Molecular-Marker-Mediated Characterization of Favorable Exotic Alleles at Quantitative Trait Loci in Maize

M. Ragot,* P. H. Sisco, D. A. Hoisington, and C. W. Stuber

ABSTRACT

Exotic maize (*Zea mays* L.) germplasm, shown to be useful for developing improved temperate cultivars, has remained little used partly because of many inherent shortcomings. Five F_2 populations, developed from South American and U.S. germplasm, were used to detect favorable factors of exotic origin at quantitative trait loci (QTL) with isozymes and RFLPs. A number of traits of agronomic importance, including grain yield, were measured on F_2 individuals and/or F_3 families grown in several environments. Many QTLs, mostly with small effects, were identified. Major QTLs for grain yield and number of ears per plant were located on chromosomes 3 and 6. Stability of QTLs across environments was high. Favorable alleles of exotic origin were found at QTLs for several traits including grain yield and number of ears per plant. Most of these alleles also showed undesirable effects on other traits, however. Nevertheless, the superiority of exotic alleles over adapted alleles was demonstrated clearly at a few QTLs, reaffirming the usefulness of exotic germplasm for temperate maize breeding.

For most cultivated species, especially maize, the amount of genetic variability currently used in breeding programs represents only a small proportion of the existing genetic diversity. A survey of commercial maize varieties (Goodman, 1985) revealed that only about 4% of the total U.S. maize acreage was planted with cultivars containing some (10–25%) non-U.S. germplasm. Furthermore, because breeders often intermate or self-fertilize plants in elite varieties and then select the best progeny, high levels of relatedness are found among the parents of current elite cultivars. Efforts have been made to enhance the germplasm base of the U.S. maize crop through the use of exotic germplasm [as defined by Hallauer (1978) and Goodman (1985)].

Exotic sources of favorable genetic factors for a number of agronomically important traits, including grain yield, have been identified (Stuber, 1978; Holley and Goodman, 1988). Development of purely exotic maize populations adapted to northern environments has been successfully achieved (Hallauer and Sears, 1972; Hallauer, 1978; Holley and Goodman, 1988). However, comparisons between partially exotic and adapted germplasm for their agronomic performance gave mixed results. While Holley and Goodman (1988) reported the development of testcrosses with 50% exotic germplasm performing comparably with commercial checks, Gerrish (1983), Crossa et al. (1987), and Crossa and Gardner (1987) found that populations containing only adapted germplasm yielded significantly higher than partially exotic populations. Although it has been demonstrated that exotic sources of maize germplasm contain desirable characteristics, their use has remained limited. Two potential causes of this limited use are (i) the difficulty of reliably identifying favorable exotic sources, and (ii) the presence of deleterious linkages between favorable and unfavorable genes in exotic germplasm (Goodman, 1985).

Because genetic factors underlying quantitative trait expression can be studied individually through the intermediate of linked qualitative factors (Sax, 1923; Tanksley et al., 1982; Stuber et al., 1980; Keim et al., 1990), molecular markers should prove useful for identifying favorable factors in exotic maize germplasm, and transferring them into adapted germplasm. QTLs have been identified for a number of traits in various crop species including tomato (*Lycopersicon* spp.) (Paterson et al., 1988; Tanksley and Hewitt, 1988), soybean (*Glycine* spp.) (Keim et al., 1990), and maize (see Stuber, 1992 for a review). Significant associations between changes in allele frequencies at isozyme loci and selection for improved grain yield were shown to exist in adapted maize germplasm (Stuber et al., 1980), demonstrating the utility of using molecular markers for tagging QTLs.

In this study, we used isozymes and RFLPs to detect and characterize QTLs for a number of agronomically important traits, including grain yield, in five different partially exotic populations. Our goal was to identify favorable factors of exotic origin that could be used to broaden the genetic base of U.S. maize germplasm and participate in continuing improvement of agronomic performance. Because of our interest in detecting all QTLs present, we used single-factor analyses based on the general linear model (Soller and Brody, 1976), interval mapping (Lander and Botstein, 1989), and selective genotyping (Lander and Botstein, 1989; Darvarsi and Soller, 1992) for the identification of QTLs. By evaluating not only F_2 individuals but also their selfed F_3 progeny grown at four locations, we were able to investigate the stability of QTLs across genetic structures and environments. Comparisons among populations provided a basis for the assessment of QTL stability across genetic background, which will be discussed elsewhere.

MATERIALS AND METHODS

Plant Material

A number of maize populations were derived from the breeding program outlined below, which involved both temper-

M. Ragot, Ciba Seeds, CH-4002 Basel, Switzerland; P.H. Sisco, Dep. of Crop Science and USDA-ARS, North Carolina State Univ., Raleigh, NC 27695-7620; D.A. Hoisington, CIMMYT, Apartado Postal 6-641, 06600 Mexico DF, Mexico; and C.W. Stuber, Dep. of Genetics, Dep. of Crop Science and USDA-ARS, North Carolina State Univ., Raleigh, NC 27695-7614. Part of a dissertation submitted by the senior author to the graduate school of the North Carolina State Univ. in partial fulfillment of the Ph.D. degree. Received 5 Nov. 1993. *Corresponding author (ragotm@abru.cg.com).

Published in Crop Sci. 35:1306–1315 (1995).

Abbreviations: cM, centimorgan; QTL, quantitative trait locus; LOD, Likelihood of Odds; RFLP, restriction fragment length polymorphism; SS, sum of squares.

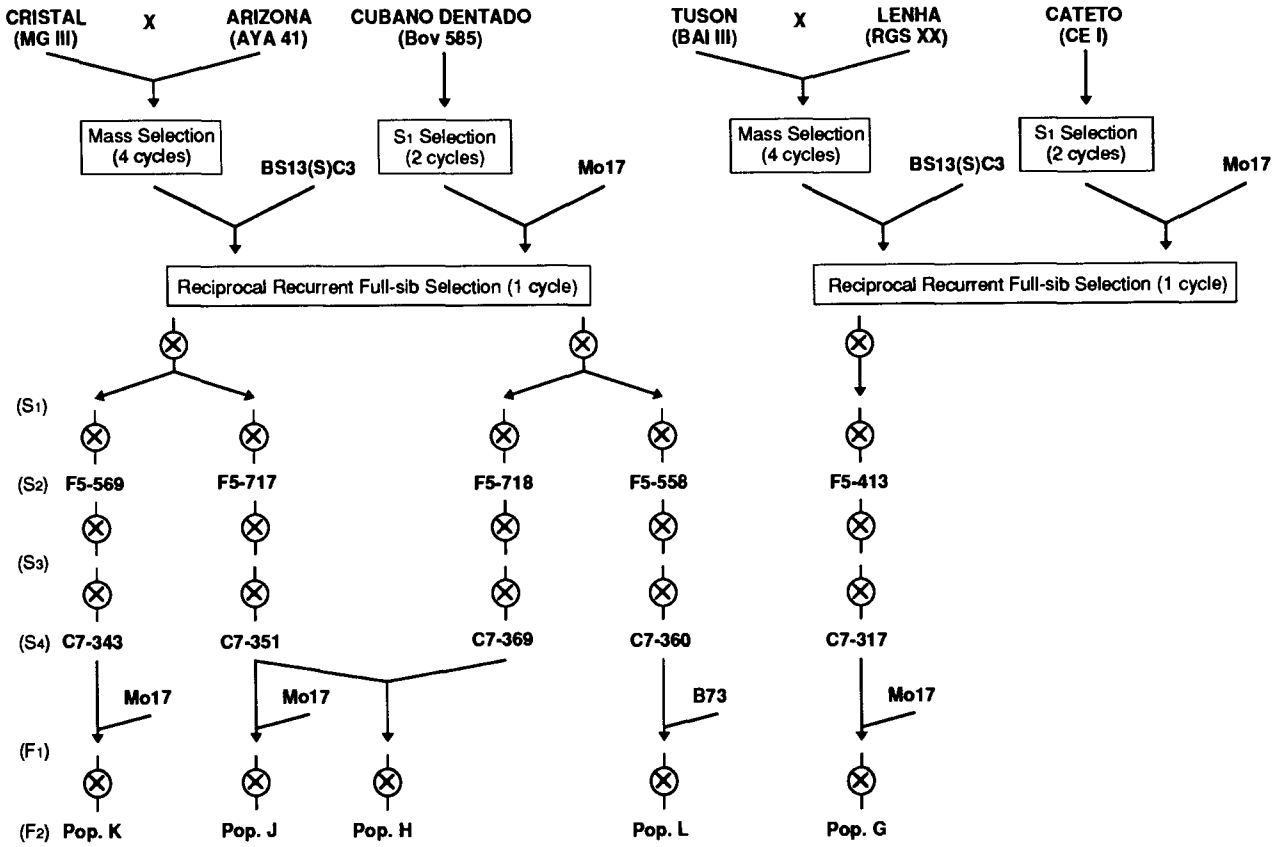

Fig. 1. Breeding program and procedures used to develop the five F_2 maize populations studied.

ate (U.S.) and exotic (Latin American) germplasm (Fig. 1). The exotic racial collections involved in the development of the five populations studied herein were Cateto (CE I), Cristal (MG III), Lenha (RGS XX), and Tuson (BAI III) from Brazil; Arizona (Aya 41) from Peru; and Cubano Dentado (Bov 585) from Bolivia. These collections are described in detail by Paterniani and Goodman (1977) (Brazilian collections), Grobman et al. (1961) (Peruvian collection), and Ramirez et al. (1960) (Bolivian collection). Exotic racial collections underwent several cycles of testing and selection for grain yield, earliness, and reduced plant height in North Carolina (Stuber, 1978), before being crossed to either BS13(S)C3, a selected population from the Iowa Stiff Stalk Synthetic, or Mo17, a widely used inbred line from the Lancaster heterotic group (Stuber, 1986). After one cycle of reciprocal recurrent full-sib selection for grain yield and, to a lesser extent, reduced plant height and resistance to lodging, these partially exotic populations were used to develop inbred lines by single-seed-descent. S_4 lines were either intermated or crossed to B73, a widely used inbred line from the Iowa Stiff Stalk Synthetic group, or to Mo17. F_1 hybrids were evaluated at several locations in 1988. They also were selfed to produce F_2 populations. The five populations studied (referred to as G, H, J, K, and L) were selected from the group of F_2 populations based on both the agronomic performance of their corresponding F_1, and their number of polymorphic isozyme loci (Table 1). F_2 individuals from each of the five selected populations were later selfed to develop F_3 families. Not all F_2 individuals produced enough F_3 seed for family evaluation, resulting in fewer F_3 families phenotyped than F_2 individuals (Table 1). This was accentuated in population K because of male sterility, later found to be the result of *cms-S* inherited from Cristal (Ragot et al., 1992).

Phenotyping

For each F_2 population, approximately 400 seeds (Table 1) were germinated in a growth chamber; the plants were sampled for isozyme genotyping and transplanted in the field at Clayton, NC, where they were evaluated in the summer of 1989. For all field evaluations (both F_2 and F_3 generations), the five populations were considered as five separate experiments and, unless otherwise specified, were all treated identically. Traits

Table 1. Genotyping and phenotyping designs used for the study of five F_2 maize populations from crosses of Latin American and U.S. germplasm.

	Genotyping				Phenotyping		
	No. of mapped loci		No. of F_2 individuals		F_2	F_3	
Population	Isozymes	RFLPs	Isozymes	RFLPs	No. of individuals	No. of families	No. of locations
G	12	23	404	56	396	352	4
H	12	34	436	384	419	387	4
J	12	26	428	58	423	395	4
K	10	30	353	54	324	256	3
L	10	31	366	56	352	308	4

recorded on F_2 individuals included both vegetative and reproductive characteristics (Table 2).

F_3 families were grown in two-row plots with one replication at each of four locations in North Carolina (Clayton, Lewiston-Woodville, Plymouth, and Kinston) in the summer of 1990. Because of insufficient seed availability, population K was not grown at Kinston. Within populations, F_3 families were randomly grouped into sets of 24 families. Set composition was kept constant across locations. At each location, sets were randomly assigned to blocks. Sets from a given population were grown on contiguous blocks, and populations were grown separately from one another. At each location and within each set, F_3 families were randomly assigned to experimental plots. F_3 phenotypes were obtained either from single plant or plot measurements. They included a number of traits previously recorded on F_2 individuals, as well as others of major agronomic importance, such as grain yield (Table 2).

Genotyping

Isozymes and RFLPs were used as genetic markers. Isozyme genotypes were obtained from coleoptilar sections, following the procedures described by Stuber et al. (1988). RFLP analyses of the parental lines and of BS13(S)C3 were conducted with radio-labeled probes (Feinberg and Vogelstein, 1983), as reported by Sisco (1991), except that genomic DNA was digested only with restriction endonucleases *Bam*HI, *Eco*RI, and *Hin*dIII. RFLP analyses of F_2 individuals involved non-radioactive techniques described in detail by Hoisington (1992). RFLP probes were obtained from Brookhaven National Laboratory (BNL), the University of Missouri at Columbia (UMC), and Native Plants, Inc. (NPI).

Informative polymorphisms were identified by comparing the marker genotypes of the two parents (B73, Mo17, or S_2 lines from which the S_4 lines had been derived) of each F_2 population. More than 100 RFLP probes were tested, each with the three restriction endonucleases listed above. For each population, each F_2 individual was genotyped at all polymorphic isozyme loci (Table 1). For RFLPs, a number of probe-enzyme combinations were selected (Table 1) from among all informative polymorphisms, the criteria being the simplicity of their RFLP patterns and the coverage of the maize genome. One to seven informative polymorphisms were identified and selected on each chromosome for all populations except population G where no informative marker was found on chromosome 7. Each F_2 individual from population H was genotyped at each of the selected RFLP loci. Only selected F_2 individuals from populations G, J, K, and L were genotyped with RFLPs, according to the strategy of selective genotyping proposed by Lander and Botstein (1989). Selective genotyping was based on average F_3 grain yield (A-GY). Only those F_2 individuals whose average F_3 grain yield was among the 7.5 to 10.5% most extreme (high and low) phenotypes were genotyped with RFLPs (Table 1).

Data Analysis

Phenotypic means, variances, and correlations were computed for each of the five populations and all traits listed in Table 2, by SAS (SAS Institute, 1989).

Segregation ratios of individual markers were determined at all segregating marker loci for each of the five populations studied. Chi-square values were computed for population H to test for deviations from the expected 1:2:1 (codominant markers) or 3:1 (dominant markers) frequencies. Because populations G, J, K, and L were selectively genotyped at RFLP loci, RFLP segregation ratios could not be meaningfully tested for goodness-of-fit to any expected ratio. Therefore Chi-square values were not computed for those populations.

Linkage maps were constructed from pairwise and multipoint linkage analyses, performed with MAPMAKER (Lander et al., 1987). RFLP loci were mapped with respect to each other and to isozyme loci based on both linkage analyses and previous knowledge (Coe, 1992; Burr and Burr, 1991). Whenever the chromosomal location of an RFLP marker determined by linkage analyses was in contradiction with previous knowledge, the marker was mapped to the location provided by Coe (1992) unless there was strong evidence (LOD score greater than 5.0 for linkage to adjacent markers) to support the map location resulting from de novo linkage analyses. Map distances (Kosambi, 1944) were calculated by MAPMAKER (Lander et al., 1987). For populations G, J, K, and L, maps were constructed by both previous knowledge (Coe, 1992; Burr and Burr, 1991) and the map of population H. Results from linkage analyses were used only as indicators to confirm map locations (they could not be used directly to construct maps because populations G, J, K, and L had been selectively genotyped, most likely resulting in biased linkage estimates). When map distances

Table 2. Quantitative traits measured on F_2 plants and/or F_3 families of five F_2 maize populations.

Trait abbreviation	Trait name and description	Measured on F_2s†	Locations where measured on F_3s†
DTP	Number of days to pollen shed = number of days from planting to beginning of pollen shed (F_2), or number of days from sowing to 50% of individuals shedding pollen (F_3)	Yes	Cl, Le, Pl
DTS	Number of days to silking = number of days from planting to top ear silk emergence (F_2)	Yes	–
EAR	Number of ears per plant = number of ears per plant, calculated from the number of ears and the number of plants on a plot	No	Cl, Le, Pl
EHT	Ear height = distance between ground and top ear node at maturity	Yes	Cl, Le, Pl‡
GY	Grain yield = grain yield at harvest, adjusted for a standard grain moisture	No	Cl, Le, Pl, Ki
H2O	Grain moisture = ear moisture at harvest, calculated from ear weight at harvest and dry ear weight (F_2), or grain moisture at harvest (F_3)	Yes	Cl, Le, Pl, Ki
KR	Number of kernel rows = number of kernel rows on top ear	Yes	
LA	Leaf area = area of top ear leaf, calculated from leaf length and width	No	Cl, Le, Pl§
LN	Number of leaves = number of leaves above top ear	Yes	Cl, Le, Pl§¶
PHT	Plant height = distance between ground and tassel tip at maturity	Yes	Cl, Le, Pl
TB	Number of tassel branches = number of tassel branches, including main and secondary branches	Yes	–

† F_2 measurements were single-plant measurements whereas F_3 measurements were, unless otherwise indicated, single-plot measurements. In some instances the prefix A has been added to the trait symbols to specify F_3 data averaged over all locations (Cl, Clayton; Le, Lewiston-Woodville; Pl, Plymouth; and Ki, Kinston).

‡ Plot average based on 10 individual plant measurements.

§ Plot average based on five individual plant measurements.

¶ Trait not recorded at any location for populations K and L.

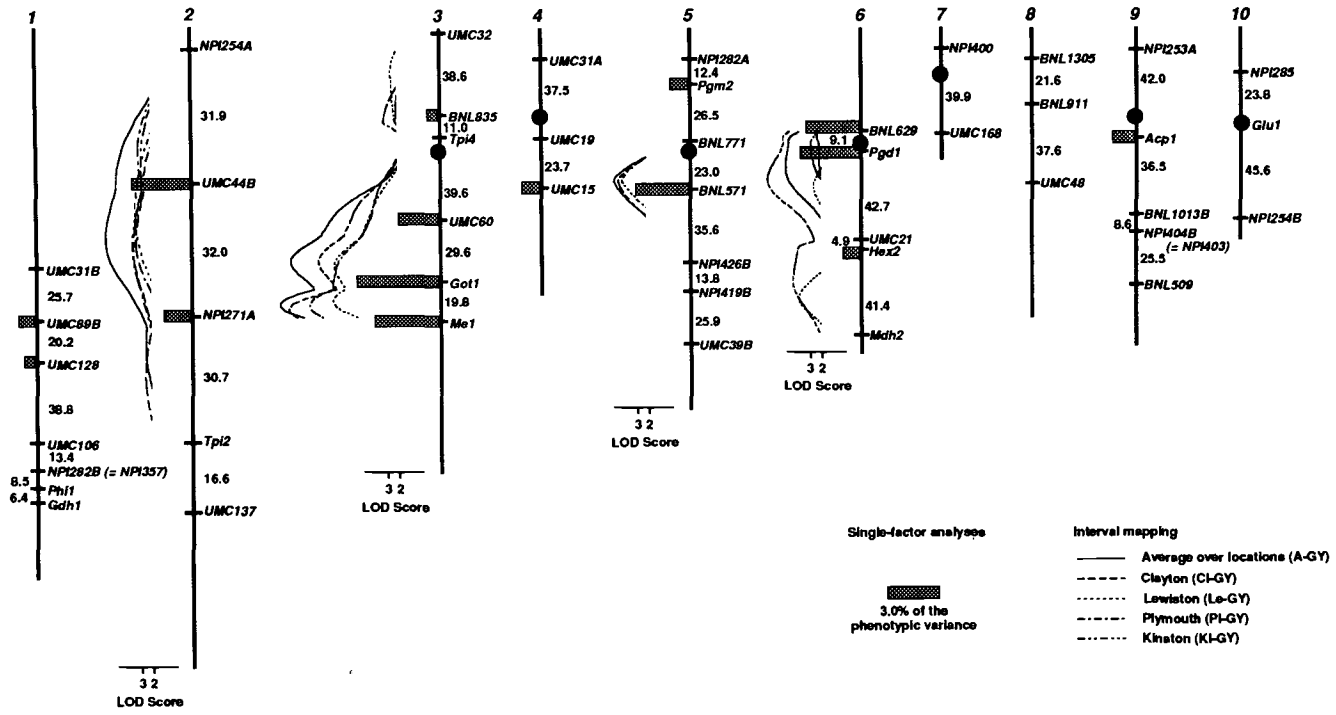

Fig. 2. QTL likelihood map, and map location and magnitude of effects of QTLs detected by single-factor analyses, for grain yield of F_3 families of population H. Interval mapping is represented by QTL likelihood plots showing LOD score curves exceeding the threshold of 2.0. Single-marker analysis is shown by bars protruding from the chromosome, whose length indicates the estimated phenotypic effect ($_sR^2$). Map distances between adjacent markers are given in centimorgans (Kosambi, 1944).

between two loci were not directly available from population H the means of the distances from other populations were used as the best estimates available.

Single-factor QTL analyses were performed only on data from population H. Because it is based on the standard linear model, the single-factor approach could not be used for the analysis of any dataset obtained through selective genotyping. Single-factor analyses involve comparisons of phenotypic means of marker genotypes and were performed for each marker locus by techniques of analysis of variance with SAS (SAS Institute, 1989). The model used to detect the presence of QTLs based on F_3 phenotypes was

$$P_{ijkl} = \mu + L_i + S_j + M_k + (L \times M)_{ik} + (S \times M)_{jk} + (L \times S)_{ij} + (L \times S \times M)_{ijk} + E_{ijkl} \quad [1]$$

where P = phenotype, L = location, S = set, M = marker genotype, and E is the error term. All sources of variation except the marker genotype were considered to be random effects. Because each set's genetic composition was constant across locations, sets could thus be considered as genetic entities, rather than environmental groupings. Therefore, sets were considered as factorial to, rather than nested within locations. Because the variances of $(L \times M)$ and $(L \times S \times M)$ were usually not significantly different from zero, the mean square of $(S \times M)$ was used as the error term for the computation of the F-statistic used to test significance of the marker source of variation, at the $\alpha = 0.01$ level. Significance at one marker locus was interpreted as the presence of a QTL in the vicinity of that locus. A second model of analysis of variance was used to account for variation among F_3 families:

$$P_{ijk} = \mu + L_i + S_j + F/S_{jk} + (L \times S)_{ij} + (L \times F/S)_{ijk} \quad [2]$$

where P, L, and S are as defined above, and F/S represents the variation among F_3 families within a set. All sources of variation were considered to be random effects. For each marker locus detecting the presence of a QTL we calculated

Table 3. Phenotypic means and standard deviations of F_3 generation averaged over F_2s and locations for all traits recorded on the five F_2 maize populations.

Trait (unit)	Population				
	G	H	J	K	L
A-DTP (count)	67.6/1.62†	70.4/1.76	69.7/2.09	67.4/1.62	69.3/2.08
A-EAR (count)	1.2/0.15	1.8/0.28	1.9/0.37	1.9/0.27	1.8/0.31
A-EHT (m)	0.70/0.13	0.82/0.13	0.86/0.15	0.68/0.11	0.89/0.17
A-GY (t ha^{-1})	3.23/0.78	3.31/0.90	3.57/1.06	4.77/0.85	3.77/0.95
A-H2O (g kg^{-1})	157/10.2	153/9.3	150/7.9	138/7.5	148/9.6
A-LA (m^2)	0.054/0.006	0.063/0.007	0.057/0.007	0.059/0.006	0.062/0.006
A-LN (count)	6.3/0.42	6.5/0.48	6.6/0.50	–	–
A-PHT (m)	2.16/0.21	2.30/0.26	2.42/0.19	1.94/0.19	2.42/0.26

† Mean/standard deviation.

Table 4. Map position and characterization of the QTLs identified by single-factor or interval mapping analyses for averages over environments of traits measured on F_3 families of population H.

		Single-factor analyses†					Interval mapping‡					
Trait	Ch§	Marker¶	$_sR^2$ (%)	$_sR^{*2}$ (%)	a#	d††	Interval¶	Closest marker	Peak LOD score	$_iR^2$ (%)	a'‡‡	d'§§
DTP	1	*UMC89B*	3.8	8.0	–	–	*UMC31B-UMC128*	*UMC89B*	3.65	4.5	−0.28	−0.61
DTP	2	*Tpi2*	1.3	7.5	–	–	–	–	–	–	–	–
DTP	3	*Got1*	1.9	9.0	***	**	–	–	–	–	–	–
DTP	4	*UMC15*	1.7	12.5	***	NS	–	–	–	–	–	–
DTP	5	*NPI419B*	1.8	8.7	***	NS	–	–	–	–	–	–
DTP	6	*UMC21*	1.0	10.1	***	NS	–	–	–	–	–	–
DTP	6	*Hex2*	1.6	10.7	***	NS	–	–	–	–	–	–
DTP	9	*Acp1*	3.6	13.1	***	NS	–	–	–	–	–	–
DTP	9	*NPI404B*	4.1	18.9	***	NS	*NPI253A-BNL5.09*	*BNL10.13B*	4.24	11.2	−0.82	−0.16
EAR	1	*UMC106*	2.1	6.2	–	–	–	–	–	–	–	–
EAR	3	*UMC60*	2.8	8.1	–	–	*Tpi4-Got1*	*UMC60*	4.99	18.5	−0.17	0.02
EAR	3	*Got1*	1.6	9.4	***	NS	–	–	–	–	–	–
EAR	3	*Me1*	3.5	13.9	***	NS	*Got1-End*¶¶	*Me1*	5.97	7.3	−0.11	0.03
EAR	4	*UMC15*	1.5	9.5	**	**	–	–	–	–	–	–
EAR	6	*BNL6.29*	5.3	16.5	***	NS	–	–	–	–	–	–
EAR	6	*Pgd1*	5.2	17.7	***	NS	*End-Mdh2*	*Pgd1*	9.89	35.3	0.24	0.02
EAR	6	*UMC21*	1.0	9.5	*	*	–	–	–	–	–	–
EAR	6	*Hex2*	1.6	11.6	***	NS	–	–	–	–	–	–
EHT	1	*UMC89B*	3.7	7.1	–	–	*UMC31B-UMC128*	*UMC89B*	4.69	8.7	−0.01	−0.02
EHT	1	*UMC128*	9.0	5.2	–	–	–	–	–	–	–	–
EHT	2	*Tpi2*	5.6	11.3	–	–	*NPI271-End*	*Tpi2*	6.76	10.3	0.02	−0.02
EHT	2	*UMC137*	1.8	6.7	–	–	–	–	–	–	–	–
EHT	3	*BNL8.35*	1.2	10.5	***	NS	–	–	–	–	–	–
EHT	3	*Tpi4*	1.6	10.5	***	NS	*UMC32-Got1*	*Tpi4*	3.58	14.1	−0.03	−0.01
EHT	3	*UMC60*	0.9	7.0	–	–	–	–	–	–	–	–
EHT	3	*Got1*	1.1	7.4	NS	***	–	–	–	–	–	–
EHT	3	*Me1*	1.3	11.7	***	NS	–	–	–	–	–	–
EHT	4	*UMC15*	1.3	9.2	**	NS	–	–	–	–	–	–
EHT	5	*BNL5.71*	2.6	11.6	***	NS	*BNL7.71-NPI426B*	*BNL5.71*	3.72	4.6	0.01	−0.01
EHT	5	*NPI419B*	1.6	6.5	NS	***	–	–	–	–	–	–
EHT	9	*Acp1*	1.9	11.9	NS	***	–	–	–	–	–	–
EHT	9	*BNL10.13B*	1.5	7.2	–	–	–	–	–	–	–	–
EHT	9	*NPI404B*	1.9	10.5	***	NS	–	–	–	–	–	–
GY	1	*UMC89B*	0.9	5.4	–	–	–	–	–	–	–	–
GY	1	*UMC128*	0.6	2.7	–	–	–	–	–	–	–	–
GY	2	*UMC44B*	3.4	11.8	***	*	*NPI254A-Tpi2*	*UMC44B*	5.95	13.8	0.65	0.55
GY	2	*NPI271A*	1.5	9.7	***	NS	–	–	–	–	–	–
GY	3	*BNL8.35*	0.6	8.9	**	NS	–	–	–	–	–	–
GY	3	*UMC60*	2.3	7.2	–	–	*Tpi4-Got1*	*UMC60*	11.25	27.4	−1.06	0.36
GY	3	*Got1*	4.7	15.6	***	*	–	–	–	–	–	–
GY	3	*Me1*	3.7	15.5	***	NS	*Got1-End*	*Me1*	12.19	17.9	−0.85	0.40
GY	4	*UMC15*	1.0	10.2	**	**	–	–	–	–	–	–
GY	5	*Pgm2*	1.0	7.8	***	NS	–	–	–	–	–	–
GY	5	*BNL5.71*	2.9	14.6	*	***	*BNL7.71-NPI426B*	*BNL5.71*	4.88	6.1	−0.20	0.61
GY	6	*BNL6.29*	2.9	12.3	***	NS	–	–	–	–	–	–
GY	6	*Pgd1*	3.3	11.1	***	NS	*End-UMC21*	*Pgd1*	5.14	14.2	0.77	−0.05
GY	6	*Hex2*	1.0	7.5	***	NS	–	–	–	–	–	–
GY	9	*Acp1*	1.2	9.8	NS	***	–	–	–	–	–	–
H2O	3	*UMC60*	0.4	4.5	–	–	*Tpi4-Me1*	*UMC60*	10.48	36.4	7.6	−7.3
H2O	3	*Got1*	2.1	12.9	–	–	–	–	–	–	–	–
H2O	3	*Me1*	0.8	10.6	***	NS	–	–	–	–	–	–
H2O	5	*Pgm2*	0.6	13.2	–	–	–	–	–	–	–	–
H2O	5	*BNL5.71*	0.7	8.9	–	–	*BNL7.71-NPI426B*	*BNL5.71*	3.07	3.8	0.7	−3.6
H2O	5	*NPI419B*	0.8	4.1	***	NS	–	–	–	–	–	–
H2O	9	*Acp1*	2.4	13.3	***	NS	–	–	–	–	–	–
H2O	9	*BNL10.13B*	1.7	7.8	–	–	*NPI253A-BNL5.09*	*BNL10.13B*	8.87	32.1	−6.9	−5.9
H2O	9	*NPI404B*	3.7	15.9	***	*	–	–	–	–	–	–
LA	1	*UMC89B*	0.8	8.5	–	–	–	–	–	–	–	–
LA	2	*NPI254A*	3.0	14.4	***	NS	–	–	–	–	–	–
LA	2	*UMC44B*	1.9	13.7	***	*	*End-End*	*UMC44B*	5.54	12.4	−0.003	0.001
LA	2	*NPI271A*	1.8	11.1	***	NS	–	–	–	–	–	–
LA	2	*Tpi2*	2.2	10.2	–	–	–	–	–	–	–	–
LA	3	*BNL8.35*	1.6	10.9	***	NS	–	–	–	–	–	–
LA	3	*Tpi4*	2.1	10.0	***	***	–	–	–	–	–	–
LA	3	*UMC60*	1.9	6.6	–	–	–	–	–	–	–	–
LA	3	*Got1*	3.9	13.9	***	*	*UMC32-End*	*Got1*	5.98	13.4	−0.003	0.001
LA	3	*Me1*	2.2	12.0	***	NS	–	–	–	–	–	–
LA	5	*BNL5.71*	2.0	10.8	***	*	*BNL7.71-NPI426B*	*BNL5.71*	4.52	5.6	−0.002	0.001
LA	5	*UMC39B*	2.6	5.8	–	–	*NPI419B-UMC39B*	*End*##	3.06	4.0	0.001	0.002
LA	6	*BNL6.29*	1.9	11.3	NS	***	–	–	–	–	–	–
LA	6	*UMC21*	2.1	10.4	***	NS	–	–	–	–	–	–
LA	6	*Hex2*	2.2	10.3	***	NS	–	–	–	–	–	–

continued

Table 4. continued.

		Single-factor analyses†					Interval mapping‡					
Trait	Ch§	Marker¶	${}_sR^2$ (%)	${}_sR^{*2}$ (%)	a#	d††	Interval¶	Closest marker	Peak LOD score	${}_iR^2$ (%)	a'‡‡	d'§§
LA	*8*	*BNL9.11*	2.0	9.6	***	**	–	–	–	–	–	–
LA	*9*	*Acp1*	3.5	12.9	***	***	*NPI253A-BNL10.13B*	*Acp1*	3.12	3.7	0.001	0.002
LA	*10*	*Glu1*	1.1	8.6	**	NS	–	–	–	–	–	–
LN	*1*	*UMC89B*	5.9	10.8	–	–	*UMC31B-Phi1*	*UMC89B*	7.15	13.1	−0.27	−0.04
LN	*1*	*UMC128*	2.7	7.7	–	–	–	–	–	–	–	–
LN	*1*	*UMC106*	1.9	8.2	–	–	–	–	–	–	–	–
LN	*1*	*NPI282B*	1.5	10.4	NS	***	–	–	–	–	–	–
LN	*1*	*Gdh1*	6.3	3.0	–	–	–	–	–	–	–	–
LN	*2*	*Tpi2*	2.0	12.0	–	–	–	–	–	–	–	–
LN	*2*	*UMC137*	1.2	6.3	–	–	*NPI271A-End*	*UMC137*	3.21	7.0	−0.16	−0.09
LN	*3*	*BNL8.35*	1.3	10.3	***	NS	–	–	–	–	–	–
LN	*3*	*Got1*	2.5	10.9	***	NS	–	–	–	–	–	–
LN	*5*	*NPI282A*	1.0	8.4	*	**	–	–	–	–	–	–
LN	*5*	*BNL5.71*	2.6	10.2	*	***	–	–	–	–	–	–
LN	*5*	*NPI419B*	1.5	8.3	***	NS	–	–	–	–	–	–
LN	*6*	*Mdh2*	3.1	12.0	***	NS	*Hex2-End*	*End*	4.06	4.7	0.14	0.03
LN	*9*	*Acp1*	4.4	12.2	***	**	*NPI253A-BNL10.13B*	*Acp1*	4.90	6.7	−0.16	0.10
PHT	*1*	*UMC89B*	5.0	7.7	–	–	*UMC31B-UMC128*	*UMC89B*	6.86	11.7	−0.05	−0.02
PHT	*1*	*UMC128*	2.8	9.0	–	–	–	–	–	–	–	–
PHT	*2*	*Tpi2*	1.0	7.7	–	–	–	–	–	–	–	–
PHT	*3*	*BNL8.35*	1.2	9.0	***	NS	–	–	–	–	–	–
PHT	*3*	*Me1*	0.9	9.4	*	**	–	–	–	–	–	–
PHT	*4*	*UMC15*	1.4	10.4	***	NS	–	–	–	–	–	–
PHT	*5*	*NPI282A*	1.3	8.2	***	NS	–	–	–	–	–	–
PHT	*5*	*Pgm2*	1.0	9.9	***	NS	*NPI282A-BNL5.71*	*Pgm2*	3.42	7.0	0.04	−0.005
PHT	*5*	*BNL7.71*	0.9	7.1	–	–	–	–	–	–	–	–
PHT	*9*	*Acp1*	1.6	12.2	NS	***	–	–	–	–	–	–

*, **, ***, and NS indicate respectively: significance at $P = 0.05$, 0.01, and 0.001, and non-significance.

† For single-factor analyses, QTLs are identified by the markers which detected significant ($P < 0.01$) marker-trait associations. The magnitude of the effect is given by ${}_sR^2$ and ${}_sR^{*2}$ at the marker locus.

‡ For interval mapping, QTL locations are indicated by the chromosome on which the QTLs lie, and the interval containing both the QTL and the chromosomal region around the QTL where LOD score >2.0. The magnitude of the effect of the QTL (i.e., at the peak LOD score) is given by ${}_iR^2$.

§ Chromosome.

¶ Map position of all marker loci are shown in Fig. 2.

Test of significance of the additive value.

†† Test of significance of the dominance value.

‡‡ Additive value of the male parent allele.

§§ Dominance value.

¶¶ The LOD score at the most distal marker was larger than 2.0, suggesting that the interval around the QTL where LOD score >2.0 extends beyond the most distal marker.

LOD scores were increasing towards the most distal marker, suggesting that the peak LOD score was reached beyond the most distal marker. The values of peak LOD score, ${}_iR^2$, a' and d' are those at the most distal marker.

the following two quantities (Stuber et al., 1992):

$$ {}_sR^2 = \frac{\text{SS}[M]}{\text{SS}[\text{Total}] - \text{SS}[\text{L}]} $$

where sums of squares were obtained from Model [1], and

$$ {}_sR^{*2} = \frac{\text{SS}[M] + \text{SS}[(S \times M)]}{\text{SS}[F/S]} $$

where SS[M] and SS[($S \times M$)] were obtained from Model [1], and SS[F/S] was obtained from Model [2]. The quantity ${}_sR^2$ represents the proportion of total within-location phenotypic variance that can be attributed to a single QTL at the marker locus. The quantity ${}_sR^{*2}$ is the proportion of total genetic variance that can be attributed to a single QTL at this locus (Stuber et al., 1992). Single-factor analyses based on F_2 phenotypes were done with the following model:

$$ P_{ij} = \mu + M_i + E_{ij} \qquad [3] $$

where P, M, and E are as defined earlier. Each QTL based on F_2 phenotypes was characterized by the quantity:

$$ R^2 = \frac{\text{SS}[M]}{\text{SS}[\text{Total}]} $$

which is similar to ${}_sR^2$ defined for QTLs based on F_3 measurements.

Whenever significant marker-trait associations were detected at several linked marker loci, all of these associations were assumed to be detecting the presence of a single QTL.

QTL likelihood maps were constructed for each population by the method of interval mapping (Lander and Botstein, 1989) with MAPMAKER/QTL. For population H, analyses were performed on all traits listed in Table 2. For populations G, J, K, and L, because of selective genotyping based on average F_3 grain yield, only traits that showed a phenotypic correlation of at least 0.3 ($P < 0.001$) with average F_3 grain yield were analyzed. Similar analyses were also conducted on a restricted dataset constructed from population H by selecting F_2 individuals based on average F_3 grain yield, in a manner analogous to the selective genotyping performed on the other populations. A LOD score threshold of 3.0 was used to declare the existence of a QTL (Stuber et al., 1992). Each QTL was characterized by the value:

$$ {}_iR^2 = 1 - \frac{\sigma^2}{\sigma_P^2} $$

calculated at the point of maximum LOD score, where σ_P^2 is the total phenotypic variance and σ^2 the phenotypic variance not controlled by this QTL. Results also were represented as QTL likelihood plots (Paterson et al., 1988), omitting regions with LOD scores lower than 2.0 (Fig. 2).

Results from analyses of different traits or environments

were compared by computing the Spearman rank correlation (LOD score correlation) between the LOD scores calculated every 2 centimorgans (cM) along the genome (distances based on Haldane's mapping function). Unless otherwise indicated, all LOD score correlations presented were significantly different from zero at the $\alpha = 0.001$ level.

The effects of environments on the identification of QTLs were assessed from single-factor analyses on population H by testing the significance of the ($L \times M$) source of variation in Model [1]. For interval mapping analyses, the presence/absence of QTLs was compared across locations and LOD score correlations were computed for all possible pairwise combinations between F_3 individual-location or average phenotypes. Genotype × environment interactions were assessed from the ($L \times S$) term in Model [1] with the variance of ($L \times S \times M$) as the error variance.

The type of gene action was assessed by calculating additive and dominance values for all significant QTLs. Contrasts were computed to test the significance of additive and dominance values for QTLs identified by single-factor analyses on population H. In cases where significant additive gene action was observed, the origin of the favorable allele was identified and reported.

RESULTS AND DISCUSSION

Marker Genotypes

Marker loci showing significant ($P < 0.05$) deviations from the expected genotypic frequencies were found on almost all chromosomes. They represented 17, 26, 25, 50, and 40% of the loci analyzed on populations G, H, J, K, and L, respectively. There were no differences between isozyme and RFLP loci. The presence of distorted segregation ratios, which we detected, is in agreement with what has been observed in other maize marker studies (Edwards et al., 1987).

Marker locations (Fig. 2, and data not shown) were generally in agreement with the maps of Coe (1992) and Burr and Burr (1991). Most of the differences observed between our maps and the above mentioned were consistent with known duplications of chromosomal segments: *1L-4S* (UMC23) and *3L-2L* (UMC2) (Coe, 1992; Burr and Burr, 1991), *1S-9L* (NPI404) (Koester, 1992), and *1L-5S* (NPI282) (V. Turner, 1989, personal communication) (*1L* and *4S* refer to the long arm of chromosome *1* and the short arm of chromosome *4*, respectively; duplicated loci are identified by adding the letter "B" to the probe name). We also observed a number of yet undocumented map positions: *UMC89B* on *1L*; *NPI426B*, *NPI419B*, and *UMC39B* on *5L*; *BNL10.13B* on *9L* (Fig. 2); and *NPI271B* on *3S* (data not shown). The finding of such map positions may be related to the partially exotic nature of the germplasm used in this study.

Phenotypic Variation

Phenotypic means and variances were computed for all traits and populations (Table 3). Grain yield (GY) was the most variable trait for all locations and populations. Grain moisture (H2O) and number of leaves (LN) showed limited variability with coefficients of variation rarely exceeding 10%. The least variability was found for number of days to pollen shed (DTP) and number of days to silking (DTS), whose coefficients of variation rarely reached 3% (data not shown).

Phenotypic correlations between grain yield and number of ears per plant (EAR) were consistently high ($P < 0.001$) except for population K where the correlation coefficient between grain yield and number of ears per plant measured at Lewiston was only 0.25 (data not shown). Ear height (EHT) and plant height (PHT) also were highly correlated, both across populations and environments. Correlations between F_2 phenotypes and averages of the corresponding F_3 phenotypes were always significantly different from zero except for grain moisture in populations G, J, K, and L (data not shown). However, measures of grain moisture were different between F_2 plants and F_3 families; the former being total ear moisture, including kernels and cob, and the latter being a direct measure of grain moisture.

Detection and Characterization of QTLs

QTLs were detected on all chromosomes except chromosome *7* (Tables 4 and 5; Fig. 2). Only one QTL was identified on chromosome *8*, and only two on chromosome *10*. The apparent paucity of QTLs on chromosomes *7* and *10* might have been caused by the lack of polymorphism, and therefore the limited number of marker loci genotyped on these chromosomes. On the contrary, given the number of markers analyzed on chromosome *8*, the detection of a single QTL on this chromosome likely reflects a lack of genetic effect. The highest concentrations of QTLs were found on *1L*, *3L*, *5L*, and around the centromere of chromosome *9*. QTLs for grain yield and QTLs for number of ears per plant were often detected in the same chromosomal regions (*3L* and *6L*). To a lesser extent, the same observation could be made for plant height and ear height (Table 4). Due to the use of a limited number of markers it was not possible to determine, in those regions containing multiple QTLs, the degree of linkage between these QTLs. Whether due to linkage or pleiotropy, the identification of both QTLs for grain yield and QTLs for number of ears per plant in the same chromosomal regions provided some genetic explanation for the high phenotypic correlations observed between these two traits.

QTLs were detected for all traits at least in one environment. A maximum of 36.4% of the total phenotypic variance ($_iR^2$) was explained by a single QTL [see grain moisture (H2O) in Table 4]. Values of $_iR^2$ were largely over-estimated for QTLs detected by selective genotyping (Table 5), and therefore were not taken in consideration for this discussion. Minimum values observed for the percentages of phenotypic ($_iR^2$) and genotypic ($_sR^{*2}$) variances explained by variation at individual QTLs were 3.7 [see leaf area (LA) in Table 4] and 2.7% [see grain yield (GY) in Table 4], respectively. In an F_2 population of tomato of size 350, Patterson et al. (1991) reported similar values for the smallest detectable effects. More than 40% of the QTLs detected in population H explained less than 10% of the phenotypic variance, reaffirming the polygenic basis of quantitative variation (Falconer, 1989), and showing agreement with observations of

Table 5. Map position and characterization of QTLs identified by interval mapping and selective genotyping for populations G, J, K, and L.

Pop	Trait	Ch†	Interval‡	Peak LOD score	$_iR^2$ (%)§	Closest marker	a¶	d#
G	A-GY	*4*	*UMC15-Cat3*	3.18	69.9	*Cat3*	−1.49	0.17
G	A-GY	*5*	*BNL5.71-UMC39B*	3.46	30.0	*UMC39B*	0.23	−1.31
G	A-GY	*10*	*UMC130-Glu1*	4.45	6.4	*Glu1*	0.40	0.32
J	A-LA	*3*	*BNL8.35-Got1*	5.06	28.7	*UMC60*	−0.005	−0.001
J	A-EAR	*3*	*E8-UMC60*	4.83	5.5	*Tpi4*	−0.12	0.00
J	A-EAR	*4*	*UMC31A-UMC15*	4.82	24.4	*UMC15*	−0.25	0.05
J	A-GY	*1*	*UMC128-NPI282B*	3.71	35.8	*UMC128*	1.20	1.08
J	A-GY	*4*	*UMC31A-UMC15*	9.11	39.7	*UMC15*	−1.37	0.91
J	A-GY	*6*	*Pgd1-Mdh2*	8.53	27.9	*Hex2*	1.00	1.00
K	A-EAR	*2*	*UMC44B-NPI212B*	3.79	45.8	*NPI271A*	−0.14	0.29
K	A-EAR	*3*	*UMC32-E8*	4.04	41.6	*E8*	−0.19	0.19
K	A-EAR	*3*	*E8-UMC60*	5.80	44.4	*BNL8.35*	−0.23	0.12
K	A-EAR	*6*	*UMC59-BNL6.29*	4.44	60.3	*UMC59*	−0.10	0.39
K	A-EAR	*6*	*BNL6.29-UMC132*	3.30	62.7	*UMC132*	0.12	0.39
K	A-EAR	*6*	*UMC132-Mdh2*	3.61	59.6	*UMC132*	0.11	0.38
K	A-EHT	*1*	*UMC31B-Acp4*	14.65	38.5	*Pgm1*	0.04	0.005
K	A-PHT	*1*	*UMC31B-Acp4*	12.49	38.0	*Amp1*	0.07	0.005
K	A-PHT	*9*	*End*††-BNL10.13B	5.15	8.9	*End*‡‡	0.03	0.01
L	A-GY	*3*	*BNL8.35-End*	7.83	11.2	*End*	0.74	0.24
L	A-EAR	*6*	*UMC59-UMC21*	4.67	9.1	*BNL6.29*	−0.13	0.05
L	A-EAR	*9*	*End-BNL10.13B*	4.70	30.2	*Acp1*	−0.24	0.01

† Chromosome.
‡ Interval containing both the QTL and the chromosomal region around the QTL where LOD score >2.
§ Magnitude of the effect at the QTL (i.e., at the peak LOD score).
¶ Additive value of the male parent allele.
# Dominance value.
†† The LOD score at the most distal marker was larger than 2.0, suggesting that the interval around the QTL where LOD score >2 extends beyond the most distal marker.
‡‡ LOD scores were increasing towards the most distal marker, suggesting that the peak LOD score was reached beyond the most distal marker. The values of peak LOD score, $_iR^2$, *a* and *d* are those at the most distal marker.

Stuber et al. (1992) for grain yield in maize, and Paterson et al. (1991) for fruit size, soluble solids concentration, and pH in tomato. It is likely that additional QTLs with small effects might have been detected if larger populations had been analyzed.

Additive gene action was detected at most QTLs and was prevalent over dominance (Tables 4 and 5). At QTLs for grain yield, however, significant dominance effects were often observed. The otherwise apparent lack of significant dominance deviations might be the consequence of the inability to detect their presence based on F_2 genotypes and F_3 phenotypes (Paterson et al., 1991), rather than the result of their absence.

The effect of environments on the identification and characterization of QTLs has been a concern shared by many (Edwards et al., 1987; Stuber et al., 1987; Paterson et al., 1988; Stuber et al., 1992). Paterson et al. (1991) showed that different QTLs were detected in tomato for fruit size, soluble solids concentration and fruit pH, when analyses were based on phenotypes obtained from either of two environments in California or one in Israel. In maize, Stuber et al. (1992) did not find conclusive evidence for the presence of marker × environment interactions from data gathered on backcross families evaluated in six different environments. However, effects of the environments were clearly demonstrated when mapping QTLs for resistance to gray leaf spot in maize (Bubeck et al., 1993).

In this study, comparisons of presence or absence of QTLs detected by interval mapping showed that from a total of 70 QTLs detected in all populations and for all traits analyzed, 21.4% were detected at all locations, 34.3% at two or more but not all locations, and 44.3% at a single location. Fifty percent of the LOD score correlations between single-environment F_3 phenotypes and the corresponding averages over locations were larger than 0.8 and 45% were between 0.5 and 0.8 (data not shown). Over all traits and populations, 51% of the LOD score correlations between single-environment phenotypes for each trait analyzed and in each population (e.g., between F_3 grain yield measured at Clayton and F_3 grain yield measured Lewiston) were larger than 0.5 (data not shown). Interestingly, also about 50% of the corresponding phenotypic correlations were larger than 0.5. Significant ($P < 0.05$) marker × environment interactions, as revealed by the study of the ($L \times M$) source of variation in Model [1] of single-factor analyses, were identified only in four out of 99 significant ($P < 0.01$) marker-trait associations in population H (data not shown), i.e., merely 4% of the time, or no more than expected by chance. All of the above observations clearly show that the environment affected very little the detection of QTLs, although small effects were noted in the interval mapping analyses. However, the existence of interactions between QTLs and environments could not be ruled out. For some of the QTLs detected in several environments, relative magnitudes of their effects varied across environments.

The differences observed between F_2 and F_3 generations for presence/absence of QTLs may reflect nothing more than environmental variation, given that F_2 and F_3 individuals were grown in different environments (years and locations).

Favorable Factors of Exotic Origin

For each locus detecting the presence of a QTL for a given trait, the origin of the favorable allele at the

Table 6. Favorable alleles of exotic origin at QTLs for agronomic traits with desirability being defined as high grain yield, low ear height, high leaf area, large number of leaves, low plant height, small number of days to pollen shed, small number of days to silking, large number of ears per plant, and low grain moisture.

Pop†	Trait	Ch‡	Closest marker locus	Origin of favorable exotic allele	Effect§	Origin of adapted allele¶	Traits for which exotic allele is unfavorable#
G	GY	*4*	*Cat3*	Lenha × Tuson	0.13 t ha^{-1}	Mo17	–
G	GY	*10*	*Glu1*	Lenha × Tuson	0.07 t ha^{-1}	Mo17	–
H	DTP	*3*	*Got1*	Arizona × Cristal	−0.39 day	Mo17	LN
H	EAR	*3*	*Got1*	Arizona × Cristal	0.05 ear	Mo17	LN
H	EAR	*6*	*UMC21*	Cubano Dentado	0.05 ear	BS13(S)C3	DTP-LA
H	EAR	*6*	*Hex2*	Cubano Dentado	0.07 ear	BS13(S)C3	DTP-LA-LN
H	EHT	*3*	*Me1*	Cubano Dentado	−0.03 m	BS13(S)C3	EAR-GY-H2O-LA
H	GY	*3*	*Got1*	Arizona × Cristal	0.41 t ha^{-1}	Mo17	LN
H	GY	*6*	*UMC21*	Cubano Dentado	0.12 t ha^{-1}	BS13(S)C3	DTP-LA
H	GY	*6*	*Hex2*	Cubano Dentado	0.17 t ha^{-1}	BS13(S)C3	DTP-LA-LN
H	LA	*3*	*Got1*	Arizona × Cristal	0.002 m^2	Mo17	LN
H	LN	*6*	*Mdh2*	Cubano Dentado	0.14 leaf	BS13(S)C3	LA
H	PHT	*3*	*Me1*	Cubano Dentado	−0.05 m	BS13(S)C3	EAR-GY-H2O-LA
K	EAR	*3*	*BNL8.35*	Arizona × Cristal	0.19 ear	Mo17	–

† Population.
‡ Chromosome.
§ The effect of an exotic allele is defined as half the difference between the mean value of F_3 families which have a homozygous exotic genotype and the mean value of F_3 families which have a homozygous adapted genotype at the marker locus.
¶ Donor of the adapted allele to which the exotic allele was compared.
# Traits on which the exotic allele at the marker locus indicated in the fourth column has significant deleterious effects.

marker locus was determined. An exotic allele was declared favorable for a given trait if, within the population where it was found, the mean performance of individuals homozygous for this allele was better than that of individuals homozygous for the allele of the elite inbred line found in this population. Of the 70 QTLs identified over all populations and traits, 12 (17%) had favorable alleles of exotic origin (Table 6), clearly demonstrating the value of exotic germplasm. Favorable exotic factors were identified for all traits but grain moisture, including characteristics such as plant and ear height and number of days to pollen shed, which are known to be weaknesses of exotic germplasm. Exotic factors that were favorable for some traits were often deleterious to other traits. In one instance, however, an exotic factor (the factor from Arizona × Cristal at *Got1* in population H) had favorable effects on all traits, including grain yield and number of days to pollen shed, known to be negatively correlated.

The identification of exotic factors on *3L (GOT1)* and *6L (UMC21* and *Hex2)* associated with increases in both grain yield and number of ears per plant, together with the high phenotypic and LOD score correlations observed between these two traits, strongly suggested the presence of either tightly linked or pleiotropic QTLs in these two chromosomal regions. Similarly, exotic factors at QTLs for plant and ear height, located on *3L*, were responsible for reduced values for both traits, also suggesting tight linkage or pleiotropy between these QTLs. Fine mapping, using a large number of close markers (Paterson et al., 1990), may assist in the determination of the tightness of linkage between these QTLs. Distinction between very tight linkage and pleiotropy may necessitate the isolation and cloning of the gene or genes involved in those QTLs.

With effects on grain yield as high as 0.41 t ha^{-1} (Table 6), and the occurrence of broken deleterious linkages, exotic factors should be useful for improving U.S. germplasm, provided favorable factors can be distinguished from deleterious ones. Exotic factors identified in this study could be introgressed into adapted germplasm by repeated backcrosses. However, to limit the risks of simultaneously introgressing deleterious characteristics, QTLs may need to be located more precisely than was done for the purpose of this preliminary survey. Nevertheless, the relatively large chromosomal segments containing favorable QTLs identified in this study most likely did not contain major deleterious characteristics, as seen from the increase in grain yield they produced.

ACKNOWLEDGMENTS

This research was partly supported by a fellowship from Ciba Seeds Ltd. to M. Ragot. Thanks are being extended to Drs. G. Gay, T. Islieb and T. Stalker for critical reading of the manuscript, and B. Weir for advice on correlations among LOD scores.

REFERENCES

Bubeck, D.M., M.M. Goodman, W.D. Beavis, and D. Grant. 1993. Quantitative trait loci controlling resistance to gray leaf spot in maize. Crop Sci. 33:838–847.

Burr, B., and F.A. Burr. 1991. Recombinant inbreds for molecular mapping in maize. Trends Genet. 7:55–60.

Coe, E. 1992. Working maps. Maize Genet. Coop. Newsl. 66:150–159.

Crossa, J., and C.O. Gardner. 1987. Introgression of an exotic germplasm for improving an adapted maize population. Crop Sci. 27:187–190.

Crossa, J., C.O. Gardner, and R.F. Mumm. 1987. Heterosis among populations of maize (*Zea mays* L.) with different levels of exotic germplasm. Theor. Appl. Genet. 73:445–450.

Darvarsi, A., and M. Soller. 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theor. Appl Genet. 85:353–359.

Edwards, M.D., C.W. Stuber, and J.F. Wendel. 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics 116:113–125.

Falconer, D.S. 1989. Introduction to quantitative genetics. Longman Scientific and Technical, Harlow, U.K.

Feinberg, A.P., and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction fragments to high specific activity. Anal. Biochem. 132:6–13.

Gerrish, E.E. 1983. Indications from a diallel study for interracial maize hybridization in the corn belt. Crop Sci. 23:1082–1084.

Goodman, M.M. 1985. Exotic maize germplasm: Status, prospects, and remedies. Iowa State J. Res. 59:497–527.

Grobman, A., W. Salhuana, and R. Sevilla, in collaboration with P.C. Mangelsdorf. 1961. Races of maize in Peru. Their origins, evolution and classification. National Academy of Sciences-National Research Council, no. 915. National Academy of Sciences, National Research Council, Washington, DC.

Hallauer, A.R. 1978. Potential of exotic germplasm for maize improvement. p. 229–247. *In* D.B. Walden (ed.) Maize breeding and genetics. Wiley, New York.

Hallauer, A.R., and J.H. Sears. 1972. Integrating exotic germplasm into Corn-Belt maize. Crop Sci. 12:203–206.

Hoisington, D.A. 1992. Laboratory protocols. CIMMYT Applied Molecular Genetics Laboratory. Mexico, D.F., Mexico.

Holley, R.N., and M.M. Goodman. 1988. Yield potential of tropical hybrid maize derivatives. Crop Sci. 28:213–218.

Keim, P., B.W. Diers, T.C. Olson, and R.C. Shoemaker. 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735–742.

Koester, R.P. 1992. Identification of quantitative trait loci controlling maturity and plant height using molecular markers in maize (*Zea mays* L.). Ph.D. thesis, North Carolina State University (DA9221518).

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12:172–175.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181.

Paterniani, E., and M.M. Goodman. 1977. Races of maize in Brazil and adjacent areas. CIMMYT, Mexico, D.F., Mexico.

Paterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H. Rabinowitch, S.E. Lincoln, E.S. Lander, and S.D. Tanklsley. 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127:181–197.

Paterson, A.H., J.W. deVerna, B. Lanini, and S.D. Tanksley. 1990. Fine mapping quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics 124:735–742.

Paterson, A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln, and S.D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726.

Ragot, M., P.H. Sisco, M.M. Goodman, and C.W. Stuber. 1992. A Brazilian source of *cms*-S. Maize Genet. Coop. Newsl. 66:92–93.

Ramirez, R., E., D.H. Timothy, E. Diaz B., and U.J. Grant, in collaboration with G.E. Nicholson, E. Anderson, and W.L. Brown. 1960. Races of maize in Bolivia. National Academy of Sciences-National Research Council, no. 747. National Academy of Sciences, National Research Council, Washington, DC.

SAS Institute. 1989. SAS/STAT user's guide. Version 6, Fourth Edition, Vol. 1 and 2. SAS Institute Inc., Cary, NC.

Sax, K. 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:522–560.

Sisco, P.H. 1991. Duplications complicate genetic mapping of *Rf4*, a restorer gene for cms-C cytoplasmic male sterility in corn. Crop Sci. 31:1263–1266.

Soller, M., and T. Brody. 1976. On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor. Appl. Genet. 47: 35–39.

Stuber, C.W. 1978. Exotic sources for broadening genetic diversity in corn breeding programs. Proc. Annu. Corn Sor. Res. Conf. 33: 34–47.

Stuber, C.W. 1986. Use of exotic sources of germplasm for maize improvement. p. 19–31. *In* O. Dolstra and P. Miedema (ed.) Breeding of silage maize. Pudoc, Wageningen, the Netherlands.

Stuber, C.W. 1992. Biochemical and molecular markers in plant breeding. Plant Breed. Rev. 9:37–61.

Stuber, C.W., M.D. Edwards, and J.G. Wendel. 1987. Molecular-marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop Sci. 27:639–649.

Stuber, C.W., S.E. Lincoln, D.W. Wolff, T. Helentjaris, and E.S. Lander. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839.

Stuber, C.W., R.H. Moll, M.M. Goodman, H.E. Schaffer, and B.S. Weir. 1980. Allozyme frequency changes associated with selection for increased grain yield in maize (*Zea mays* L.) Genetics 95:225–236.

Stuber, C.W., J.F. Wendel, M.M. Goodman, and J.S.C. Smith. 1988. Techniques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L.) North Carolina Agric. Res. Ser. Tech. Bull. 286.

Tanksley, S.D., and J. Hewitt. 1988. Use of molecular markers in breeding for soluble solids content in tomato – a re-examination. Theor. Appl. Genet. 75:811–823.

Tanksley, S.D., H. Medina-Filho, and C.M. Rick. 1982. Use of naturally occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49:11–25.