favour of the QTL on chromosome 3, which alone explained more than 25% of the phenotypic variance of the population. Besides these

Table 2. *Genetic characteristics of QTLs involved in the pigment composition of the third leaf of maize seedlings developed at sub-optimal temperature (Exp. 1) and detected above a LOD threshold of 2.5*

Chr, chromosome number; cM, position of the peak of the QTL in centimorgans; V(%), % of phenotypic variance explained by the QTL; A, additivity expressed in the unit of the trait; V+A+Z, xanthophyll cycle pool (violaxanthin+antheraxanthin+zeaxanthin); Z/(V+A+Z), proportion of the xanthophyll cycle pool in the form of zeaxanthin.

Trait	Chr	cM	Nearest marker	LOD score	V(%)	A
Chl *a+b* (μmol m^{-2})	3	68	*npi114*	3.37	4.91	17.49
		289	*umc317*	3.75	5.97	17.54
					9.85	
Chl *a/b* (mol mol^{-1})	3	72	*umc10*	4.47	6.51	0.23
β-carotene/lutein (mol mol^{-1})	3	71	*umc10*	3.96	8.49	0.029
	7	118	*umc80*	2.68	4.16	–0.023
					12.84	
Neoxanthin/chl *a+b* (mol mol^{-1})	–	–	–	–	–	–
Lutein/chl *a+b* (mol mol^{-1})	3	73	*umc10*	3.00	6.09	–0.013
β-carotene/chl *a+b* (mol mol^{-1})	–	–	–	–	–	–
Violaxanthin/chl *a+b* (mol mol^{-1})	–	–	–	–	–	–
Antheraxanthin/chl *a+b* (mol mol^{-1})	–	–	–	–	–	–
Zeaxanthin/chl *a+b* (mol mol^{-1})	3	76	*umc10*	4.06	7.02	–0.026
V+A+Z/chl *a+b* (mol mol^{-1})	3	80	*umc10*	2.97	6.12	–0.026
Z/(V+A+Z) (mol mol^{-1})	3	73	*umc10*	3.05	4.41	–0.046

four loci, no other QTL was detected with a LOD threshold of 2.5 in leaves 1, 2, 3, and 4 at all developmental stages.

Figure 4 shows the impact of the presence of positive or negative alleles at the four target QTLs on the phenotype of the RILs grown at 15 °C. As suggested from the results presented in Table 3, the allelic composition at the QTLs had little influence on the ΦPSII of leaves 1 and 2. By contrast, leaves 3 and 4 of lines carrying favourable alleles at all four loci had a quantum yield of electron transport at PSII up to four times higher than leaves of lines carrying unfavourable alleles. The data presented in Fig. 4 also indicate that the effect of positive alleles on the plant phenotype was weaker than the effect of negative alleles when compared to the mean value of the RIL population. This result may be due to a sampling effect or may indicate genetic interactions between the QTLs, epistasis. The second hypothesis was investigated by comparing ΦPSII in lines with different allelic combinations. The most significant interaction was observed between the QTL on chromosome 3 and the other QTLs (Fig. 5). The average ΦPSII of leaves 3 and 4 from lines carrying a positive allele at the QTL on chromosome 3 (dotted line in Fig. 5A) was substantially higher than the ΦPSII of leaves from lines carrying a negative allele at this locus (dotted line in Fig. 5B). Adding positive or negative alleles at the loci on chromosomes 1, 2 and 9 had only a marginal effect on the ΦPSII of lines with a positive allele at the QTL on chromosome 3 (Fig. 5A). By contrast, the allelic composition at QTLs on chromosomes 1, 2 and 9 had a large influence on the ΦPSII of lines with a negative allele at the locus on chromosome 3, especially when considering the effect of three QTLs together (Fig. 5B). In the latter case, the simultaneous presence of positive alleles at the QTLs on chromosomes 1, 2 and 9 restored ΦPSII to a level close to what observed in lines with a positive allele at the QTL on chromosome 3. From those results it can be concluded that the QTL on chromosome 3 presents epistatic interactions with the other QTLs involved in the expression of the ΦPSII measured in leaves developed at low temperature.

Discussion

The RILs used in this study showed a large variation in cold-tolerance, as judged from their broad variation of CO_2 fixation (Fig. 2). When compared with tolerant lines, the more sensitive lines grown at low temperature were also characterized by an increase in photoinhibition (indicated by F_v/F_m, Fig. 2B), confirming previous results obtained with different cultivars (Aguilera *et al*., 1999) and with lines bred for contrasting cold-tolerance using chlorophyll fluorescence (Fracheboud *et al*., 2000). Cold-tolerance was positively correlated with the content of chlorophyll and with the ratios chlorophyll *a/b* and β-carotene/lutein and inversely correlated with the amount of zeaxanthin on a chlorophyll basis (Fig. 3). This is in good agreement with the results of Haldimann (1998) on maize genotypes of different origin grown at sub-optimal temperature. Light-harvesting complexes are known to contain both chlorophyll *a* and *b*, whilst the reaction centre cores of PSI and PSII contain only chlorophyll *a* (Yamamoto and Bassi, 1996). Therefore, the lower chlorophyll *a/b* ratio of sensitive lines compared with tolerant lines may reflect a lower number of reaction centres per light-harvesting

Table 3. *Genetic characteristics at four loci detected for the quantum yield of electron transport at PSII in maize seedlings grown at sub-optimal temperature (Exp. 2)*

V(%), % of phenotypic variance explained by the QTL.

Leaf number	Days at 15 °C	Chromosome 1: 146 cM		Chromosome 2: 138 cM		Chromosome 3: 70 cM		Chromosome 9: 62 cM	
		LOD	V(%)	LOD	V(%)	LOD	V(%)	LOD	V(%)
1	–0.1	0.03	0.0	0.05	0.0	0.17	0.0	0.93	0.5
	0.1	0.32	0.9	0.02	0.3	0.02	0.3	0.14	1.8
	1	0.13	0.3	0.13	0.9	0.09	0.0	0.00	0.8
	4	0.00	0.0	0.05	0.5	0.00	0.1	0.41	0.0
2	1	0.02	0.0	0.10	0.6	1.74	1.4	0.00	0.6
	4	0.31	1.1	0.52	1.3	0.26	0.1	0.19	0.0
	8	0.93	2.6	0.31	1.4	1.06	0.8	0.22	0.0
	12	1.16	2.4	1.65	4.3	0.39	0.7	0.40	0.2
3	8	3.61	6.0	0.31	2.5	10.15	18.4	0.36	0.1
	12	2.02	4.5	1.37	5.2	9.90	21.5	0.91	0.2
	19	0.69	1.8	1.71	5.8	10.21	20.4	1.04	1.4
	26	0.11	0.5	0.93	3.2	9.13	20.2	0.94	1.6
4	19	1.83	3.6	0.31	1.6	16.98	27.7	0.25	0.8
	26	0.63	1.5	1.04	3.6	13.81	23.3	0.17	0.7

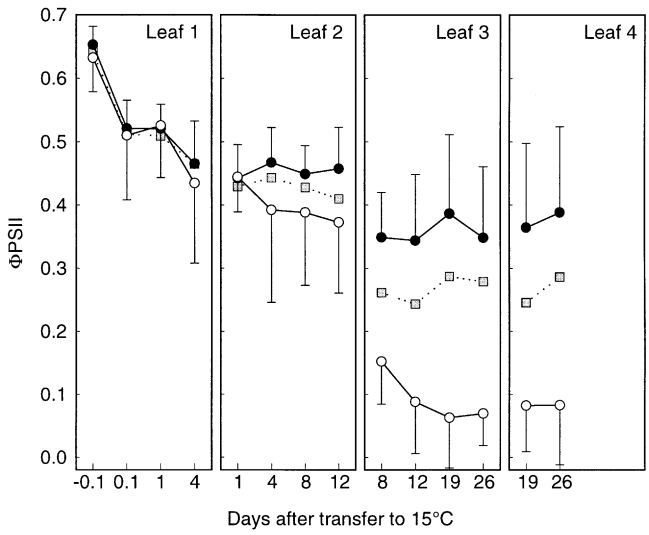

Fig. 4. Quantum yield of electron transport in recombinant inbred lines of maize over time at 15 °C during experiment 2. Grey squares represents the mean values of the whole RIL population (n=218–229) while black and white circles correspond to the phenotypic mean of RILs homozygotes with the favourable (n=14–17) and non-favourable allele, respectively (n=12–17), at the nearest marker (see Table 1 for markers names) to the four QTL peaks detected for ΦPSII on chromosomes 1 (146 cM), 2 (138 cM), 3 (70 cM), and 9 (62 cM); Error bars represent standard deviation of lines with favourable and unfavourable alleles at theses QTLs.

complex. This hypothesis is confirmed by the similar relationship between CO_2 fixation and the ratio between β-carotene and lutein (Fig. 3C), since β-carotene is mainly associated with the reaction centres of PSI and PSII while lutein is associated with light-harvesting complexes (Lee and Thornber, 1995; Yamamoto and Bassi, 1996). The tolerant lines were also characterized by a lower accumulation of the xanthophyll zeaxanthin compared to the sensitive lines (Fig. 3D). Zeaxanthin, known to be involved in the dissipation of excess absorbed energy in various plant species (Demmig-Adams and Adams, 1996), has been shown to be of particular importance for the reduced photosynthetic efficiency of maize at low temperature (Fryer *et al*., 1995). Thus, as pointed out by Haldimann (1998), the higher zeaxanthin content of sensitive lines compared with tolerant lines may reflect a higher requirement for energy dissipation due to a lower photosynthetic rate.

The phenotypic correlations between photosynthetic traits (Fig. 2) and the identification of QTLs from measurements on leaf 3 (Table 1), were generally in good agreement. The best correlation was obtained for CO_2 fixation and ΦPSII (Fig. 2A) which showed essentially the same QTLs (Table 1) for both leaves developed at 15 °C and 25 °C. This provides additional genetic evidence of the tight relationship between the two traits, as observed in previous studies (Edwards and Baker, 1993; Leipner *et al*., 1999).

The QTL on chromosome 3 around 70 cM was detected for most of the parameters in leaves developed at 15 °C and seems to be of major importance because it expresses a large percentage of the phenotypic variance. The phenotype resulting in a differential expression at this QTL might be due to a single or a cluster of genes. Interestingly, this QTL was the only QTL detected for F_o, the intrinsic fluorescence emitted by the leaf under the very weak modulated measuring beam and which, therefore, does not

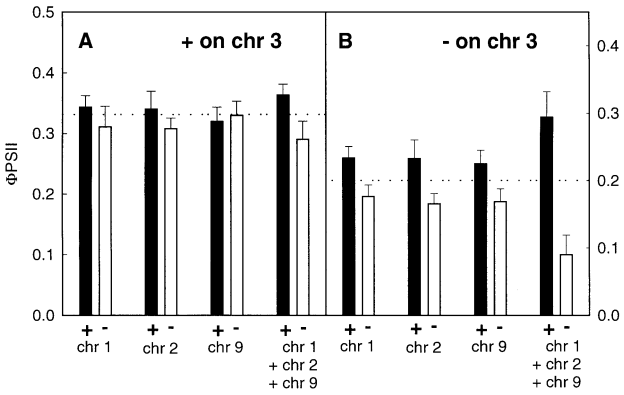

Fig. 5. The effect of allelic composition on ΦPSII in maize leaves developed at low temperature (experiment 2) from RILs homozygote from the parent carrying a favourable (A) or an unfavourable (B) allele at the nearest marker (see Table 1 for markers names) to the QTL on chromosome 3 combined with favourable (back bars) or unfavourable (white bars) alleles at the QTLs on chromosome 1 (chr 1), chromosome 2 (chr 2), chromosome 9 (chr 9) or the combined alleles on chromosomes 1, 2 and 9 (chr 1+chr 2+chr 9). The dotted lines represent the average ΦPSII of RILs with a favourable (A) or an unfavourable (B) allele at the QTL on chromosome 3. The data represent the average ±STD of all measurements of leaves 3 and 4.

involve any photochemical processes, but rather reflects modifications of the structure of the thylakoid membrane. In this respect, the susceptible lines with high F_o have a similar phenotype as a class of photosynthetic maize mutants, described as high chlorophyll fluorescence mutants (see Miles, 1994, for a review). Some of these mutants have been characterized and were shown to lack several polypeptides of PSI and PSII (Heck *et al*., 1999). In addition, the major QTL on chromosome 3 of leaves developed at 15 °C was the main QTL detected for pigment composition (Table 2) and a positive allele at this QTL is sufficient to confer a relatively high level of tolerance, independent of the alleles present at the other significant QTLs (Fig. 5A). Taken together, these observations suggest that the responsible gene(s) at this QTL is involved in the early development of the chloroplast at low temperature. An investigation of the maize genome database (www.agron.missouri.edu) indicated the presence of an interesting candidate gene for this QTL, *tha1* (thylakoid assembly protein 1). The analysis indicates that the peak of the QTL for CO_2 fixation and ΦPSII is located 2.5 cM before the marker *umc10* in the map, which is almost exactly the position of *tha1*, located 2.2, 2.3 and 2.6 cM before *umc10* on the maps umc 96, umc 98 and Pioneer composite 1999, respectively. Interestingly, the *tha1* mutation induces a reduction of the levels of PSII, PSI and cytochrome bf polypeptides (Barkan *et al*., 1995). The *tha1* gene functions in the targeting of certain proteins into the thylakoid (Voelker and Barkan, 1995). This hypothesis is in agreement with the observation that some polypeptides encoded by the chloroplast genome are particularly affected by low growth temperature in maize (Nie and Baker, 1991; Robertson *et al*., 1993). The QTL on chromosome 3 was also probably detected in leaves developed at 25 °C at 83 cM (Table 1). However, it is not relevant for the rate of photosynthesis in these leaves since F_o is not correlated with photosynthesis of leaves developed at 25 °C (Fig. 2) and since the LOD score at this loci for CO_2 fixation is very low (0.47).

The effects of the QTLs detected for CO_2 fixation and ΦPSII on chromosomes 1, 2 and 9 in leaves developed at 15 °C were different in lines with a positive or negative allele at the QTL on chromosome 3 (Fig. 5). In combination, positive alleles at these QTLs can partially restore electron transport in lines with a negative allele at the QTL on chromosome 3 (Fig. 5B) implying that they might restore the development of functional chloroplasts at low temperature. Furthermore, their effect seemed to be cumulative, suggesting that they function on independent pathways. The second QTL for CO_2 fixation and ΦPSII which appeared to be specific to low temperature was located on chromosome 2 at 137–138 (Table 1). A possible candidate gene at this locus is ssu2 (ribulose bisphosphate carboxylase small subunit 2), found *c*. 3–4 cM before the marker *umc98* on the map Pioneer composite 1999.

The two additional QTLs detected on chromosomes 1 and 9 for CO_2 fixation and ΦPSII in leaves developed at 15 °C were also probably detected for these traits in leaves developed at 25 °C, implying that they are important for the rate of photosynthesis independently of growth temperature, although the QTL on chromosome 9 seemed more important in leaves developed at 25 °C than for leaves developed at 15 °C (Table 1). The maize database did not reveal a good candidate gene for these QTLs.

Although the results of stomatal resistance indicate that there was no cold-induced water stress in these experiments, the QTLs on chromosomes 1, 2 and 9 correspond to QTLs associated with flowering and yield parameters that were identified in F_3 families of this material under drought conditions (Ribaut *et al*., 1996, 1997). This may indicate that certain genes are involved in the tolerance to both stresses as suggested by Hughes and Dunn (1996). These may, for example, be involved in the defences against oxidative damage, which were proposed to be important for cold-tolerance (Kingston-Smith *et al*., 1999) and water-stress tolerance (Dellongo *et al*., 1993) in maize. Additional experimentation is necessary to validate the presence of putative common mechanisms of plant defence under cold and drought stresses.

Acknowledgements

We are very grateful to Damien Liberherr for his assistance in constructing the RFLP linkage map. Part of this work was supported by the Swiss National Science Foundation, grant No. 3100-058910.99.

References

Aguilera C, Stirling CM, Long SP. 1999. Genotypic variation within *Zea mays* for susceptibility to and rate of recovery from chill-induced photoinhibition of photosynthesis. *Physiologia Plantarum* **106,** 429–436.

Austin DF, Lee M, Veldboom LR, Hallauer A. 2000. Genetic mapping in maize with hybrid progeny across testers and generations: grain yield and grain moisture. *Crop Science* **40,** 30–39.

Barkan A, Voelker R, Mendelhartvig J, Johnson D, Walker M. 1995. Genetic-analysis of chloroplast biogenesis in higher-plants. *Physiologia Plantarum* **93,** 163–170.

Dellongo OT, Gonzalez CA, Pastori GM, Trippi VS. 1993. Antioxidant defences under hyperoxygenic and hyperosmotic conditions in leaves of 2 lines of maize with differential sensitivity to drought. *Plant and Cell Physiology* **34,** 1023–1028.

Demmig-Adams B, Adams III W. 1996. Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. *Planta* **98,** 460–470.

Edwards GE, Baker NR. 1993. Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynthesis Research* **37,** 89–102.

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

Fracheboud Y, Iannelli MA, Pietrini F, Massacci A. 2000. Photoprotection in maize at suboptimal temperature. In: Parente G, Frame J, eds. *COST action 814*: *crop development for the cool and wet regions of Europe*. Brussels: Office for the Official Publications of the European Communities, 115–120.

Fryer MJ, Andrews JR, Oxborough K, Blowers DA, Baker NR. 1998. Relationship between CO_2 assimilation, photosynthetic electron transport, and active O_2 metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiology* **116,** 571–580.

Fryer MJ, Oxborough K, Martin B, Ort DR, Baker NR. 1995. Factors associated with depression of photosynthetic quantum efficiency in maize at low growth temperature. *Plant Physiology* **108,** 761–767.

Genty B, Briantais JM, Baker NR. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990,** 87–92.

Gilmore AM, Yamamoto HY. 1991. Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C18 high-performance liquid chromatographic column. *Journal of Chromatography* **543,** 137–145.

Haldimann P. 1998. Low growth temperature-induced changes to pigment composition and photosynthesis in *Zea mays* genotypes differing in chilling sensitivity. *Plant, Cell and Environment* **21,** 200–208.

Haldimann P. 1999. How do changes in temperature during growth affect leaf pigment composition and photosynthesis in *Zea mays* genotypes differing in sensitivity to low temperature? *Journal of Experimental Botany* **50,** 543–550.

Haldimann P, Fracheboud Y, Stamp P. 1995. Carotenoid composition in *Zea mays* developed at sub-optimal temperature and different light intensities. *Physiologia Plantarum* **95,** 409–414.

Heck DA, Miles D, Chitnis PR. 1999. Characterization of two photosynthetic mutants of maize. *Plant Physiology* **120,** 1129–1136.

Hoisington D, Khairallah M, González-de-León D. 1994. *Laboratory protocols: CIMMYT applied molecular genetics laboratory*, 2nd edn. Mexico, DF: CIMMYT.

Hughes MA, Dunn MA. 1996. The molecular biology of plant acclimation to low temperature. *Journal of Experimental Botany* **47,** 291–305.

Jiang C, Zeng ZB. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* **140,** 111–127.

Kingston-Smith AH, Harbinson J, Foyer CH. 1999. Acclimation of photosynthesis, H_2O_2 content and antioxidants in maize (*Zea mays*) grown at sub-optimal temperatures. *Plant, Cell and Environment* **22,** 1071–1083.

Kraja AT, Dudley JW. 2000. QTL analysis of two maize inbred line crosses. *Maydica* **45,** 1–12.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1,** 174–181.

Lebreton C, Lazicjancic V, Steed A, Pekic S, Quarrie SA. 1995. Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. *Journal of Experimental Botany* **46,** 854–865.

Lee ALC, Thornber JP. 1995. Analysis of the pigment stoichiometry of pigment–protein complexes from barley (*Hordeum vulgare*): the xanthophyll cycle intermediates occur mainly in the light-harvesting complexes of photosystem I and photosystem II. *Plant Physiology* **107,** 565–574.

Lee M. 1995. DNA markers and plant breeding programs. *Advances in Agronomy* **55,** 265–344.

Leipner J, Fracheboud Y, Stamp P. 1997. Acclimation by suboptimal growth temperature diminishes photooxidative damage in maize leaves. *Plant, Cell and Environment* **20,** 366–372.

Leipner J, Fracheboud Y, Stamp P. 1999. Effect of growing season on the photosynthetic apparatus and leaf antioxidative defences in two maize genotypes of different chilling tolerance. *Environmental and Experimental Botany* **42,** 129–139.

Leipner J, Stamp P, Fracheboud Y. 2000. Artificially increased ascorbate content affects zeaxanthin formation but not thermal energy dissipation or degradation of antioxidants during cold-induced photooxidative stress in maize leaves. *Planta* **210,** 964–969.

Miles D. 1994. The role of high chlorophyll fluorescence photosynthesis mutants in the analysis of chloroplast thylakoid membrane assembly and function. *Maydica* **39,** 35–45.

Moon HG, Brewbaker JL, Lu XW. 1999. Major QTLs for disease resistance and other traits identified in recombinant inbred lines from tropical maize hybrids. *Maydica* **44,** 301–311.

Nie GY, Baker NR. 1991. Modifications to thylakoid composition during development of maize leaves at low growth temperatures. *Plant Physiology* **95,** 184–191.

Ribaut J-M, Hoisington DA, Deutsch JA, Jiang C, González-de-León D. 1996. Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. *Theoretical and Applied Genetics* **92,** 905–914.

Ribaut J-M, Jiang C, González-de-León D, Edmeades GO, Hoisington DA. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection. *Theoretical and Applied Genetics* **94,** 887–896.

Ribaut J-M, Poland D. (eds) 2000. *Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments*. A strategic planning workshop held at CIMMYT, El Batan, Mexico, 21–25 June 1999. Mexico DF: CIMMYT.

Robertson EJ, Baker NR, Leech RM. 1993. Chloroplast thylakoid

protein changes induced by low growth temperature in maize revealed by immunocytology. *Plant, Cell and Environment* **16,** 809–818.

Stirling CM, Nie GY, Aguilera C, Nugawela A, Long SP, Baker NR. 1991. Photosynthetic productivity of an immature maize crop: changes in quantum yield of CO_2 assimilation, conversion efficiency and thylakoid proteins. *Plant, Cell and Environment* **14,** 947–954.

Voelker R, Barkan A. 1995. Two nuclear mutations disrupt distinct pathways for targeting proteins to the chloroplast thylakoid. *EMBO Journal* **14,** 3905–3914.

Welz HG, Geiger HH. 2000. Genes for resistance to northern corn leaf blight in diverse maize populations. *Plant Breeding* **119,** 1–14.

Yamamoto HY, Bassi R. 1996. Carotenoids: localization and function. In: Ort DR, Yocum CF, eds. *Advances in photosynthesis,* Vol. 4. *Oxygenic photosynthesis: the light reactions*. Dordrecht, Boston: Kluwer Academic Publishers, 539–563.

Zeng ZB. 1994. Precision mapping of quantitative trait loci. *Genetics* **136,** 1457–1468.