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Genetic analysis of adaptation differences between highland and lowland tropical maize using molecular markers

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Abstract Molecular-marker loci were used to investigate the adaptation differences between highland and lowland tropical maize. An F_2 population from the cross of two inbred lines independently derived from highland and lowland maize germplasm was developed, and extracted $F_{3:4}$ lines were phenotype in replicated field trials at four thermally diverse tropical testing sites, ranging from lowland to extreme highland (mean growing season temperature range 13.2–24.6°C). Traits closely related with adaptation, such as biomass and grain yield, yield components, days from sowing to male and female flowering, total leaf number, plant height and number of primary tassel branches (TBN), were analyzed. A large line × environment interaction was observed for most traits. The genetic basis of this interaction was reflected by significant, but systematic, changes from lowland to highland sites in the correlation between the trait value and genomic composition (designated by the proportion of marker alleles with the same origin). Joint analysis of quantitative trait loci (QTLs) over sites detected 5–8 QTLs for each trait (except disease scores, with data only from one site). With the exception of one QTL for TBN, none of these accounted for more than 15% of the total phenotypic variation. In total, detected QTLs accounted for 24–61% of the variation at each site on average. For yield, yield components and disease scores, alleles generally favored the site of origin. Highland-derived alleles had little effect at lowland sites, while lowland-derived alleles showed relatively broader adaptation. Gradual changes in the estimated QTL effects with increasing mean site temperature were observed, and paralleled the observed patterns of adaptation in highland and lowland germplasm. Several clusters of QTLs for different traits reflected the relative importance in the adaptation differences between the two germplasm types, and pleiotropy is suggested as the main cause for the clustering. Breeding for broad thermal adaptation should be possible by pooling genes showing adaptation to specific thermal regimes, though perhaps at the expense of reduced progress for adaptation to a specific site. Molecular marker-assisted selection would be an ideal tool for this task, since it could greatly reduce the linkage drag caused by the unintentional transfer of undesirable traits.

Key words Maize · Adaptation · Tropical · Highland and lowland · QTL mapping

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Introduction

Although maize as a species has an extremely broad range of adaptation (Fischer and Palmer 1983), there are distinct germplasm groupings which show specific adaptation to four broad classes of environments: lowland tropics (considered here to be < 1200 masl), mid-elevation tropics and subtropics (1200–1800 masl), the highlands (1800–2800 masl) (Eagles and Lothrop 1994) and temperate environments (maximum photoperiod during growth > 14 h). Much of this adaptation is undoubtedly due to varying levels of disease resistance and to photoperiod sensitivity (Ellis et al. 1992; Edmeades et al. 1994).

In the absence of major differences in disease incidence and photoperiod, however, there appear to be fundamental differences between highland- and lowland-adapted genotypes in their response to temperature. These can be a major cause of genotype × environment interactions when highland and lowland genotypes are evaluated together in their environments of origin. In a controlled environment study of two highland and three non-highland cultivars, for example, Ellis et al. (1992) reported that the optimum temperature for time from sowing to tassel initiation was 20–22°C in the highland

genotypes, and around 30°C for the others. In a separate study cool-adapted highland cultivars, when grown in warm lowland environments (T_{mean}=28°C), suffered an 83% decline in biomass and a 92% decline in harvest index compared with their performance in an environment with a T_{mean} of 17°C (Lafitte and Edmeades 1997). When lowland genotypes were grown in a cool highland environment in the same study, biomass production was unaffected, but grain yield and harvest index declined by 54 and 57%, respectively. Lafitte and Edmeades (1997) concluded that the primary reasons for the poor performance of highland genotypes in warm environments were supraoptimal temperature effects on ear formation and partitioning. These were accompanied by reductions in radiation interception and radiation-use efficiency. Other studies of highland, lowland and Corn Belt Dent hybrids grown as young plants in controlled environments have shown superior growth of highland hybrids versus the others at a mean temperature of 11°C (23.0 vs 10.6 g plant^{-1}), roughly equal growth at 22.5°C (24.8 vs 29.0 g plant^{-1}) and inferior growth at 32.5°C (6.2 vs 9.7 g plant^{-1}) (Hardacre and Eagles 1989). The capacity of highland genotypes to emerge more rapidly from soils as cool as 13°C has also been documented (Hardacre and Turnbull 1986; Eagles and Lothrop 1994), and there is evidence that highland germplasm continues to fill grain to lower temperatures than Corn Belt Dent germplasm (Newton and Eagles 1991).

One postulated basis for differences in thermal adaptation among species has been the existence of species-specific ‘thermal kinetic windows‘ for key enzymes (Burke et al. 1988), defined as the temperature range at which the activities of key enzymes are above a threshold level (Burke et al. 1988). Initially it was thought that this could account for differences in adaptation between highland- and lowland-adapted genotypes, but one study of a candidate enzyme has cast doubt on whether enzyme thermal kinetics can predict thermal adaptation (Turner et al. 1994).

These studies pose the question of whether it is possible to combine tolerance to cool temperatures with good yields in warm environments. If the two types of adaptation are not mutually exclusive, broad thermal adaptation might be achieved by selection in germplasm composed of genotypes that have evolved under diverse thermal regimes. In a study of highland-, temperate- and lowland-adapted varieties, and 100 full-sib families of the population ‘Largo del Dia‘ that was created by blending germplasm with diverse thermal adaptation, genotypes were evaluated in seven environments ranging from cool to hot (Lafitte et al. 1997). The authors concluded that selection for broad thermal adaptation within Largo del Dia resulted in a cultivar with stable but relatively low grain yield over a wide range of temperatures, and that the temperate genotype was also broadly adapted.

A major breeding goal of CIMMYT’s Maize Program is the development of cultivars with broad adaptation and stable production over an array of environments which vary spatially and temporally in thermal characteristics. Maize varieties in the tropics may be increasingly exposed to a wide range of temperatures if the global warming trend results in more variable weather over time (Mearns 1995). In addition, tropical maize is being increasingly grown in cool winter months under irrigation, especially in Asia, and adaptation of a single cultivar to both summer and winter conditions should result in more stable annual yields. A clear understanding of the genetic basis of thermal-adaptation differences between cool- and warm-adapted cultivars could enhance our ability to select for stable yields in both.

The objective of the present study was, with the aid of molecular markers, to identify genomic segments and allelic effects associated with differences in adaptation to thermally diverse environments in an F_2 population derived from a cross between two lines, one adapted to the tropical highlands and another adapted to the tropical lowlands. This permitted the identification of genomic regions responsible for adaptation differences between highland and lowland tropical maize, and provided information on whether alleles contributing to performance in one thermal regime also contribute in a similar manner in a different regime. This could make possible the combination of alleles conferring specific thermal adaptation in a single genotype that should perform well under a wide array of temperatures.

Materials and methods

Plant material

Two inbred lines, one from a lowland population (S_8, from CIMMYT Population 21) and another from a highland population (S_5, from CIMMYT Pool 1), were extracted by continuous selfing. Population 21, a late white dent lowland tropical population, is derived from Tuxpeño Crema I as described by Johnson et al. (1986). Pool 1 was composited from germplasm collected in the highlands of Peru, Mexico, Colombia, Bolivia and Ecuador, with some introgression of teperate germplasm (Eagles and Lothrop 1994). Both populations underwent a number of cycles of recurrent selection for improved agronomic performance in the lowland and highland tropics, respectively, before inbreeding began. Inbred line extraction was based on superior *per se* and test-cross performance in the target environment. The cross between the two lines was made in 1991 in the Tlaltizapan (TL) winter season (see Table 1 for site details) and F_1 plants were self-pollinated in El Batan (BA) in the summer of 1992. Seeds of two F_2 ears were planted out as spaced tagged plants in TL the following winter season. Leaf samples from 307 individual F_2 plants were collected shortly before flowering for the determination of marker genotypes, but only 196 plants were selfed to produced F_3 seed. Finally, $F_{3:4}$ lines were produced by making plant-to-plant crosses among 16 plants within each line in each of two summer environments where these lines were considered to be reasonably well adapted (BA and TL). Equal amounts of seed per line from each location were bulked to provide the seed used in subsequent field evaluations during the summer of 1993 at BA, TL and Toluca (TO), and at Poza Rica (PR) in the following winter.

Field trials and trait measurement

A total of 196 $F_{3:4}$ lines were evaluated in 1993 at four CIMMYT experiment stations, each representing a different thermal regime. The major characteristics of the four environments are listed in Table 1. The Poza Rica summer environment was not included in this

Table 1 Environmental characteristics of four experimental sites in Mexico where $F_{3:4}$ maize lines were phenotyped. Location codes are: PR = Poza Rica winter; TL = Tlaltizapan summer; BA = EI Batan summer; TO = Toluca summer. Meterological data were collected for the crop cycle at standard meterorological stations within 1 km of the experimental plots unless otherwise specified

Characteristics	Site			
	PR	TL	BA	TO
Latitude (°N)	21	19	20	20
Photoperiod (h)[a]	11.4	13.5	13.7	13.5
Elevation (masl)	60	940	2240	2650
Average T_{max} (°C)[b]	26.4	31.9	24.7	22.0
Average T_{min} (°C)[b]	16.1	17.3	8.9	4.4
Average temp. (°C)[b]	21.3	24.6	16.8	13.2
Radiation (MJ m^{-2} d^{-1})[c]	11.9	22.7	18.9	18.2
Accum. rad. (MJ m^{-2})	1422	2564	3138	3333
Crop cycle (d)[d]	125	115	165	183
Crop cycle (°Cd)[e]	1578	1635	1514	1313
Ratio (radiation: thermal time) for crop cycle				
[MJ m^{-2} (°Cd)$^{-1}$]	0.90	1.57	2.07	2.54
Soil type	Sandy loam	Calcareous vertisol	Alluvial loam	Alluvial loam

[a] Estimated date of tassel initiation
[b] Mean for growing season from sowing to harvest
[c] Radiation measured within 1 km of site for PR and TL; 8 km from site for BA; and for TO estimated from hours of bright sun received at TO, converted to radiation using the ratio of hours of bright sun to radiation received at BA
[d] Duration from sowing to harvest; at TO harvest occurred during grain filling 2 weeks after a frost of –4.3°C defoliated the crop
[e] Thermal time from sowing to harvest [daily T_{max} and T_{min} values individually corrected to a T_{base} = 8°C; values of T_{max} > 30°C corrected as $(30 - (T_{max} - 30))$]

study because of difficulties in controlling the incidence of leaf and ear disease with fungicide in non-adapted germplasm in this hot, humid environment. Parental lines were not included in the field evaluations. Fertilizer levels applied were 150–200 kg N and 26–35 kg P ha^{-1}. Weeds and insects were controlled by chemical methods as needed, and had no effect on crop performance. Foliar diseases did not occur in TL, and were partially controlled by the application of Tilt fungicide at the other sites. Nevertheless, there was sufficient development of foliar disease at PR to warrant a visual score (1 = no disease, to 9 = heavily diseased) of the severity of *Exserohilum turcicum* (TUR), *Helminthosporium maydis* (HM) and *Puccinia polysora* (RUST) on two occasions during grain filling.

The design used at each site was an alpha (0,1) lattice in two replications, with an incomplete block size of 14. Plot size was a single row 2.5 m long, and within- and between-row plant spacings were 0.20 m and 0.75 m. The target density was 67 000 plants ha^{-1}, obtained by oversowing and thinning. Traits measured and analyzed are summarized with their acronyms, mean values and standard deviations in Table 2. These are thought to be indicators of the major differences in adaptation and morphology between highland and lowland populations. Leaves were identified on five plants per plot by removing the last 5 cm of the tip of leaf numbers 5 and 10 with a scissors before the lower leaves had senesced. Total leaf number (LFN) was obtained by counting all leaves on these marked plants shortly after flowering. Time from sowing until 50% of plants exhibited anthers (MF) and silks (FF) was recorded, and the anthesis-silking interval (ASI) calculated as (FF-MF). Shortly after, flowering plant height (PH) was measured on five plants per plot as the distance from the ground to the point of insertion of the flag leaf. Three-weeks after flowering the number of primary branches (including the main spike) on the tassel (TBN) was counted on five plants per plot. For measures of grain yield (YLD) and biomass (BIOM), border plants were removed to give a net harvested area per plot of 1.5 m^2. Since there was a wide range of maturities among the lines, final harvest took place on several occasions at PR, TL and BA. At these sites, ears were removed from all plants in the harvested area and ears and plants counted. The rest of the plant was cut at ground level, and chopped into pieces less than 10 cm in length. All plant parts were then dried to constant weight at 80°C in a forced air oven, the shelled grain biomass recorded, and the cobs added to the weight of the non-grain biomass. Two representative samples of 100 kernels each were counted, dried again to constant weight at 80°C, and weighed (HKW). Harvest index (HDX) was calculated as the ratio of YLD to BIOM. At TO, a high cool site (Table 1), BIOM was measured during grain filling on a single date 3–9 weeks after flowering because a frost (–4.2°C) defoliated the crop on October 5 (183 days after sowing). Grain yield and its components could not, therefore, be estimated at that site.

Analysis of variation among lines within and between sites

Since the incomplete block within each replicate is a random factor in the alpha-lattice design, a mixed-model procedure was used for the analysis of variation among lines. Sites were assumed to be fixed with four specific thermal regimes, and all other factors, including replicate, block within replicate, line and line by site interactions, to be random. By the PROC MIXED procedure (SAS 1988), variance components of line and line by site interactions were estimated and tested for their significance in the presence of the effects of replicate and block within replicate. Broad-sense heritabilities were then estimated from the variance components. Correlation coefficients among traits within each site were also calculated.

Marker-genotype determination and linkage-map construction

Genomic DNA was extracted from 307 F_2 plants and two parental lines. DNA was purified, quantified, digested with two restriction enzymes (*Eco*R1 and *Hin*dIII), separated on agarose gels (0.7%) and transferred to nylon membranes (Hybond N, Amersham) by Southern blotting. Labeled probes were used to detect polymorphisms. Details of the protocols are given in Hoisington et al. (1994). About 150 probes from the University of Missouri Columbia (UMC) and the Brookhaven National Laboratory (BNL) were used to screen the two parental lines. The best 86 probes were chosen for the genotype determination of all F_2 plants and the linkage map for the population was constructed using the software MAPMAKER 2.0 (Lander et al. 1987). Each marker locus was tested for segregation distortion from a 1:1 ratio for the two parental alleles.

Markers of different origin were counted for each line and the proportion used to represent the genomic composition with respect to its origin. Variation among lines due to genomic composition was analyzed by the correlation between trait value and the proportion of alleles of one source (in this case the highland parent) which represent the average effects of all alleles of one origin relative to those from the other parent. Heterozygosity of each line was estimated as the proportion of marker loci with heterozygous genotypes, and correlations with traits were also calculated.

QTL mapping over multiple sites

Composite interval mapping, CIM, (Zeng 1994; Jansen and Stam 1994) was used for QTL analysis. Because the main purpose of this investigation was to investigate the expression of alleles of different origin (highland or lowland) over selected environments, the extension of the CIM procedure to joint analysis of multiple traits and environments by Jiang and Zeng (1995) fits the purpose of this study especially well. The analysis was performed on the subset of 161 individuals for which both molecular and phenotypic data on lines were available. The phenotypic value for each line used for QTL analysis was its least square mean from the mixed-model procedure (SAS 1988), assuming both replicate and block within replicate as random and the lines at each site as fixed.

The models and procedure

The CIM procedure can assume different models (Zeng 1994), depending on the number and position of the markers employed as cofactors. Four models were used in this study: (1) simple interval mapping (Lander and Botstein 1989 or Modell III of Zeng 1994 but with multiple traits), which was only used for selecting markers as cofactors in the following CIM procedure; (2) CIM with only unlinked markers as cofactors (Modell II of Zeng 1994), which gave the smallest residual variance and was supposed to have the highest power for QTL detection in the absence of multiple QTLs linked in repulsion; (3) CIM with the selected markers and cofactors and two markers flanking the interval under test but at least 30 cM away from that interval (Model I of Zeng 1994); (4) same as (3) but with 20 cM as the minimum distance. By model (1), whenever a likelihood ratio (LR) value exceeded the threshold, the nearest marker was then selected, and model (2) was used to increase the QTL detecting power and models (3) and (4) to eliminate any possible ''ghost QTLs'' (Martinez and Curnow 1992). The need to use models (3) and (4) will be shown in the results.

Whenever a LR value reached the threshold under any of the above models, a QTL was considered identified, unless it was proved to be a ghost QTL by further analysis. A ghost QTL was identified when a QTL was suggested by model (1) and/or (2) with a peak in the LR profile, but later disappeared when evaluated under model (3) and/or (4).

Separate analyses for each site and joint analysis over all sites were performed. A QTL was considered identified when the LR was significant at a single site and/or in the joint analysis over all sites (for a detailed discussion of the comparison between the two analyses, see Jiang and Zeng 1995). The estimation of the position and effects of the QTL was made at the significant LR peak.

The type of gene action was estimated from the absolute values of the ratio (DR) of the estimated dominance effects to that of the additive effects, but only for those QTLs and sites that were significant in separate analyses. Since the traits were measured as the $F_{3:4}$ family mean, the estimated dominance effects have been multipied by two to satisfy its definition in an F_2 generation. The criterion used for classification followed Stuber et al. (1987): additive if DR < 0.2, partial dominance if 0.2 ≤ DR < 0.8, dominance if 0.8 ≤ DR < 1.2, and overdominance if DR ≥ 1.2.

Critical value used for QTL detection and test of QTLs by site interaction

A LOD score of 3.0 was adopted in this study, which is equivalent to a LR value of 13.82 and corresponds to a significance level of $P = 0.0032$ with $df = 3$ at one site. With this significance level, the corresponding critical LR value for the joint test is 21.42 for three sites with $df = 7$, and 24.80 for four sites with $df = 9$. With this significance level, the genome-wise significance level (or type-I error rate) has an upper limit of 0.003276 per marker interval, or 0.24 in this study, since 76 marker intervals were scanned for significant QTLs. The lower limit of this test can be estimated as 0.003276 per marker interval, or 0.03 with ten independent chromosomes. Given these considerations, QTL by site interactions could be tested, since the test position was fixed by joint mapping (Jiang and Zeng 1995). LR values of 9.49 and 12.59 were used for comparisons among three and four sites with $df = 4$ and 6, respectively, since the numbers of parameters in the full models are seven and nine for three and four sites while the reduced model has only three parameters.

Variance explained by a QTL and by all QTLs at each site

The estimation of the proportion of phenotypic variance associated with a QTL was obtained by regression of the trait value on the expected number of copies of the alleles from the highland source at the QTL, which was calculated from the genotype of the flanking markers and the recombination frequencies between the QTL and the markers. This regression was performed for each detected QTL and for all QTLs at each site. The average variance explained over sites was also calculated for each trait.

Results

Crop performance at the four sites

The means of 161 lines for all 13 traits are presented in Table 2. The largest and smallest yields of above-ground

Table 2 Traits measured and their means and standard deviations by location. Location codes are the same as in Table 1. Genotypic effects were significant ($P < 0.05$) for all traits

Trait	Description	Mean (± SD)			
		PR	TL	BA	TO
Yield and yield components					
BIOM	Aboveground biomass(g m^{-2})	408 ± 86	865 ± 185	1011 ± 153	544 ± 145
YLD	Grain yield (g m^{-2})	133 ± 51	219 ± 91	340 ± 81	NA
HDX	Harvest index (g g^{-1})	0.31 ± 0.07	0.24 ± 0.06	0.34 ± 0.06	NA
HKW	100 kernel weight (g)	15.1 ± 2.3	16.1 ± 2.1	16.4 ± 2.4	NA
Phenology					
MF	Sowing to 50% anthesis (days)	64.9 ± 4.1	60.1 ± 2.8	90.9 ± 3.8	142.1 ±4,7
FF	Sowing to 50% silking (days)	NA	62.7 ± 3.8	93.9 ± 4.5	144.4 ± 5.9
ASI	Anthesis-silking interval, FF-MF (days)	NA	2.64 ± 2.2	2.98 ± 2.4	2.37 ± 2.8
Morphology (per plant basis)					
LFN	Total leaf number	16.2 ± 1.0	20.2 ± 0.9	20.2 ± 1.0	18.6 ± 1.0
TBN	Tassel primary branch no.	5.0 ± 3.3	10.2 ± 5.1	11.5 ± 5.1	9.0 ± 4.8
PH	Plant height (cm)	121 ± 14	176 ± 16	152 ± 16	126 ± 14
Foliar disease scores (1 = no disease; 9 = very diseased)					
HM	*Helminthosporium maydis*	4.27 ± 1.30	NA	NA	NA
RUST	*Puccinia polysora*	4.18 ± 1.24	NA	NA	NA
TUR	*Exserohilum turcicum*	2.69 ± 0.97	NA	NA	NA

biomass and grain were found at BA and PR, respectively. Mean temperatures at these sites were 16.8 and 21°C, respectively. Large differences among sites were also found for MF, FF and in total season length (Tables 1, 2), but when MF was converted to thermal time it occurred at 847, 840, 852 and 1052°Cd after sowing at PR, TL, BA and TO, respectively (data not shown). These data suggest a direct effect of cold temperature in delaying flowering at TO, and in fact the minimum temperature fell below 10°C on 95% of the nights during the growing season at TO versus 58% at BA and 5% at PR and TL. Radiation received and radiation per unit thermal time were largest at TO [3333 MJ m^{-2}; 2.54 MJ m^{-2} $(°Cd)^{-1}$], but interception and efficiency of use of that radiation was apparently much reduced by cool night temperatures. Plant height also varied among sites, plants being shortest at PR and TO, and tallest at TL. TBN and LFN were least at PR, the site with the shortest natural photoperiods, followed by TO (Table 2). Traits showing the least differences across sites were HDX, HKW and ASI.

Table 3 Estimates of variance components, genetic variance (σ^2_g), genotype × site interaction ($\sigma^2_{g\times e}$), their ratio ($\sigma^2_{g\times e}/\sigma^2_g$) and heritabilities for each trait by the mixed-model procedure

Trait	σ^2_g	$\sigma^2_{g\times e}$	σ^2_e	$\sigma^2_{g\times e}/\sigma^2_g$	h^2 [a]
BIOM	5678	5147	21 000	0.91	0.51
YLD	1779	1638	4775	0.92	0.59
HDX (×10)	0.16	0.18	0.28	1.15	0.71
HKW	1.77	1.59	3.66	0.90	0.65
MF	8.47	3.49	6.43	0.41	0.79
FF	11.87	7.18	7.73	0.60	0.83
ASI	1.92	1.18	5.48	0.61	0.53
LFN	0.46	0.25	0.39	0.55	0.79
TBN	17.10	1.84	5.19	0.11	0.88
PH	134	38	92	0.28	0.79
HM	0.93		1.20		0.44
RUST	0.90		1.16		0.44
TUR	0.54		0.83		0.39

[a] $h^2 = \dfrac{\sigma^2_g + \sigma^2_{g\times e}}{\sigma^2_g + \sigma^2_{g\times e} + \sigma^2_e / 2}$

Variation among lines

The standard deviations among lines are also presented in Table 2. Among-line variation for all traits and sites was inspected for continuity and, as a consequence, an approximately normal distribution has been assumed for the analysis of variation among lines and for QTL mapping for most traits. Only TBN in PR showed a skewed distribution towards a low number of tassel branches.

Variance components among lines and interaction with sites were statistically significant for all traits (Table 3). Line by site interaction variances for yield and yield components were largest for all traits relative to the among-line variance, with a ratio of about 1 for all four yield-related traits. Heritabilities of line mean performance within site for BIOM and YLD were 0.51 and 0.59, the lowest among all traits, with the exception of disease scores which were recorded at only one site. TBN and PH presented least G × E intraction, with a ratio of 0.11 and 0.28 relative to among-line variance and a heritability of 0.88 and 0.79, respectively.

Genomic composition from the highland source ranged from 0.3 and 0.7 with an average of 0.47 for alleles of highland origin. A significant correlation was found between the trait value and genomic composition of each line for all traits except PH (Table 4). In addition, directional changes in sign and/or magnitude of the coefficient from lowland to highland sites were obvious for yield components, flowering traits and for LFN. For example, significant negative correlations for BIOM, YLD and HDX in PR and TL (T_{mean} = 21.3–24.6°C) all become close to zero in BA (T_{mean} = 16.8°C) and were positive for BIOM in TO (T_{mean} = 13.2°C). Note that if the proportion of lowland instead of highland alleles is used, all the correlations will have the same magnitude but opposite sign. Some inconsistencies in flowering traits and LFN in PR were noted, and are perhaps due to the effect of the short photoperiod and cold nights of the winter season that favored early flowering in lines carrying a high proportion of highland alleles. High negative correlations were also observed for two of the disease scores in PR, confirming the common observation that

Table 4 Correlation coefficients between the trait and the proportion of the highland alleles and the level of heterozygosity at all marker loci in each F_2 individual. $P < 0.05$ (italic) and $P < 0.01$ (**bold**)

Trait	Correlation with genomic composition				Correlation with heterozygosity			
	PR	TL	BA	TO	PR	TL	BA	TO
BIOM	**–0.39**	**–0.32**	–0.03	**0.23**	**0.21**	**0.17**	**0.28**	**0.19**
YLD	**–0.39**	**–0.35**	0.04		*0.16*	*0.16*	**0.22**	
HDX	**–0.31**	**–0.29**	0.06		0.13	0.11	0.05	
HKW	**–0.30**	–0.12	0.00		0.09	0.04	0.06	
MF	**–0.21**	0.13	**–0.32**	**–0.45**	–0.02	–0.05	0.07	0.00
FF		*0.19*	**–0.22**	**–0.41**		–0.07	0.00	–0.01
ASI		*0.15*	0.09	–0.12		–0.06	–0.11	–0.02
LFN	*–0.17*	0.06	–0.13	**–0.33**	0.11	0.05	0.13	0.10
TBN	*–0.19*	–0.13	*–0.16*	*–0.15*	–0.06	–0.02	–0.02	–0.01
PH	–0.12	0.06	–0.12	–0.05	*0.15*	0.09	*0.17*	**0.24**
HM	**0.42**				–0.13			
RUST	–0.06				0.00			
TUR	**0.26**				–0.05			

Table 5 correlation coefficients calculated between traits among family means measured at four sites in Mexico. Values in italics are significant at $P < 0.05$; values in **bold** are significant at $P < 0.01$

Trait		BIOM	YLD	HDX	HKW	MF	FF	ASI	LFN	TBN	PH	HM	RUST
YLD	(PR)	**0.87**											
	(TL)	**0.80**											
	(BA)	**0.71**											
HDX	(PR)	**0.58**	**0.89**										
	(TL)	**0.40**	**0.80**										
	(BA)	0.13	**0.78**										
HKW	(PR)	**0.39**	**0.45**	**0.44**									
	(TL)	**0.27**	**0.25**	0.14									
	(BA)	**0.20**	**0.44**	**0.43**									
MF	(PR)	**0.33**	0.10	–0.08									
	(TL)	–0.07	**–0.37**	**–0.49**	–0.04								
	(BA)	**0.23**	*–0.19*	**–0.46**	**–0.34**								
	(TO)	*–0.16*											
FF	(TL)	**–0.20**	**–0.52**	**–0.64**	–0.03	**0.84**							
	(BA)	0.13	**–0.28**	**–0.49**	**–0.47**	**0.86**							
	(TO)	**–0.21**				**0.88**							
ASI	(TL)	**–0.28**	**–0.44**	**–0.48**	0.00	0.14	**0.65**						
	(BA)	–0.12	**–0.24**	**–0.22**	**–0.37**	0.09	**0.58**						
	(TO)	*–0.17*				**0.21**	**0.64**						
LFN	(PR)	**0.51**	**0.31**	0.11	**0.28**	**0.65**							
	(TL)	**0.33**	0.07	*–0.17*	0.11	**0.44**	**0.33**	–0.02					
	(BA)	**0.44**	0.12	**–0.23**	–0.08	**0.56**	**0.49**	–0.07					
	(TO)	–0.03				**0.70**	**0.65**	**0.21**					
TBN	(PR)	–0.06	*–0.18*	**–0.28**	*–0.19*	0.05			*0.18*				
	(TL)	0.04	0.03	0.02	*–0.15*	*–0.18*	–0.03	**0.21**	–0.05				
	(BA)	0.00	–0.13	**–0.20**	–0.13	*0.15*	*0.18*	0.12	*0.18*				
	(TO)	–0.11				*0.15*	**0.23**	**0.22**	**0.27**				
PH	(PR)	**0.54**	**0.27**	0.03	0.11	**0.55**			**0.57**	–0.04			
	(TL)	**0.33**	0.05	*–0.19*	0.11	**0.49**	**0.40**	0.04	**0.50**	–0.04			
	(BA)	**0.60**	**0.21**	**–0.22**	–0.07	**0.54**	**0.47**	0.05	**0.63**	–0.03			
	(TO)	**0.44**				**0.25**	*0.19*	–0.01	**0.37**	*–0.17*			
HM	(PR)	**–0.50**	**–0.47**	**–0.36**	**–0.31**	**–0.22**			**–0.27**	–0.05	*–0.17*		
RUST	(PR)	*–0.15*	–0.08	–0.02	*0.15*	**–0.38**			**–0.26**	*–0.15*	*–0.16*	*0.18*	
TUR	(PR)	*–0.18*	*–0.18*	–0.12	**–0.23**	–0.05			–0.06	**–0.24**	0.03	**0.28**	0.12

highland genotypes are relatively vulnerable to disease in lowland environments. A high proportion of highland alleles were consistently associated with less branching of the tassel.

Correlations between each trait and the level of heterozygosity of each F_2 individual were also calculated, and significance was found for BIOM, YLD and PH at almost all sites (Table 4). This result is consistent with commonly observed positive effect of heterozygosity on height and yield, regardless of the environment.

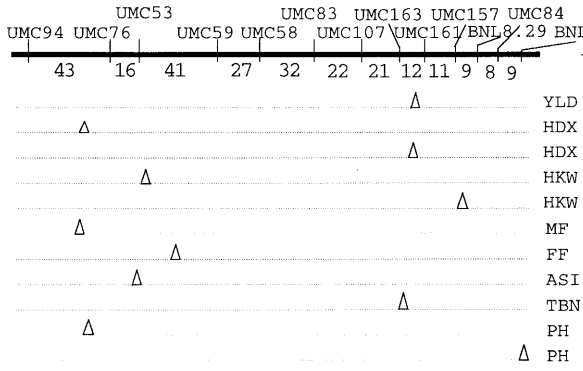
Correlations among traits within each site are listed in Table 5. The highest positive correlations were found among yield components, BIOM, YLD and HDX. As expected, almost all traits except TBN showed a significant correlation within BIOM. Relatively high negative correlations were also found between ASI and YLD and HDX. TBN also showed a generally negative correlation with YLD, HDX and HKW. Large differences in the magnitude of correlations among sites were observed for some traits, reflecting again significant line by site interactions. Because these correlations are based on the total effects of the whole genome, no distinction can be made between pleiotropy and linkage as the underlying causes unless a genomic analysis is performed with the aid of molecular markers.

Linkage map and allelic segregation

Figure 1 shows the linkage map for the 86 RFLP markers employed in this study. Although there are a few gaps of more than 50 cM, the map is comparable to other published maps (e.g. Ribaut et al. 1996), with a genomic coverage of 1709 cM and an average interval size of 22 cM. Ten out of 86 markers showed significant

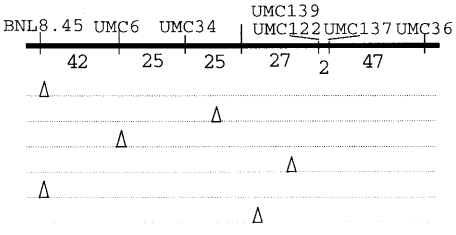
Fig. 1 RFLP linkage map and QTL plots of tropical maize from the analysis of an F_2 population by separate analysis at each stie and joint analysis (*JM*) over sites that involved testing of QTL by site interaction ($Q \times E$). The *horizontal axis* in the center of each plot represents the chromosome with both markers and distances (cM) indicated. The *triangles* indicate the map positions of the likelihood ratio (LR) peaks. The *values* on the right of each plot are the proportion of phenotypic variation explained (R^2) by the QTL in a regression analysis in the population of 161 $F_{3:4}$ lines evaluated at four testing sites, PR, TL, BA and TO. *Dir* indicates the direction of the estimated additive effect of a QTL [*H*: the substitution (additive) effect of a lowland allele by a highland allele is positive, *L*: the substitution (additive) effect of a highland allele by a lowland allele is positive]. *DR* represents the dominance ratio (*A*: additive, *P*: partial dominance, *D*: dominance, *O*: over dominance, upper cases *P, D and O* indicate a positive effect to increae the trait value, *lower cases p, d and o* indicate a negative effect to decrease the trait value). * indicates significance ($P < 0.05$) when LR is compared with threshold

Chromosome 1



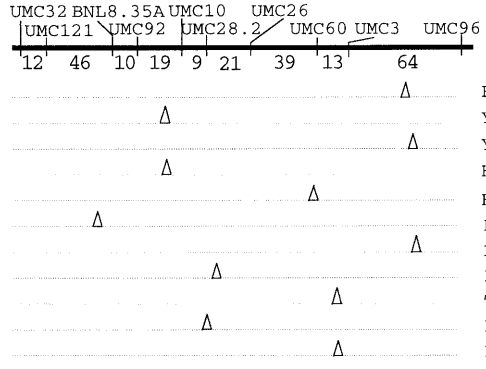
	R^2(%)(Dir,DR) PR	TL	BA	TO	LR JM	LR QxE
YLD	8.2(L,P)*	5.1(L,O)*	0.1(H)		S	S
HDX	9.9(L)	13.4(L,D)*	3.4(L)		S	NS
HDX	*6.8(L)*	*19.1(L,O)**	*4.5(L)*		*NS*	*S*
HKW	12.6(L,D)*	3.0(H)	5.6(L,O)*		S	S
HKW	10.4(L,O)*	0.5(H)	1.0(H)		S	S
MF	7.8(H,A)*	14.1(H,p)*	0.2(H)	2.6(H)	S	S
FF		26.1(H,A)*	2.3(H)	0.5(L)	S	S
ASI		20.1(H,A)*	7.2(H)	1.0(H)	S	NS
TBN	*0.2(H)*	*0.4(H)*	*0.3(H)*	*24.5(H,O)**	*S*	*S*
PH	9.5(H,O)*	1.2(H)	2.0(L)	0.7(L)	S	S
PH	7.6(L,A)*	3.2(L)	1.7(L)	2.0(L)	S	NS

Chromosome 2



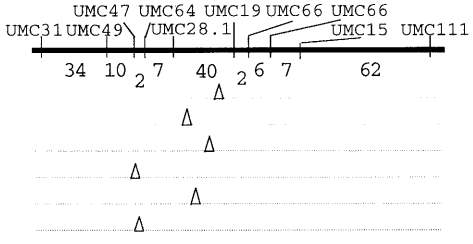
	R^2(%)(Dir,DR) PR	TL	BA	TO	LR JM	LR QxE
BIOM	3.6(L)	8.1(L,O)*	0.2(H)	6.2(H,A)*	S	S
ASI		12.5(L,A)*	7.7(L,A)*	1.2(L)	S	NS
TUR	*14.2(H,o)**				*S*	
FLN	1.8(H)	7.0(H,O)*	8.0(H,A)*	2.1(H)	S	S
TBN	21.0(L,A)*	14.2(L,P)*	24.7(L,P)*	29.6(L,P)*	S	S
TBN	3.6(L)	1.2(H)	0.1(H)	0.1(H)	S	S

Chromosome 3



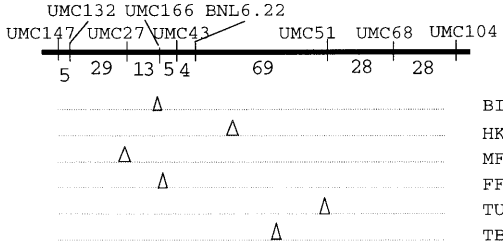
	R^2(%)(Dir,DR) PR	TL	BA	TO	LR JM	LR QxE
BIOM	6.7(L,o)*	5.1(L)	3.7(L)	5.0(L)	NS	NS
YLD	11.1(L,D)*	8.5(L)	3.9(L)		S	NS
YLD	8.5(L,o)*	1.7(L)	3.9(H)		S	S
HDX	13.0(L,P)*	8.5(L)	1.7(L)		S	S
HKW	5.9(L)	10.1(L,D)*	0.4(L)		S	NS
HM	22.3(H,D)*				S	
RUST	6.9(H,o)*				S	
FLN	1.1(H)	1.0(L)	0.3(H)	1.3(L)	S	S
TBN	3.9(H)	4.8(H,O)*	4.4(H)	6.5(H)	NS	NS
PH	2.7(L)	1.0(H)	1.0(L)	0.5(L)	S	S
PH	1.4(L)	6.2(L)	4.8(H)	8.1(L)	S	S

Chromosome 4



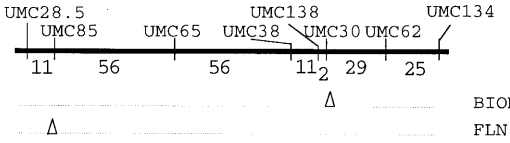
	R^2(%)(Dir,DR) PR	TL	BA	TO	LR JM	LR QxE
HDX	*2.7(L)*	*8.8(L,P)**	*4.5(L)*		*NS*	*NS*
MF	0.1(H)	3.5(H,A)*	0.1(H)	2.4(H)	S	S
ASI		2.8(H)	7.6(H,p)*	1.2(L)	S	S
RUST	8.2(L,O)*				S	
FLN	1.8(H)	8.5(H,P)*	6.1(H,O)*	3.3(H,P)	S	S
PH	2.0(H)	9.5(H,P)*	7.2(H,P)*	2.9(H)	S	S

Chromosome 5



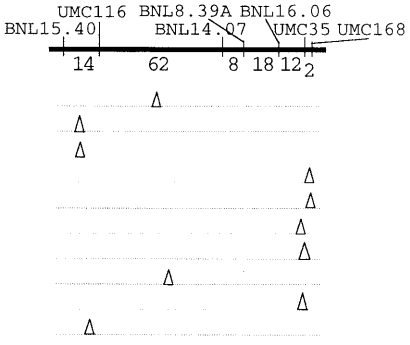
	R^2(%)(Dir,DR) PR	TL	BA	TO	LR JM	LR QxE
BIOM	4.3(L)	0.2(L)	6.7(H)	10.2(H,A)*	S	S
HKW	4.0(L)	1.1(L)	5.3(H)		S	S
MF	*17.0(L,A)**	*9.9(L)*	*10.1(L,d)**	*8.2(L)*	*S*	*S*
FF	*10.1(L,p)**	*10.1(L,o)**	*2.5(L)*		*S*	*NS*
TUR	9.5(H,A)*				S	
TBN	6.2(H,o)*	5.5(H,P)*	4.8(H)	9.4(H,p)*	S	NS

Chromosome 6



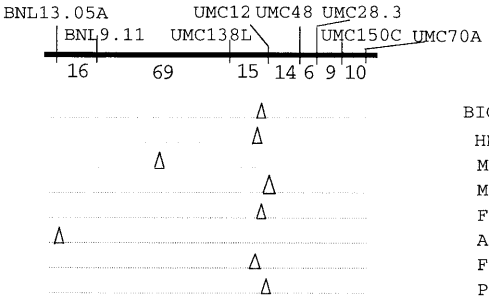
	R^2(%)(Dir,DR)				LR	
	PR	TL	BA	TO	JM	QxE
BIOM	0.5(L)	3.3(L)	4.0(L)	2.9(H)	S	S
FLN	3.0(H)	1.2(H)	5.0(H,A)*	0.8(H)	S	NS

Chromosome 7



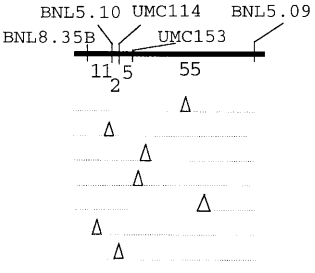
	R^2(%)(Dir,DR)				LR	
	PR	TL	BA	TO	JM	QxE
BIOM	*5.3(H,O)**	*3.1(H)*	*8.6(H)*	*4.3(H)*	*NS*	NS
YLD	7.0(H,O)*	1.3(H)	3.3(H)		NS	*NS*
HDX	8.7(H,O)*	0.1(H)	1.7(H)		S	S
HDX	0.2(L)	4.8(L,D)*	0.2(L)		NS	S
HKW	0.3(H)	2.1(L)	4.5(H,D)*		S	S
MF	0.6(L)	7.8(H,d)*	1.8(H)	0.5(L)	S	S
FF		*6.0(H,p)**	*2.6(H)*	*0.6(H)*	*NS*	*NS*
ASI		6.4(L,A)*	10.5(L,o)*	6.0(L)	S	S
FLN	1.7(L)	7.7(H)	5.4(H)	0.1(L)	S	S
TBN	37.4(L,d)*	44.6(L,d)*	29.3(L,d)*	20.6(L,d)*	S	S

Chromosome 8



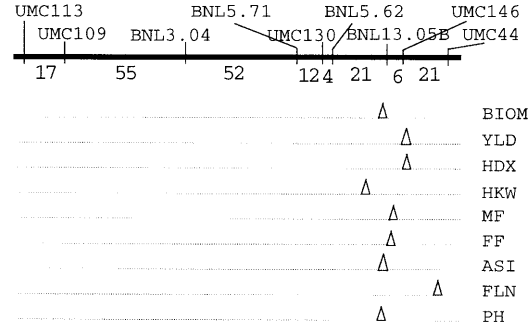
	R^2(%)(Dir,DR)				LR	
	PR	TL	BA	TO	JM	QxE
BIOM	10.7(L,O)*	7.8(L)	8.8(L,O)*	2.1(L)	S	NS
HDX	2.0(L)	0.4(H)	13.5(H,p)*		S	S
MF	4.2(L,o)*	4.5(L)	3.7(L,o)*	4.9(L,o)*	S	S
MF	6.2(L,P)*	8.1(L,P)*	18.2(L,P)*	17.2(L,P)	S	S
FF		2.4(L)	10.6(L,D)*	13.9(L,P)*	S	S
ASI		0.7(L)	2.4(H)	2.8(H)	S	S
FLN	7.8(L,A)*	5.3(L)	20.3(L,P)*	19.4(L,P)*	S	S
PH	10.0(L,O)*	13.2(L,P)*	17.6(L,D)*	13.5(L,O)*	S	S

Chromosome 9



	R^2(%)(Dir,DR)				LR	
	PR	TL	BA	TO	JM	QxE
HKW	5.0(H)	4.0(H)	9.6(H,p)*		S	S
MF	1.0(H)	1.1(H)	14.6(L,P)*	5.7(L,A)*	S	S
FF		0.9(H)	22.1(L,A)*	4.8(L)	S	S
ASI		2.5(L)	9.4(L,d)*	0.8(L)	S	S
RUST	5.6(H,o)*				S	
FLN	9.3(H,O)*	3.7(H)	1.9(L)	1.6(L)	S	S
PH	2.5(H)	0.9(L)	8.6(L,O)*	1.9(L)	S	S

Chromosome 10



	R^2(%)(Dir,DR)				LR	
	PR	TL	BA	TO	JM	QxE
BIOM	9.9(L,P)*	7.3(L,O)*	12.2(L,O)*	5.5(L)	S	S
YLD	5.8(L)	11.7(L,P)*	8.9(L)		S	S
HDX	5.4(L)	9.7(L,A)*	3.4(L)		NS	NS
HKW	8.4(L,D)*	0.3(L)	2.4(L)		S	NS
MF	3.6(L,p)*	1.8(L)	1.9(L)	1.1(L)	S	S
FF		5.9(H,p)*	0.9(H)	0.9(L)	S	S
ASI		8.0(H,d)*	11.4(H,o)*	0.3(L)	S	S
FLN	2.4(L)	1.4(H)	1.1(L)	2.6(L)	S	S
PH	7.5(L,P)*	1.7(L)	5.0(L,O)*	4.7(L,A)*	S	S

Fig. 1 (Continued)

($P < 0.05$) segregation distortions, which were skewed in favor of lowland alleles for one marker on each of chromosomes 1 and 3, four markers on chromosome 9, and in favor of highland alleles for two adjacent markers each on chromosomes 5 and 10. However, allelic frequencies for all marker loci were within the range of 0.40–0.60. Seven markers showed segregation for individuals from only one F_1 plant but not another, two each on chromosomes 1, 6 and 10, and one on chromosome 2.

Numbers of QTLs detected

The position and characteristics of the QTLs identified by CIM over four sites are presented in Fig. 1 for all traits. Five to eight QTLs were detected for each trait, except for disease scores that were measured only at PR, where only one, three and two QTLs were significant for HM, RUST and TUR, respectively. Each detected QTL was usually significant only at one or two sites and very few were significant at three or four sites. This varied with traits: the number of QTLs significant at one site only versus two or more sites were 17 to 4 for yield components, 10 to 10 for flowering traits and 7 to 9 for morphological traits. The lack of significance of the same QTL in different sites parallels the change in the estimated effects over sites, as well as the test of QTL by site interaction. In addition, there were nine QTLs significant under joint analysis over sites but non-significant in any of the separate analyses at single sites. Seven QTLs were not significant under model (2) but were detected with model (3) and/or (4) by using flanking markers as cofactors. Likelihood profiles of these QTLs were all carefully inspected before they were accepted. Two pairs of linked QTLs were detected for HDX and PH, and one pair was found for YLD, HKW, MF and TBN. All QTL pairs detected on the same chromosome were at least 100 cM apart and their interaction with sites can then be tested independently.

Magnitude of the QTL effects

The R^2 value from the regression analysis [or from the estimated effects for QTLs detected only under model (3) or (4)] for each QTL at each site is listed in Fig. 1, along with the direction of the estimated effects. For comparison among QTLs, R^2 values averaged over sites were also calculated, and ranged from 1.3 to 33.0% of the total phenotypic variance (Table 6). R^2 values for yield components were all less than 9%, and generally lowest among all traits. Traits with QTL and R^2 values between 10 and 15% included MF, LFN, PH, and TUR. The largest R^2 value was found for TBN. Two QTLs were found on chromosome 2 and 7 with a total R^2 value of more than 50%. This result suggests that these are major genes. One QTL for HM also had an R^2 value of 22.0%. The R^2 values for the same QTL changed dramatically over sites, mirroring the pattern of QTL by site interaction. For the QTL with the largest effect on TBN (on chromosome 7) the R^2 value decreased from 44.6% in TL to 20.6% in TO.

The detected QTLs often showed a progressive change in effect from lowland (or highland) sites to highland (or lowland) sites, usually paralleling the mean temperatures of the sites. For example, for the QTL of HDX on chromosome 3, the value of R^2 was 13.0% in PR (T_{mean} = 21.3°C) and only 1.7% in BA (T_{mean} = 16.8°C). For two QTLs for ASI on chromosomes 1 and 2, the R^2 values were estimated as 20.1% and 12.5% in TL (T_{mean} = 24.6°C) but declined to 7.2% and 7.7% in BA, and accounted for only 1.0% and 1.2% of the phenotypic variation in TO (T_{mean} = 13.2°C).

In addition to the percent of phenotypic variance explained by each QTL, we calculated multilocus estimates of the percentage of the variance explained by all detected QTLs for each trait (Table 6). The total R^2 ranged from 24% for YLD to 61% for TBN. Again, QTLs for yield components had the lowest total R^2 values.

Table 6 Total numer of significant QTLs detected across sites and at each site, the origin of alleles providing positive additive effects (in parentheses: L=lowland; H=highland), and the estimated QTL effect (R^2) averaged over sites for each trait

Trait	No. of QTLs	Significant QTLs (origin of QTL with positive additive effect)				R^2 (%)	
		PR	TL	BA	TO	Range	Total
BIOM	7	4 (3L, 1H)	2 (2L)	2 (2L)	2 (2H)	2.7–8.7	34.5
YLD	5	4 (3L, 1H)	2 (2L)	0	NA[a]	3.8–8.8	24.3
HDX	8	2 (1L, 1H)	5 (5L)	1 (1H)	NA	1.7–8.9	34.3
HKW	7	3 (3L)	1 (1L)	3 (1L, 2H)	NA	2.3–7.1	31.7
MF	8	5 (1L, 4H)	4 (3L, 1H)	4 (4L)	3 (3L)	1.5–12.4	45.3
FF	6	NA	4 (1L, 3H)	3 (3L)	1 (1L)	2.6–9.6	38.0
ASI	7	NA	4 (2L, 2H)	5 (3L, 2H)	0	2.0–9.4	35.0
LFN	8	2 (1L, 1H)	2 (2H)	4 (1L, 3H)	1 (1L)	2.5–13.2	35.0
TBN	6	3 (3L)	4 (3L, 1H)	2 (2L)	4 (3L, 1H)	1.3–33.0	60.8
PH	8	4 (3L, 1H)	2 (1L, 1H)	4 (3L, 1H)	2 (2L)	1.3–13.6	40.3
HM	1	1 (1H)	NA	NA	NA	22.3	22.3
RUST	3	3 (1L, 2H)	NA	NA	NA	5.6–8.2	16.4
TUR	2	2 (2H)	NA	NA	NA	9.5–14.7	11.4

[a] NA signifies variable was not measured at that site

Direction of the QTL effects

Lowland and highland maize populations are the products of strong disruptive selection for adaptation to environments with low and high temperature, but especially to low values of T_{min} during the growing season. It is expected, therefore, that alleles from lowland lines should be more adapted to lowland environments and highland-derived alleles to highland environments. The QTL-mapping results of this study generally support this prediction. A typical example in terms of additive effects is the QTL on chromosome 2 for BIOM, which was significant with a R^2 value of 8.1% at TL (T_{mean} = 24.6°C) with the positive effect from the lowland parent allele. The same QTL was also significant at TO (T_{mean} = 13.2°C) with a R^2 value of 6.2% but here it favored the highland allele. Similar changes were found for most of the QTLs for yield components that showed significant QTL by site interaction.

If only QTL effects significant at a single site are considered (see Table 6), among 31 QTLs for yield components (at 27 different loci with some QTLs significant in more than one site), 23 were detected in PR and TL and eight in BA and TO (note that there was no grain yield data from TO). Among 23 QTL effects significant iPR and TL, 20 (87%) were in favor of the lowland alleles (with positive effects). Among the eight QTL effects significant in BA and TO, five (63%) favored highland alleles. In general lowland alleles showed a broader adaptation than highland alleles. In 16 of 27 QTL loci (59%) the direction of the estimated additive effects remained the same over all sites, though many were non-significant. Of these, all but four favored the lowland alleles, while only one for each of BIOM, YLD, HDX on chromosome 7 and HKW on chromosome 9 favored highland alleles.

Adaptation to specific sites here is directly measured by yield itself and its components. The association of other traits with adaptation was sometimes less obvious. Among six QTLs detected for the three disease scores at PR, all but one favor the lowland alleles as a means of increasing levels of resistance. Clearly, selection pressure against these predominantly lowland dieseases has been much higher in lowland than in highland germplasm, and resistant alleles are more likely to be obtained from lowland than from highland germplasm. For phenological traits, on the other hand, all lowland alleles delayed flowering in BA and TO, while the effects of highland alleles were to hasten flowering, probably to avoid cold temperatures and early frost in highland environments. Although an increase in MF may be associated with an increase in BIOM, it was associated with a reduction in HDX and in YLD at TL and BA (Table 5). Thus the association between QTLs for MF and adaptation was not clear. Although ASI was highly negatively correlated with yield, desirable alleles for short ASI in each site came from both parents with similar frequency, suggesting that there has been similar selection pressure for this trait in both types of germplasm.

In summary, the estimated QTL effects for phenological traits changed over sites only in magnitude, while the QTL effects for yield components changed in magnitude and direction as well. For morphological traits, such as LFN and TBN, about half of the QTLs had significnt effects at more than one site. For these QTLs no change in direction over sites was observed, though the QTLs had relatively large effects.

By inspection of the dominance ratio, 3 out of 31 QTL effects (10%) that were significant in at least one site for yield components were additive, 14 (45%) partially dominant to dominant, and 14 (45%) overdominant. Among QTLs that were partially dominant to overdominant, all but four, one for each yield trait, gave positive estimates for their dominant effects in a direction that would enhance adaptation regardless of the environment. For all other traits, 15 out of 72 (21%) QTL effects significant in a single site were additive, 36 (50%) were partially dominant to dominant, and 21 (29%) overdominant. The direction of the dominance effects were to reduce ASI (six of seven QTL effects), FF (three of four QTL effects) and MF (7 of 11 QTL effects), and to increase PH (all eight QTL effects). No tendencies were found for QTL effects on TBN and disease score. Obviously dominance effects generally increase the vigor of a line, and this was reflected in increased yield and its components.

QTL clusters

Many clusters of QTLs for different traits can be found along the genome by inspecting the estimated QTL positions in Fig. 1. Four regions were found to be relatively important for adaptation differences, with significant effects on several traits at more than one site. They included: (1) a region at the beginning of chromosome 1 between marker *umc94* and *umc53* which had the largest effects on HDX, FF and ASI among all detected QTLs, and favored the lowland allele; (2) a region at the beginning of chromosome 7 around marker *bnl15.40* which showed a major effect on TBN and significant effects on YLD and HDX in PR in favor of the highland allele; (3) a region on chromosome 8 between marker *umc138L* and *umc48* which had the largest effects on MF, LFN and PH, and which favored the lowland allele for BIOM in BA and the highland allele for HDX in BA; (4) the end region of chromosome 10 between marker *bnl5.62* and *umc44* which had the largest effects on BIOM and YLD, favored the lowland allele for these traits, and had relatively large effects on other traits as well.

Although predominant effects were not found in these four regions, the total R^2 values averaged over all sites are 28%, 24% and 27% for BIOM, YLD and HDX, respectively. The segment on chromosome 10 was stable over sites, and seems important for adaptation to a broad range of thermal regimes. Alleles from the lowland parent in this segment increased BIOM, YLD, HDX, HKW, MF, LFN and PH at all sites and decreased the values of

FF and ASI at TL and BA. The largest distances between estimated QTL positions for different traits within this segment were 20 cM between the QTLs of HKW and YLD, and 32 cM between the QTLs for LFN and HKW.

QTL clusters form the genetic basis of correlations between traits. For those yield components in this study that showed the largest correlation, linked QTLs were found on chromosomes 1, 3, 7, 8 and 10. For flowering traits, linked QTLs were found on chromosomes 1, 4, 5, 7, 8, 9 and 10. The significant positive correlation of BIOM with LFN and PH can be explained partially by QTLs on chromosomes 3, 8 and 10. The significant correlation between HM score and yield components at PR was linked with the presence of the only QTL for HM (R^2 = 22%) that was detected on chromosome 3 and linked with a QTL for YLD and HDX about 27 cM away from that site. Even though the distinction between close linkage and pleiotropy is still unable to be made in most of these cases, pleiotropic effects for some QTLs are expected, and can be easily understood from the physiological relationship that exists among these traits.

Discussion

Environmental factors influencing maize adaptation

Most of the observed differences in crop performance among sites are related to the duration and velocity of growth, and the efficiency with which radiation was converted to biomass and grain under the different temperature regimes. The duration from sowing to flowering was extended from 60 days in the warmest site (TL), to 142 days at the coolest (TO), and photoperiod differences between these sites were minor (Tables 1 and 2). The largest biomass and grain yields were observed in BA followed by TL, suggesting that in this population of lines the optimal temperature for production was one with average temperatures of around 17–20°C. Other studies confirm this general conclusion. Cooper (1979) reported that when maize adapted to the highland tropics was planted over a range of altitudes (average air temperatures 15–22°C), maximum grain yields were associated with a mean air temperature of only 15°C, and crop growth rates were more closely related to the total radiation received than to temperature. Wilson et al. (1973), in a similar trial, reported maximum grain yields at a mean temperature of 20.5°C. Comparisons of yields at different elevations in Mexico led to similar conclusions (Goldsworthy and Colegrove 1974; Goldsworthy et al. 1974). Temperate and warm-adapted hybrids, observed in environments with mean temperatures of 18–29°C, gave maximum grain yields at an average temperature of about 20°C (Muchow 1990), in general agreement with the controlled environment studies reported by Hardacre and Eagles (1989). Simulation suggested that the principal reason for yield differences among thermally diverse environments was not variation in incident daily radiation, but simply variation in the duration over which the crops intercepted the radiation (Muchow et al. 1990). High temperatures accelerate leaf senescence and thus reduce the opportunity to capture radiation. Hardacre and Turnbull (1986) reported that leaf area durations of two Corn Belt hybrids were greatest at 20°C.

A good index of potential yield of a site would be the ratio of radiation received to elapsed thermal time. This ratio was in the order of PR < TL < BA < TO (Table 1), and for PR, TL and BA this paralleled the order of yield increases (Table 2). We believe that this ratio explains much of the low grain yields observed in the tropics when compared with cooler temperate zones (see for example Chaing 1981), and explains why yields in the low-radiation PR site were lower than would be expected simply from its temperature regime. Toluca, with the highest ratio of radiation to thermal time but second lowest yields, was a notable exception to this relationship. We conclude that temperatures of 5°C or less, which occurred on 45% of the nights during crop growth at this site, resulted in impaired chlorophyll production (chlorosis was very obvious in some lines) and damage to the photosynthetic mechanism of the crop (Miedema 1982), leading to a much reduced radiation-use efficiency.

Total leaf number and TBN were smallest in PR, and may be an effect of the short photoperiod (Table 1) at that site (Ellis et al. 1992). The reduction in LFN at PR was not, however, reflected in reductions in real or thermal time to MF. Lafitte et al. (1997) reported a general decline in LFN with increasing mean temperature in similar studies at the same sites, but this was not evident at TO in the present experiment. Plant height was shortest at PR and TO and greatest at TL, a pattern among sites which has been previously observed among full-vigor cultivars (CIMMYT, unpublished data). Despite previous reports (Lafitte and Edmeades 1997; Lafitte et al. 1997), temperature did not have a marked effect on the mean value of HDX at PR, TL and BA, nor on ASI, although HDX could not be observed in the coolest site, TO. When assimilation is reduced by stress at flowering, HDX and ASI are negatively correlated (Bolaños and Edmeades 1993; Ribaut et al. 1996), suggesting that there were not large differences in assimilate availability around the flowering period at PR, TL and BA.

Comparison of results of QTL mapping with line performance

Previous experiments with highland and lowland cultivars grown in highland, lowland and mid-altitude sites have clearly shown a dramatic cultivar × environment interaction for yield, yield components and flowering traits (Eagles and Lothrop 1994; Lafitte and Edmeades 1997; Lafitte et al. 1997). Those studies showed that highland cultivars were generally characterized by rapid emergence from cool soils, earliness to flower, relatively fewer leaves, few tassel branches, a lower leaf area index, and shorter plant height relative to their lowland counterparts. A marked decline in HDX was identified by the

authors as the main reason for the low grain yield of highland-adapted cultivars in hot lowland environments (T_{mean} = 28°C). The results of the present experiment generally agree with these observations. Grain yield, BIOM and HDX showed the largest G × E variance relative to the corresponding genetic variances (Table 3). Kernel weight was the least affected by environments (Table 3), and is in general much less affected by the environment than kernel number (Fischer and Palmer 1983). In general, the presence of highland alleles was associated with earliness to flower, fewer leaves and lower biomass and grain production (Table 4). Harvest index appeared to be the factor driving differences in specific line adaptation, and this varied from 0.06 to 0.48 in the three environments at which it was measured. The decline in HDX of highland cultivars in lowland sites, previously reported by Lafitte and Edmeades (1997), was also evidenced here as a negative correlation between HDX and the proportion of highland alleles in the genome at PR and TL, but not at BA (Table 4). Had these lines been evaluated in the hot PR summer environment it is likely this correlation would have been much larger. Since ASI is considered to be an indicator of the partitioning of current assimilates to the developing ear at flowering (Bolaños and Edmeades 1993), it is not surprising that ASI and HDX were highly correlated at these two sites.

Characteristics of QTL effects associated with adaptation

Adaptation of germplasm to a specific thermal regime is expected to be the consequence of long-term natural and artificial selection resulting in allelic substitution at many loci. The results of this experiment reveal no major gene for yield components, which are considered here to be a direct measure of adaptation. Each detected QTL accounted for less than 10% of the total phenotypic variation in the population over sites. Although most of the detected QTLs showed significant effects at only one or two sites, the total effects of the identified QTLs accounted for about 60% of the genetic variation for BIOM, about 40% for YLD, and about 50% for HDX and HKW in each site, where this proportion was determined by dividing the R^2 value by the heritability of each trait.

An important observation is that a few chromosome regions present significant effects on several traits, including yield components, flowering and morphological traits. These clustered QTLs may have important effects on developmental processes in different environments, and consequently, pleiotropic effects on several traits may well be detected. A clustering of QTLs for several traits was also observed by Xiao et al. (1996) in a cross between two subspecies of rice, and they suggested that the pleiotropic effects were the major reason for this phenomenon.

Although large differences in TBN between highland (with low TBN) and lowland (with high TBN) cultivars are commonly observed (Eagles and Lothrop 1994), the association of this trait with adaptation is largely unknown. In this study a QTL with major effects for TBN [R^2 = 33%] was found on chromosome 7. A significant correlation of this chromosome region with YLD and HDX was also observed. Lowland alleles in this region contributed positively to TBN, but negatively to YLD and HDX at all sites. The total distance between the QTLs for the three traits was only 3 cM, and to another QTL for BIOM only 33 cM. However, the distinction between close linkage and pleiotropy cannot be made in either case.

Progressive, systematic changes in estimated QTL effects were found for most QTLs for almost all traits, and these paralleled thermal differences among sites. Most of these changes were only in magnitude, and an exchange of favorable alleles from one source to the other as the T_{mean} of sites increased only occurred for a few QTLs of yield components. Most alleles for yield components expressed positively and most strongly in environments closest to their origin, and alleles with major effects in the environment of their origin often had relatively minor effects elsewhere. Some alleles tended to be favorable in all sites, and more often they were from the lowland than from the highland parent, a finding consistent with the observation that highland maize tends to be narrowly adapted (Eagles and Lothrop 1994). Taken together, these results present a strong case for a joint analysis of QTLs over sites so that trends responsible for genotype × environment interactions can be clearly distinguished. In comparisons of the QTL-mapping results from different populations and testing sites, Beavis et al. (1994) found only a few QTLs in common even when correlations of performance among sites were intermediate to high. The separate analysis of each site in this study confirms this finding. It is clear that in QTL-mapping studies the joint analysis over several environments can provide more useful information than separate analyses for making breeding decisions, especially when the major cause of environmental differences is known.

Implications of germplasm improvement

The results of this experiment suggest that biomass, grain yield and the harvest index of highland germplasm grown in environments such as PR and TL could be enhanced by the judicious introgression of specific genomic regions from lowland germplasm. This would undoubtedly lead to broader adaptation in this germplasm and an increase in its general utility, though at a potential cost of susceptibility to highland diseaes. Consistent with this hypothesis is the degree of success enjoyed by the CIMMYT Highland Maize Breeding Program in introgressing genetic variation from lowland and temperature sources into highland germplasm (Eagles and Lothrop 1994).

Experiments with cultivars indicate a smaller degree of yield loss for the lowland cultivars from exposure to

highland cool temperature than that for highland cultivars exposed to the warm tempeatures characteristic of the lowlands. This is perhaps because lowland germplasm has been exposed to a wider array of environments during development. Germplasm adapted to warmer environments, however, could still benefit from the introgression of highland traits. For example, the capacity of temperate germplasm to germinate and emerge from cool soils in spring has been enhanced by the conventional introgression of highland germplasm (Hardacre and Eagles 1989; Eagles and Lothrop 1994), though this has been hindered by linkage drag resulting in poor root quality and lodging susceptibility.

An attempt to derive a single population, Largo del Dia, with broad thermal adaptation by intercrossing tropical cultivars from diverse thermal backgrounds has met with mixed success (Lafitte et al. 1997). The resulting population exhibited an unique response of crop development to temperature and stable grain yield over a wide range of thermal environments. In any single environment, however, it was out-yielded by cultivars possessing specific adaptation to that environment, probably because alleles lacking adaptation to that environment were also present. The present study appears to confirm these results: effects of most QTLs important to adaptation gradually change with temperature, and broad adaptation is possible only across a moderate range of temperature.

Utilization of genetic variability from unadapted sources using conventional backcrossing methods has thus proved to be a difficult and rather inefficient process. The use of molecular markers to target the transfer of desirable traits, without the linkage drag of less-useful genes from other regions of the donor genome, could greatly increase the efficiency of this process (Tanksley and Nelson 1996). Marker-assisted selection could be used to transfer alleles, such as those identified here on chromosome 7, from highland to lowland germplasm to improve performance in lowland environments. Selection for the QTLs identified on chromosome 10 will, on the other hand, provide useful genetic variation when selecting for broad adaptation. Available genetic variation for broad adaptation in highland germplasm appears to be relatively limited, and this could provide the improvements in grain yield and partitioning in warmer environments that this class of germplasm requires.

In conclusion, despite the possible inadequate representation of these two major germplasm classes by the two inbred lines used in this study, results are in good agreement with previous findings from cultivar evaluations. This study has provided much more detailed information on the relative importance of genomic segments and their effects on adaptation to thermally diverse sites, and has increased our understanding of the genetic basis of adaptation differences between highland and lowland germplasm. When environmental differences are as large as those experienced in this study, distinct sets of genes (or alleles of three genes) would be required for specific adaptation, and although genetic sources for wide adaptation may exist, they will be limited. Using this information and molecular markers it is now possible to accumulate alleles providing adaptation to a specific environment in a single genotype that would then possess broad adaptation, and to do so more efficiently than by conventional means. This should enhance the stability of grain yield across environments at little or no cost to yield at specific sites. Large-scale adoption of such a strategy should, however be preceded by a broader search in more lines and populations for genomic segments with specific and/or broad adaptation that can be directly used in a breeding program.

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