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ORIGINAL PAPER

Quantitative trait loci for early plant vigour of maize grown in chilly environments

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Abstract Maize (*Zea mays* L.) is particularly sensitive to chilling in the early growth stages. The objective of this study was to determine quantitative trait loci (QTL) for early plant vigour of maize grown under cool and moderately warm conditions in Central Europe. A population of 720 doubled haploid (DH) lines was derived from a cross between two dent inbred lines contrasting in early vigour and were genotyped with 188 SSR markers. The DH lines per se and their testcrosses with a flint line were evaluated in field experiments across 11 environments in 2001 and 2002. Plants were harvested after six to eight leaves had been fully developed to assess fresh matter yield as a criterion of early vigour. Seven QTL were detected for line performance and ten QTL for testcross performance, explaining 64 and 49% of the genetic variance. Six out of seven QTL detected in the lines per se were also significant in their testcrosses. Significant QTL × environment interaction was observed, but no relationship existed between the size of the QTL effects and the mean temperature in the individual environment. The correlation between fresh matter yield and days to silking was non-significant, indicating that differences in early plant vigour were not simply caused by maturity differences. For three additional chilling-related traits, leaf chlorosis, leaf purpling, and frost damage seven, six, and five QTL were detected, respectively. Three QTL for leaf chlorosis, two for leaf purpling, and two for frost damage co-localized with QTL for fresh matter yield. Results are considered as a reliable basis for further genetic, molecular, and physiological investigations.

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Introduction

Maize (*Zea mays* L.), being a C4 plant of tropical origin, is sensitive to chilling. The optimal temperature for maize growth extends from 30 to 35°C (Miedema 1982). As temperature drops below this range, plant growth steadily declines and stops at 6–8°C. Prolonged exposure to low temperatures leads to irreversible cellular and tissue injury (Greaves 1996). In the context of this study, the term chilling refers to temperatures at which the maize plant can survive but is greatly impaired in growth, i.e., to a range of about 6–15°C. Chilling in the grain-filling stage causes yield losses and decreases the energy content of silage maize (Frei 2000). However, chilling is particularly deleterious in the early growth stages of maize where it affects various developmental and physiological processes of development (Greaves 1996; Marocco et al. 2005). Therefore, early plant vigour is regarded as an important trait for maize grown in the cool environments of Central and Northern Europe.

Chilling particularly affects photosynthesis manifesting itself in a chlorotic phenotype (Baker and Nie 1994; Foyer et al. 2002; Fryer et al. 1998; Leipner et al. 1999; Marocco et al. 2005). This is mainly caused by an overexcitation of the phytochromes and a concomitant production of oxygen radicals. Experimental results also indicate that low temperatures have a negative effect on the development of the photosynthetic apparatus (Nie and Baker 1991). Chilling-induced symptoms may also include a purple leaf coloration caused by anthocyanin (Cobbina and Miller 1987).

Maize varieties with improved early plant vigour lead to a better ground cover and thus help to minimize erosion and nitrate leaching at the beginning of the maize growing season. Classical plant breeding has resulted in a slow but steady improvement of this trait in Central Europe (Frei 2000). Faster progress should be possible with marker-assisted selection based either on mapped quantitative trait loci (QTL) or candidate genes. In maize, QTL were identified for tolerance to abiotic stress, including nitrogen deficiency (Agrama et al. 1999; Bertin and Gallais 2001) and drought (Ribaut et al. 1996). First QTL studies on chilling tolerance in maize, mainly with respect to photosynthesis, were recently published by Fracheboud et al. (2002, 2004), Hund et al. (2005) and Jompuk et al. (2005).

The objective of the present study was to determine QTL for early plant vigour and chilling related traits in a Central European elite maize mapping population. In order to obtain accurate and precise estimates of QTL statistics, a large (n = 720) population of doubled haploid (DH) lines was produced and evaluated in multi-environment field experiments in 2001 and 2002.

Materials and methods

Genetic materials and genotyping

To generate a mapping population, two European dent inbred lines (SL, TH) were used as parents. Under chilling stress, line SL shows only poor early vigour, while line TH displays a high level of chilling tolerance. The two inbred lines are proprietary to KWS SAAT AG, Einbeck, Germany and were selected from current Central European breeding material of early to medium maturity. In vivo haploid induction (Deimling et al. 1997; Röber et al. 2005) was applied to the F_1 of the cross SL × TH for DH line development. Altogether 250 induction crosses between F_1 plants and inducer line RWS (proprietary to University of Hohenheim) were made in 1998. During the winter season 1998/1999 approx. 40,000 kernels were screened for the presence of phenotypic identification markers (based on the colour mutant *R1-nj*) and 5,000 putative haploid plants were treated with colchicin for doubling the chromosome number and subsequently transplanted into the field in 1999. After a second selection process using a stem colour marker, DH plants were self-pollinated to produce DH lines. Approximately 800 DH lines were checked for homogeneity and multiplied by selfing in an off-season nursery in Chile 1999/2000. A total of 720 DH lines were crossed to a flint tester line during the summer season 2000. The tester line had been selected in preparatory field experiments (data not shown) for its ability to discriminate between the parent lines with regard to early vigour.

Total genomic DNA was extracted from bulks of ten plants per DH line using the DNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Publicly available simple sequence repeat markers (SSR, http://www.maizegdb.org) were screened for allelic differences between the parent lines SL and TH. Subsequently, all DH lines were genotyped with 188 polymorphic SSR markers evenly distributed across the chromosomes. PCR products were separated on a capillary electrophoresis system ABI3700 (Applied Biosystems/Applera, Darmstadt, Germany). All steps of the marker analysis were conducted by PLANTA GmbH (37555 Einbeck, Germany) according to standard protocols. The genetic map was established by the software package JoinMap® 3.0 (Van Ooijen and Voorrips 2001) using Haldane's mapping function.

Field experiments

The 720 DH lines were evaluated for their line per se and testcross performance in field experiments across a wide range of locations in 2001 and 2002 (Table 1). The lines were randomly subdivided into eight sets, each consisting of 90 DH lines and 10 check varieties. One set was laid out as a 10 × 10 simple lattice (two replicates), in order to obtain an estimate of the experimental error. The remaining seven sets were planted with only one replicate. In each environment, genotypes were randomized within sets and sets were randomly allotted to the experimental field.

Application of herbicides followed the standard local agricultural practice. The amount of fertilizer N varied between 140 and 180 kg ha^{-1}. Plots consisted of one row with a length of 4 m, an inter-row distance of 0.75 m, and a plant density of nine plants m^{-2}.

Environments

Test sites represent typical maize growing regions of North Germany (Einbeck, Löningen), South Germany (Lindenhof, Hohenheim, Walldorf, Eckartsweier), North-West France (Arras) and Central France (Tournoisis) (Table 1). The main criterion for choosing theses locations was to cover a wide range of temperature conditions during the

Table 1 Geographic and ecological characteristics and mean fresh matter yields of the test environments

Characteristic	Year	Arras	Eckartsweier	Einbeck	Hohenheim	Löningen	Lindenhof	Tournoisis	Walldorf
Abbreviation		ARA	EWE	EIN	HOH	LOE	OLI	TOS	WAL
Altitude (m)		89	140	130	400	36	700	120	110
Soil texture		Clay loam	Silty loam	Loam	Silty loam	Loamy sand	Loam	Loamy clay	Sandy loam
Date (day, month) of									
Sowing	2001	09.05	04.05	23.04	–	–	11.05	21.04	06.05
Harvest	2001	10.07	26.06	17.07	–	–	23.07	09.07	19.06
Sowing	2002	–	–	10.05	14.05	25.04	16.05	–	29.04
Harvest	2002	–	–	15.07	26.06	09.07	09.07	–	19.06
Rainfall (mm) April–July									
	2001	226	250	170	–	–	396	304	203
	2002	–	–	341	388	n.a.	489	–	331
Mean temperature (°C) 44 das									
	2001	16.0	16.8	13.4	–	–	13.1	16.5	17.9
	2002	–	–	16.6	17.7	14.4	15.2	–	17.2
Mean fresh matter yield (g plant^{-1})									
Lines	2001	142.1	72.6	77.8	–	–	124.9	201.6	50.7
	2002	–	–	120.0	58.2	149.8	84.0	–	63.7
Testcrosses	2001	328.9	173.9	–	–	–	240.5	91.5	146.0
	2002	–	–	240.5	131.5	297.2	222.9	–	146.9

– No experiment conducted

n.a. data not assessed

early growing season. Oberer Lindenhof was selected as the coolest and Walldorf as the warmest site based on long-term averages. Theses climatic differences were largely reflected in the mean temperatures during the first 44 days after sowing. Soil texture of experimental fields varied from a loamy sand at Löningen to a loamy clay at Tournoisis. Rainfall during the early growing period was sufficient at all locations, except at Tournoisis 2001 where additional irrigation had to be applied. Sowing dates ranged from April 21 at Tournoisis 2001 to May 16 at Lindenhof 2002 due to different weather conditions at the locations. Air temperature was measured hourly 100 cm above soil surface level at each location with a temperature data logger (Escort Junior 121, Escort Data Logging Systems LTD, Auckland, New Zealand). A survey of the experimental conditions of the individual test environments is given in Table 1.

Trait assessment

Plant fresh matter yield at the beginning of shoot elongation (six to eight leaves fully developed) was taken as early vigour criterion. Genetic materials were grown at six locations in 2001 and at five locations in 2002 for line per se performance (Table 1). Testcross trials were conducted at five locations. All plants of a plot were counted, harvested, and fresh weight was determined. Fresh matter yield was calculated in g per plant. In two environments, (Eckartsweier 2001, Lindenhof 2001) five plants out of each plot of the set with two replicates were oven-dried (at 110°C) to a constant weight to determine dry matter content. At Eckartsweier 2001, Lindenhof 2001, and Einbeck 2002, plants showed different degrees of leaf chlorosis. Further, at Arras 2001, Einbeck 2001, and Lindenhof 2001, purple leaf coloration was observed. Both traits were scored on a 1 (no symptoms) to 5 (severe stress symptoms) scale. Frost-induced leaf damage (frost damage) occurred at Lindenhof 2001. The proportion of necrotic leaf area was scored on a 1 (no damage) to 10 (100% of visible leaf area damaged) scale. Days to silking (days from sowing time to the date when 50% of the plants showed silk emergence) were recorded at 12 locations in additional testcross trials.

Statistical analysis

Lattice analyses of variance were performed for the replicated set of entries (Cochran and Cox 1957). The unreplicated sets were analysed using the method of moving averages implemented in the software package PLABSTAT (Utz 1993). For each plot a moving average was calculated including four adjacent plots on each side, one plot above and one below. Plot values were adjusted according to the following equation:

$$y_i' = y_i - b(x_i - \bar{x}),$$

where y_i' is the adjusted plot value and y_i the unadjusted plot value, x_i the corresponding moving average and $\bar{x}$ the mean of all moving averages, b refers to the coefficient of regression of the plot values on the moving averages.

At each step, outlying values were checked for plausibility and treated as missing values if gross errors were detected. Generally, genotypes with very low field emergence were dropped from the analysis.

Adjusted entry means were used in the combined analyses across environments and for the QTL analyses. Heritability coefficients were estimated with their 95% confidence intervals (Knapp and Bridges 1987) on an entry mean basis as the ratio between the estimated genetic ($\hat{\sigma}_g^2$) and phenotypic variance ($\hat{\sigma}_p^2$). In addition, heritability estimates on a plot basis were computed (Wricke and Weber 1986). The latter allows comparing traits that were recorded in a different numbers of environments. Coefficients of genetic correlation and their standard errors (SE) were calculated from the analysis of variance and covariance according to Mode and Robinson (1959). All statistical computations were performed with the PLABSTAT software (Utz 1993).

QTL analyses

QTL analyses were calculated using the software package PLABQTL (Utz and Melchinger 1996). Due to missing values, only 691 DH lines were included in the QTL analysis of lines per se and 688 DH lines in the analysis of testcrosses. All markers were utilized in the analysis; however, markers with a distance smaller than 0.11 cM were combined by the software to a 'synthetic' marker. Means across environments of DH lines per se and testcrosses were analysed by composite interval mapping to determine putative QTL. PLABQTL follows the regression approach as proposed by Haley and Knott (1992) but is extended by using cofactors. Cofactor selection was done automatically by the program, using for regression an F-value threshold of 10 to enter or to drop a cofactor from the regression model. Finally, cofactor selection was manually checked to ensure that markers for important QTL were included in the model. A LOD-threshold of 3.0 was chosen to declare the presence of a QTL. This LOD-threshold was empirically confirmed by permutation analysis (Churchill and Doerge 1994) and it corresponds to a genomewise error rate of $\alpha < 0.05$ for all traits. In a final fit, the detected QTL were used for a simultaneous multiple regression. Estimates of the percentage of $\hat{\sigma}^2$ explained by individual QTL were obtained by squaring the partial correlation coefficient between the particular QTL and the phenotypic observations. For each QTL, a one-LOD score support interval was used (Lander and Botstein 1989). QTL for different traits were declared as co-segregating QTL when their one-LOD support interval overlapped. To analyse the extent of QTL × environment interactions for fresh matter yield, those QTL estimated from the means across all environments were subsequently used to perform a simultaneous fit for each single environment. To obtain the explained part of $\hat{\sigma}^2$, the proportion of $\hat{\sigma}^2$ explained by all QTL divided by the heritability.

Generally epistasis was neglected in the QTL analyses. When additive × additive epistatic effects were included in the model, the simple additive model generally showed smaller BIC (Bayesian information criterion) values and was, therefore, chosen for the final analysis.

A fivefold cross-validation approach (Utz et al. 2000) was used to check for an overestimation of the proportion of $\hat{\sigma}_g^2$ explained by QTL. Samples comprising 80% of the entries were used in calibration runs and the remaining 20% in validation runs. Within a single cross-validation run this was done five times, such that each fifth of the dataset is successively used for validation. The cross-validations were repeated 200 times, resulting in 1,000 runs.

Results

Environmental means and genetic variability

The test environments covered a wide range of growing conditions (Table 1). The period from sowing to fresh matter yield harvest varied between 44 (Hohenheim 2002) and 86 days (Einbeck 2002). The mean temperature during the first 44 days after sowing (das) was calculated for comparing the chilling stress level in the different environments. Lowest mean temperatures were measured at Lindenhof 2001 on the Swabian Alb and highest at Walldorf in the upper Rhine Valley (Table 1). The low temperature at Lindenhof 2001 caused severe chilling stress symptoms. At both locations, Waldorf and Lindenhof, diurnal temperature variation amounted to differences between day and night temperatures of more than 20°C. Freezing temperatures occurred only in one night at Lindenhof 2001. It lasted 4 h in which temperatures dropped to a minimum of –1.2°C. This short frost period was sufficient to cause severe leaf necrosis.

It was attempted to assess fresh matter yield at the beginning of shoot elongation (six to eight leaves fully developed). However, due to unfavourable weather this was not possible in all environments. Therefore, environmental means for fresh matter yield differed greatly between environments (e.g. 95 g plant^{-1} at Hohenheim and 236 g plant^{-1} at Arras; Table 1).

Differences between the parent lines (TH and SL) of the mapping population were highly significant for fresh matter yield of both line per se and testcross performance (Fig. 1 and Table 2). Line TH had a 27% higher fresh matter yield at the line per se level, and was 3.2% higher at the testcross level. The enhanced early vigour of TH was observed in each environment (data not shown). Readings for leaf chlorosis, leaf purpling, and frost damage did not differ significantly between TH and SL.

Fresh matter yield of testcrosses was twice as high as that of lines (Table 2). Water content of fresh matter was similar in lines and testcrosses (88.3 and 89.7%, resp.). Genetic variability for water content was highly significant in the lines but non-significant in the testcrosses. Fresh and dry matter yield were tightly correlated ($r > 0.9$) in the line per se experiments.

On average, 37% of the leaf area was damaged by frost at Lindenhof 2001. Damage ranged from 1 to 89% among genotypes.

Significant genetic variation occurred between the DH lines per se as well as between their testcrosses for fresh matter yield, leaf chlorosis, leaf purpling, frost damage, and days to silking (Fig. 1 and Tables 2, 3, and data not shown, respectively). Genetic variability for fresh matter yield was about two times greater among the lines per se compared with their testcrosses, resulting in increased heritability estimates for the lines. Heritabilities in DH line per se tests were medium to high for leaf chlorosis and leaf purpling and high for fresh matter yield. For the testcrosses, high heritability estimates were observed for days to silking, while estimates for fresh matter yield were medium. Heritabilities based on a single plot basis ranged between 0.3 and 0.4 for all line per se traits. For testcross performance, a low single-plot basis heritability was estimated for fresh matter yield and a medium value for days to silking.

At the line per se level, coefficients of genetic correlation (r_g) between fresh matter yield and chilling stress associated traits were $r_g = -0.28$ (±0.07) for leaf chlorosis symptoms, and non-significant for leaf purpling and frost damage. Fresh matter yields of lines and testcrosses were only moderately correlated ($r_g = 0.34$, ±0.05) when analysed across ten environments. However, the relation was much closer ($r_g = 0.59$, ±0.06) when estimated from the three environments with the lowest mean temperature during the early growing phase only (Lindenhof 2001, 2002; Löningen 2002). No correlation existed between fresh matter yield and days to silking at the testcross level.

Quantitative trait loci

Mapping of the 188 SSR markers resulted in a map length of 1,452 cM. The average marker interval spanned 8.7 cM and individual interval estimates varied from 0.0 to 73.8 cM. The proportion of the SL genome ranged from 15 to 82% among the DH lines, averaging to 49.7%.

Between 5 and 22 QTL with a LOD > 3 were detected for the individual traits (Tables 2, 4, 5, 6). They are distributed across all ten chromosome pairs. For fresh matter yield, seven QTL, located on six chromosomes, were found in the lines per se and ten QTL on seven chromosomes in the testcrosses (Tables 2, 4). The detected QTL for fresh matter yield explained 52% of the estimated phenotypic variance ($\hat{\sigma}^2_p$) in the lines per se and 32% in the testcrosses. This corresponds to an explained part of the estimated genotypic variance ($\hat{\sigma}^2_g$) of 64 and 49%, respectively (Table 2). The cross-validation analysis resulted in a 3.1% reduction in the explained part of $\hat{\sigma}^2_g$ for line per se performance and in 10.8% for testcross performance.

Individual QTL explained between 2.2 and 33.7% of $\hat{\sigma}^2_p$ for fresh matter of lines and between 2.1 and 7.5% of

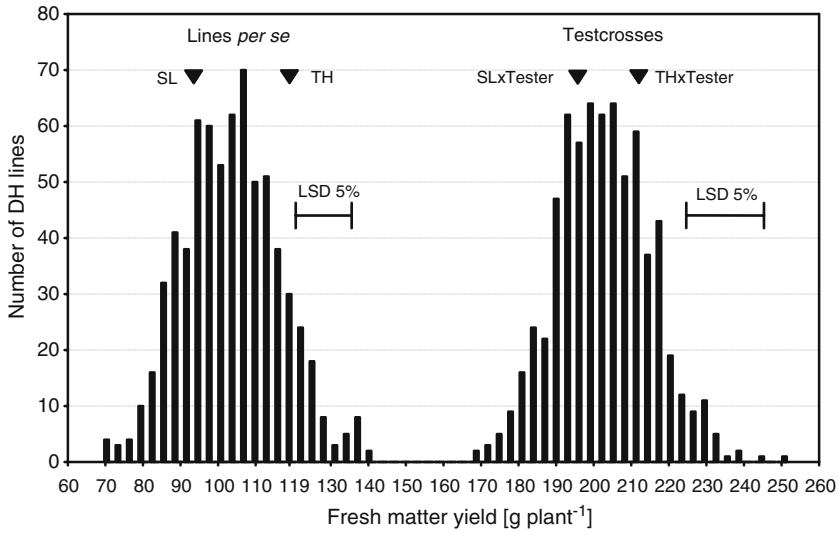

Fig. 1 Frequency distribution for fresh matter yield of 691 DH lines per se (left side) and 688 testcrosses (right side); entry means across 11, respectively, ten environments; *SL* parent line with low early vigour, *TH* parent line with high early vigour. LSD 5% = least significant difference at $P = 0.05$

Table 2 Means, coefficients of genotypic variation (CV_g), heritabilities, number of QTL, explained percentage of phenotypic ($\hat{\sigma}^2_p$) and genotypic ($\hat{\sigma}^2_g$) variance explained by all detected QTL, for fresh matter yield (FM), symptoms of leaf chlorosis (LC), leaf purpling (LP), and frost damage (FD) of lines per se and for fresh matter yield and days to silking (DS) of testcrosses; in addition, explained $\hat{\sigma}^2_g$ calculated from the calibration and validation sets of the cross-validation analysis

Material and parameter, resp.	Lines per se				Testcrosses	
	FM (g plant^{-1})	LC (scoring)	LP (scoring)	FD (scoring)	FM (g plant^{-1})	DS (days)
Number of environments	11	3	3	1	10	12
Parent line means						
SL (sensitive)	93.8	3.68	1.42	3.52	195.6	79.1
TH (tolerant)	119.0	1.20	1.76	3.17	212.2	79.0
Significance of difference	**	n.s.	n.s.	n.s.	**	n.s.
Mapping population						
Mean	104.1	2.32	1.94	3.69	202.0	79.1
CVg (%)	11.23	25.54	31.41	30.41	5.08	1.41
Heritability (all environments)	0.82	0.52	0.65	–	0.66	0.92
Heritability (1 envir., 1 replication)	0.29	0.28	0.38	0.41	0.16	0.49
Number of QTL	7	7	6	5	10	22
$\hat{\sigma}^2_p$ explained (%)	52.3	39.0	56.5	32.5	32.2	64.3
$\hat{\sigma}^2_g$ explained (%)	63.6	74.9	86.4	79.3	49.2	69.8
Cross-validation						
$\hat{\sigma}^2_g$ explained in calibration (%)	61.6	80.4	85.0	77.7	43.9	63.5
$\hat{\sigma}^2_g$ explained in validation (%)	56.8	71.6	80.3	70.3	32.8	55.4

n.s. not significant, – calculation not possible

*, ** Difference between SL and TH significant according to *F* test at the 0.05 and 0.01 probability level, respectively

Table 3 Estimated components of variance (Var. comp.) and coefficients of variation (CV%) for fresh matter yield of DH lines per se and testcrosses across 11 and 10 environments, respectively

Source of variation	Lines per se		Testcrosses	
	Var. comp.	CV%	Var. comp.	CV%
Genotypes	136.78	11.23**	105.14	5.08**
QTL	84.17	8.81**	47.28	3.40**
Residuals	52.61	6.97**	57.86	3.77**
QTL × environment	24.29	4.73**	23.95	2.42**
Error[a]	126.32	10.69	309.85	8.84

** *F* test significant at the 0.01 probability level

[a] Estimated from the replicated set of 90 DH lines and ten check varieties

testcrosses (Table 4). The three largest QTL (each with >13% explained variance) were detected in the line per se experiments on chromosomes 3, 4, and 5. The additive effect of the most important QTL for line performance (chromosome 4, position 36 cM) was 6.3% of the trial mean. For testcross performance the largest QTL was located on chromosome 2 with an additive effect of 1.8%. For five out of seven QTL detected in the line experiments and for six out of ten QTL in the testcrosses, the positive allele originated from the TH parent. For the three most significant QTL, only the parent line with better early vigour (TH) provided favourable alleles. According to the one-LOD support intervals of the QTL, six out of seven QTL detected on the line per se level were also significant in the testcrosses.

Significant QTL × environment interaction occurred for fresh matter yield in both lines per se and testcrosses (Table 3). No obvious relationship existed between the relative size of any QTL effect and the mean temperature in the individual environments. The most important QTL in the line per se showed the highest effect at Hohenheim and the lowest at Arras 2001. Low effects across all environ-

Table 4 Chromosome number, closest marker, position of the LOD peak with 1 LOD support interval, percentage of explained phenotypic variance ($\hat{\sigma}_p^2$ expl.), additive effect, and repeatability (repeat.) of QTL position in cross-validation for fresh matter yield of lines per se and testcrosses

Chromosome no.	Closest marker	Position (cM)	Support interval (cM)	LOD	$\hat{\sigma}_p^2$ expl. (%)	Add. effect[a] (g plant^{-1})	Repeat. (%)
Lines per se							
2	umc2005	120	102–128	3.0	2.2	–1.49	32
3	bnlg1047a	112	108–118	28.6	17.8	4.62	95
4	umc1117	36	34–38	61.2	33.7	6.53	98
4	bnlg292b	108	96 –122	4.8	3.3	1.89	83
5	bnlg609	74	70–78	21.8	13.9	3.73	100
6	phi075	18	14–24	7.8	5.3	2.22	95
10	umc1507	64	56–72	3.7	2.6	–1.58	32
Testcrosses							
2	dupssr21	82	78–84	10.3	6.6	3.71	100
2	umc1497	110	98–126	3.7	2.1	–2.30	42
3	umc1489	132	118–146	5.1	3.0	2.05	74
4	umc1117	38	36–50	5.2	3.2	1.97	53
4	umc1101	112	100–122	6.1	3.6	2.28	60
5	bnlg1287	32	30–34	7.3	4.6	–2.62	58
5	bnlg609	72	68–78	3.8	2.6	1.90	65
6	bnlg2243	12	6–14	11.7	7.4	3.03	45
7	bnlg2271	72	70–102	4.2	2.7	–1.77	38
8	bnlg1834	36	28–38	14.1	7.5	–3.12	86

[a] A positive value means that the allele from the chilling tolerant parent (TH) increased fresh matter yield

Table 5 Chromosome number, closest marker, position of the LOD peak with 1 LOD support interval, percentage of explained phenotypic variance ($\hat{\sigma}_p^2$ expl.), and additive effect for leaf chlorosis, leaf purpling, and frost damage for QTL of lines per se

Chromosome no.	Closest marker	Position	Support interval	LOD	$\hat{\sigma}_p^2$ expl.	Add. effect[a]
Leaf chlorosis						
1	bnlg2086	114	108–118	10.1	5.0	0.15
2	bnlg1017	28	2 –42	3.2	2.5	0.11
3	umc1489	132	114–138	14.9	8.4	–0.21
4	umc1117	36	34–38	5.3	2.8	0.11
5	bnlg1700	24	20–30	26.7	15.4	0.30
5	umc1941	70	68–74	8.2	5.5	0.16
9	bnlg1401	62	50–74	8.2	4.9	0.17
Leaf purpling						
3	bnlg1257	142	134–150	12.7	7.5	0.15
5	bnlg1046	10	4–20	13.5	7.2	0.15
7	bnlg2203	36	34–38	13.7	8.1	–0.15
9	bnlg1583	18	16–22	112.3	52.4	–0.54
9	bnlg1191	84	80–88	4.2	3.2	0.09
10	phi035	74	66–78	14.3	4.4	0.11
Frost damage						
2	umc1635	84	82–86	13.1	13.0	–0.56
3	bnlg1456	76	72–78	17.1	11.9	–0.53
5	bnlg1700	28	20–34	14.9	9.4	0.48
6	umc1413	70	56–78	7.9	4.9	0.34
7	bnlg1792	38	36–46	5.0	3.4	0.28

[a] A positive value means that the allele from the chilling tolerant parent (TH) decreased symptoms of leaf chlorosis, leaf purpling, and frost damage

Table 6 Chromosome number, closest marker, position of the LOD peak with 1 LOD support interval, percentage of explained phenotypic variance ($\hat{\sigma}^2_p$ expl.), and additive effect of QTL for days to silking of testcrosses

Chromosome no.	Closest marker	Position	Support interval	LOD	$\hat{\sigma}^2_p$ expl.	Add. effect[a]
1	umc1177	16	0–38	5.4	3.7	0.17
1	bnlg1643	176	154–186	7.1	4.8	0.17
2	bnlg2277	42	36–50	8.8	5.6	0.18
2	umc1497	104	98–114	11.0	6.3	–0.24
2	bnlg1520	142	130–144	3.5	1.4	–0.10
3	umc1746	16	0–36	4.2	3.7	–0.19
3	dupssr5	70	66–76	35.9	23.2	0.42
4	umc1550	28	20–34	6.0	4.1	–0.17
4	bnlg1189	66	62–74	3.2	2.6	–0.15
4	bnlg292b	104	90–118	4.6	3.1	–0.16
5	bnlg1700	28	22–34	3.5	2.3	–0.12
5	bnlg609	72	70–76	5.0	2.9	0.17
5	bnlg1118	96	88–96	5.4	4.3	0.20
6	nc013	102	84–118	6.5	4.5	0.20
7	bnlg2132	0	0–6	4.7	3.7	–0.15
7	bnlg1792	38	36–42	24.4	15.2	0.35
7	phi116	154	144–154	3.5	2.2	0.11
8	bnlg1131	132	124–134	9.3	5.3	0.17
9	bnlg1583	18	12–28	9.8	7.0	0.20
10	umc1152	20	4–38	3.2	2.1	–0.13
10	umc1589	54	52–56	7.5	6.5	–0.28
10	phi035	78	56–88	3.2	1.4	–0.12

[a] A positive value means that the allele from the chilling tolerant parent (TH) caused earlier flowering

ments were observed at the two QTL for line per se performance at which the positive allele originated from the SL parent.

For the three line per se traits leaf chlorosis, leaf purpling, and frost damage, seven, six, and four QTL were detected, respectively (Tables 2, 5). The simultaneous fit across all QTL explained 37.7% of $\hat{\sigma}^2_p$ for leaf chlorosis, 55.7% for leaf purpling, and 31.5% for frost damage. Three QTL for leaf chlorosis, and two for leaf purpling and frost damage, respectively, co-localized with QTL for fresh matter yield. The most important QTL for leaf chlorosis (chromosome 5, position 24 cM) explained 15.4% of $\hat{\sigma}^2_p$. For leaf purpling, a QTL on chromosome 9 (position 18 cM) explained 52.4% of $\hat{\sigma}^2_p$, corresponding to 80% of $\hat{\sigma}^2_g$. Two important QTL which explained 13.0% (chromosome 2, position 84 cM) and 11.9% (chromosome 3, position 76 cM) of $\hat{\sigma}^2_p$ were detected for frost damage symptoms. For these most significant QTL, the positive allele originated from the TH line for leaf chlorosis, but from the SL line for leaf purpling and frost damage.

The highest number of 22 significant QTL was detected for days to silking in the testcrosses (Tables 2, 6). A simultaneous fit including all QTL explained 61.9% of $\hat{\sigma}^2_p$ and 67.2% of $\hat{\sigma}^2_g$. The most important QTL was located on chromosome 3 (position 70 cM) and explained 23.2% of $\hat{\sigma}^2_p$. Six out of the 22 QTL co-localized with QTL for fresh matter yield.

Discussion

Environmental conditions

In the present experiments, plants had to cope with several periods of low temperatures during early growth. Even under conditions of high atmospheric pressure and sunshine during the day, low temperatures during the night and early morning hours should have stressed the plants. Chilly morning temperatures are particularly deleterious as they may result in oxidative stress for the plant cells caused by the combination of low temperature and high light intensity (Foyer et al. 2002). In field experiments with 12 maize inbred lines in Europe Giauffret et al. (1995) found that the temperature threshold for leaf tip appearance varied among lines from 7.1 to 12.6°C and for the elongation rate of the 9th leaf from 7.1 to 13.5°C. Such temperature ranges frequently occurred in our experiments, as well. Most published studies, however, were conducted in the north of the United States of America, where a continental climate with warmer temperatures during early plant development predominates.

The growth factors causing variation in early vigour may differ among environments. The experimental approach of our study made it impossible to fully control other environmental factors such as water and nutrient availability. However, adequate nutrient supply was ensured through fertilization. Water availability was sufficient in all environments; and experiments were kept free from pests, diseases, and weeds. Therefore, we assume that suboptimal temperatures were the most important factor impairing maize growth in our field trials.

Genetic basis of early plant vigour

Various researchers investigated the genetic variability for germination under controlled-environment or field conditions (McConnell and Gardner 1979; Mock and McNeill 1979; Revilla et al. 2000). In the present study, genotypes with reduced field emergence were excluded from the analysis to avoid confounding between the effects of chilling and seed quality. Our criterion for early plant vigour was fresh matter yield at the beginning of shoot elongation. This trait showed considerable genetic vari-

ability and involves all plant growth components affected by chilling during the early developmental stages.

According to Beavis (1998) and Utz et al. (2000), the power, accuracy, and precision of QTL mapping mainly depends on the sample size of the mapping population, the heritability of the trait, and the true number of segregating QTL. Theoretically, the large number of DH lines used in the present QTL analysis should have been sufficient to achieve a power of QTL detection of >90% considering a trait with ten QTL and a heritability of 0.6 (Beavis 1998). Indeed, cross-validation revealed a repeatability of QTL detection of 80–100% for the fresh matter yield QTL with the greatest effects (Table 4). However, for QTL with smaller effects, repeatability was much lower.

Another important feature of the detected QTL is the precision of their map position. The mean positions of the four most important QTL for fresh matter yield derived from 1,000 calibration runs showed a good agreement with results from the main analysis (data not shown).

Genotype × environment and QTL × environment interaction was significant for fresh matter yield, but the estimates of QTL effects in the individual experiments were not associated with the mean temperature of those environments. One reason for this might be that chilling stress frequently occurred even in the more favourable environments. Since no grouping of environments for chilling effects was observed, QTL analyses were conducted across all environments.

Only recently, several QTL studies on chilling tolerance in maize were reported in the literature (Fracheboud et al. 2002, 2004; Jompuk et al. 2005; Hund et al. 2004; 2005). Under field (Jompuk et al. 2005) and controlled conditions (Fracheboud et al. 2004) a major QTL was found on chromosome 6 controlling several traits related to the functioning of the photosynthetic apparatus. Although our study is also based on temperate maize breeding germplasm, this major QTL did not show up in our population. Further, Jompuk et al. (2005) reported two QTL for shoot dry weight. None of those co-localized with the three major QTL for fresh matter yield found in the present study. For leaf greenness, they detected six QTL and again no QTL co-localized with QTL for leaf chlorosis or any other trait scored here. Fracheboud et al. (2002) analysed photosynthesis traits under controlled chilling conditions in a tropical mapping population. They also did not detect the major QTL on chromosome 6 reported by Jompuk et al. (2005). A QTL position on chromosome 9, significant for CO_2 fixation, quantum yield of electron transport in photosystem II, and stomatal conductance, corresponded to a QTL for leaf chlorosis in the present study.

The different numbers and positions of QTL detected in different populations indicate that early vigour in maize is a complex quantitative trait, being dependent on the population, the environment and the developmental stage.

The relationship between line per se and testcross performance for fresh matter yield was only moderate, although six out of seven QTL detected at the line per se level were also significant in the testcrosses. These results might be due to the considerable part of the genetic variance which remained unexplained by the detected QTL. The tester line was derived from the flint maize gene pool, which is generally known for its superior chilling tolerance and good early vigour. Although, the tester line was selected for its ability to differentiate between the parent lines, interactions between the tester line and the DH lines could have influenced the relationship between line per se and testcross performance.

Surprisingly, the relationship between fresh matter yield and days to silking was non-significant. However, five out of seven QTL for fresh matter yield of lines per se and four out of ten QTL for testcross fresh matter yield showed overlap with QTL intervals for days to silking. This overlap could simply happen by chance, because the support intervals for all 22 QTL for days to silking sum up to as much as 27% of the map length. Furthermore, one of the three major QTL for fresh matter yield showed no overlap with any QTL for days to silking, and at the fresh matter yield QTL on chromosome 4, the positive allele originates from TH, while the allele for reducing days to silking comes from SL. These results indicate that differences in early vigour were not simply caused by maturity differences.

In addition to fresh matter yield, other chilling-associated traits were investigated. Significant genetic variability for symptoms of leaf chlorosis and leaf purpling only occurred in environments with very low mean temperatures. Leaf chlorosis is a well known chilling stress symptom (Foyer et al. 2002; Baker and Nie 1994; Miedema 1982). Yet the correlation between leaf chlorosis and fresh matter yield was weak. In contrast, Dolstra et al. (1994) reported coefficients of correlation of r=0.65 between photoinhibition susceptibility (measured *via* chlorophyll fluorescence) and plant height at the 10-leaf stage in 67 European maize inbred lines under early sowing conditions. In the same experiment conducted under late sowing, correlations between the two traits were only weak. Lee et al. (2002) found a positive relation between the photosynthetic parameter leaf carbon exchange and total dry weight of plants at the eight-leaf stage. This experiment included 50 inbred lines from the northern Corn Belt and Canada tested under chilling conditions in a controlled environment. Possibly the weak correlation between leaf chlorosis and fresh matter yield in our study was due to the different developmental stages under which the two traits were observed. Hodges et al. (1997) reported that some lines

altered their response to chilling from the seedling to the early growth stage. The authors concluded that chilling tolerance at these two developmental stages might be under the control of different genetic factors. However, four out of seven QTL for leaf chlorosis presented here mapped to regions for fresh matter yield. In the study of Jompuk et al. (2005), none of the two QTL for dry matter yield under conditions of early sowing showed overlap with leaf greenness.

Significant genetic variability was also observed for leaf purpling, which agrees with the study of Cobbina and Miller (1987). A major QTL, explaining 80% of $\hat{\sigma}^2_g$ was detected on chromosome 9. Interestingly, the difference between the parent lines SL and TH was not significant, which could be due to epistatic interactions. Low temperature was shown to induce anthocyanin synthesis in maize seedlings (Christie et al. 1994) eventually resulting in a purpling of leaf colour (Cobbina and Miller 1987). The reasons for increased anthocyanin production during chilling stress are not yet fully understood (Chalker-Scott 1999). At low temperatures, the uptake of phosphorus might be reduced and leaf purpling may point to phosphorus deficiency stress (Cobbina and Miller 1987). Moreover, Chalker-Scott (1999) suggested an involvement of anthocyanins in inducing cold-hardiness, as an increased concentration of solutes (e.g. anthocyanins) in the vacuole protect the cell from freezing injury. In *Pseudowintera colorata a* potential role of anthocyanins as antioxidants was also reported (Gould et al. 2002). Such a function could be beneficial during chilling stress when oxygen radicals are produced due to the overexcitation of the photosynthetic apparatus.

The anthocyanin pathway is well understood (Coe et al. 1988) and virtually all enzyme enconding genes have been isolated (Mol et al. 1998). Interestingly, the major QTL detected in our study co-localized with the mapping position of the regulatory gene *c1* as described in the MaizeGDB database (http://www.maizegdb.org/). Furthermore, the reported mapping position of the gene *r1* is within the support-interval of the QTL on chromosome 10 and the structural gene *a1* is co-localized with the QTL region on chromosome 3. However, the lack of a correlation between fresh matter yield and leaf purpling and the non-overlap of the corresponding major QTL does not indicate an important role of anthocyanins in the genetic control of early plant vigour in our experiments.

Reaction of genotypes to freezing temperatures

According to Greaves (1996), maize displays only a very limited tolerance to freezing temperatures. However, in agreement with results of the present study, Dhillon et al. (1988) reported significant genetic variability for frost injury symptoms among 64 inbred lines. The experiments were conducted in growth chambers at –2, –3, and –3.5°C for 4 h during six successive nights.

One of the two important QTL for frost damage was located near a QTL for fresh matter yield in the testcrosses, but the positive QTL alleles for fresh matter yield and frost damage did not originated from the same parent line. The correlation between the two traits was also non-significant. These results might partly be attributable to different physiological mechanisms responsible for tolerance to freezing on the one hand and to chilling on the other. Freezing injury occurs at temperatures below the freezing point of water and is caused by intracellular ice crystals or by dehydration of the protoplast (Taiz and Zeiger 1998).

Conclusion and outlook

Integrated approaches are necessary to identify the genetic and molecular mechanisms that underlie complex traits (Causse et al. 1995, Consoli et al. 2002). To achieve that goal, quantitative genetic methods such as QTL analysis have to be combined with molecular plant physiology. Our study furnished reliable QTL data for early plant vigour since they were obtained from a large mapping population and a broad range of environments. These results are presently used as a basis for further investigations to characterize the major QTL at the molecular level. Genes to be identified in those studies may serve as a starting point for retrieving natural allelic variation to improve early plant vigour in cool environments.

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