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Genetic analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphisms

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Summary. An understanding of the genetic nature underlying tolerance to low-phosphorus (low-P) stress could aid in the efficient development of tolerant plant strains. The objective of this study was to identify the number of loci in a maize (*Zea mays* L.) population segregating for tolerance to low-P stress, their approximate location, and the magnitude of their effect.

Seventy-seven restriction fragment length polymorphisms (RFLPs) were identified and scored in a maize F_2 population derived from a cross between line NY821 and line H99. The F_2 individuals were self-pollinated to produce F_3 families. Ninety F_3 families were grown in a sand-alumina system, which simulated diffusion-limited, low-P soil conditions. The F_3 families were evaluated for vegetative growth in a controlled-environment experiment. To identify quantitative trait loci (QTLs) underlying tolerance to low-P stress, the mean phenotypic performances of the F_3 families were contrasted based on genotypic classification at each of 77 RFLP marker loci.

Six RFLP marker loci were significantly associated with performance under low-P stress ($P<0.01$). One marker locus accounted for 25% of the total phenotypic variation. Additive gene action was predominant for all of the QTLs identified. Significant marker loci were located on four separate chromosomes representing five unlinked genomic regions. Two marker loci were associated with an additive by additive epistatic interaction. A multiple regression model including three marker loci and the significant epistatic interaction accounted for 46% of the total phenotypic variation. Heterozygosity per se was not predictive of phenotypic performance.

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Introduction

Low-input agriculture, particularly in lesser-developed countries, is a cost-effective strategy where soil mineral stresses limit crop production (Sanchez and Salinas 1981). The utilization of intraspecific variation for nutrient stress tolerance could permit the development of crop strains adapted to soils with low mineral content (Gerloff and Gabelman 1983). Intraspecific variation for low-phosphorus (low-P) stress has been identified previously in a number of crop species (Coltman et al. 1985; Furlani et al. 1984; Fox 1978; Whitaker et al. 1976). Tolerance to low-P stress in tomato and bean is quantitatively inherited (Coltman et al. 1987; Fawole et al. 1982b).

Soil heterogeneity makes the identification of superior plant strains difficult under field conditions (Whitaker et al. 1976). A sand-alumina system has been developed that simulates low-P soil conditions by restricting the concentration and movement of available P (Coltman et al. 1982). Phenotypic variation in maize (*Zea mays* L.) for low-P tolerance has been identified using the sand-alumina system. Subsequently, maize lines have been identified with superior performance under low-P stress (A. Da Silva, unpublished).

The advent of methods for detecting restriction fragment length polymorphisms (RFLPs) has resulted in the effective coverage of some plant genomes with genetic markers (Helentjaris et al. 1986; Landry et al. 1987; Bernatzky and Tanksley 1986), thus permitting the localization of quantitative trait loci (QTLs) and the determi-

nation of the relative magnitudes of their effect on traits of interest. Restriction fragment length polymorphisms have been used to map QTLs in tomato (Nienhuis et al. 1987; Martin et al. 1989; Paterson et al. 1988). Isozyme-based analyses of quantitative traits have been reported for maize (Edwards et al. 1987; Kahler and Wehrhahn 1986). Indirect selection for marker loci linked to QTLs of interest could enhance selection efficiency (Nienhuis et al. 1987). The localization of specific QTLs may also be the first step in the eventual cloning of loci underlying quantitative trait variation. The objective of this study was to identify RFLP markers associated with QTLs affecting tolerance to low-P stress in maize and to estimate relative effects and types of gene action exhibited by identified QTLs.

Materials and methods

Phenotypic evaluation of F_3 families

Two maize inbred lines, NY821 (provided by M. Smith, Cornell Univ., Ithaca, N.Y.) and H99 (provided by P. Crane, Purdue Univ., W. Lafayette, Ind), identified previously as being tolerant and intolerant, respectively, under low-P stress and comparable under adequate-P conditions were crossed in the field in the summer of 1987. The resultant F_1 hybrid was selfed in a winter nursery (1987 to 1988) and 150 randomly drawn F_2 plants were selfed in the field the following summer (1988) to produce F_3 families. Ninety of the F_3 families were drawn at random for further study.

Twenty seeds from each of the 90 F_3 families were germinated for 7 days in rolled paper towels moistened with tap water. Four seedlings from each family were chosen randomly from germinated seedlings and transplanted to 15-cm pots (two seedlings per pot) containing a sand-alumina mixture that simulated diffusion-limited, low-P soil conditions (Coltman et al. 1982). The sand-alumina mixture was prepared prior to transplanting according to Coltman et al. (1982) with some modification. Six 1.5-kg lots of activated-alumina (Alcoa F1, 30–50 mesh, Alcoa Co) were washed with distilled water for 30 min to remove small and broken granules. One lot of the washed alumina (1.5 kg) was added to each of six PVC tubes (100 cm × 15 cm o.d.) containing 10 l of a 200 m*M* KH_2PO_4 and 10 m*M* NaCl solution. The tubes were sealed, placed horizontally, and rotated at low speed (ca. 20 rpm) for 7 min every 3 h. This adsorption period was continued for 14 days after which the alumina was removed, rinsed with distilled water, and dried at 50 °C. Two kilograms of washed and autoclaved sand (10–30 mesh) were mixed with 50 g of dried alumina and placed into a 15-cm pot. Each pot was watered twice daily with 500 ml distilled water. This desorption period was continued for 14 days after which the maize seedlings were transplanted to pots. The solution-P concentration of the pots was approximately 20 μ*M* P at the time of transplanting and 2–3 μ*M* P at the time of harvest.

Following transplantation, the maize seedlings were grown for an additional 17 days. The pots were watered once daily with 250 ml of a nutrient solution containing the following: 7.5 m*M* N as 2.5 m*M* $Ca(NO_3)_2 \cdot 4H_2O$ and 1.25 m*M* NH_4NO_3; 3 m*M* K as KCl; 2.5 m*M* Ca as $Ca(NO_3)_2 \cdot 4H_2O$; 1 m*M* S as $MgSO_4 \cdot 7H_2O$; 1 m*M* Mg as $MgSO_4 \cdot 7H_2O$; 500 μ*M* Na as NaCl; 50 μ*M* Fe as Fe-HEDTA (HEDTA is hydroxyethylenediaminetriacetic acid, obtained from Sigma); 3.5 m*M* Cl as 500 μ*M* NaCl and 3 m*M* KCl; 23 μ*M* B as H_3BO_3; 4.5 μ*M* Mn as $MnSO_4 \cdot H_2O$; 0.38 μ*M* Zn as $ZnSO_4 \cdot H_2O$; 0.5 μ*M* Cu as $CuSO_4 \cdot 5H_2O$; and 0.05 μ*M* Mo as $(NH_4)_6Mo_7O_4 \cdot 4H_2O$. At harvest, the shoots were excised from the roots. The roots were removed from the sand and washed to remove adhering sand and alumina. Shoots and roots were dried to a constant weight at 60 °C and weighed separately.

The experiment was conducted using a randomized complete block design. There were two complete blocks in each of two repetitions of the experiment; thus each F_3 family was represented by eight plants replicated over time under the same environmental conditions. The experiment was carried out in a growth room under fluorescent and high-pressure sodium lighting (275–325 μmol m^{-2} s^{-1} at the top of the pots) with a 16-h light and an 8-h dark cycle. Temperatures ranged from 30° to 35 °C during the light period and from 20° to 23 °C during the dark period. Both parents and their F_1 hybrid were included in each repetition.

Detection of RFLPs

Secondary, immature ear shoots (ear shoots that had begun to protrude from the leaf axis) from F_2 plants grown in the field or leaf tissue from 20 bulked F_3 seedlings were lyophilized and ground in a coffee grinder. Genomic DNA was extracted using the method described by Saghai-Maroof et al. (1984). The DNA was quantified and redissolved to a concentration of 0.3 μg μl^{-1} in TE (10 m*M* TRIS pH 8, and 1 m*M* EDTA).

Approximately 5 μg of genomic DNA was digested using one of three restriction enzymes: *Eco*R I, *Eco*R V, or *Hin*d III. The DNA was digested with 12.5 U of enzyme according to the manufacturer's instructions for 4 h in the presence of 2.5 m*M* spermidine (Helentjaris et al. 1985). The DNA was loaded into a 0.8% (w/v) agarose gel (25 cm × 20 cm) in the presence of 1 × TAE buffer (40 m*M* TRIS-acetate pH 8, 10 m*M* EDTA) and electrophoresed overnight (20–25 V). The gel was stained with ethidium bromide to permit visualization of the DNA. The gel was rocked for 10 min in 500 ml 0.25 *M* HCl to depurinate the DNA and to aid in the transfer of large fragments; this was followed by 30 min in 500 ml 0.4 *M* NaOH and 0.6*M* NaCl to denature the DNA. The gel was neutralized in 500 ml 0.5*M* TRIS pH 7.5 and 1.5 *M* NaCl for 30 min. The DNA was transferred from the gel onto a nylon membrane (Micron Separations) in the presence of 25 m*M* $NaPO_4$ pH 6.5 using capillary transfer (Helentjaris et al. 1985). The membrane was washed for 15 min in 2 × SSC, blotted dry, and baked at 90 °C in vacuo for 3 h.

Plasmids (pUC8 or pUC19) containing *Pst* I-digested maize genomic DNA (500–2500 bp) inserts were obtained from D. Hoisington (Univ. of Missouri-Columbia). These genomic DNA clones identified polymorphisms in a maize population derived from crossing TX303 and CO159. Polymorphisms were subsequently mapped, and this information is available along with linkage map data as a public set of maize RFLP probes.

Plasmid DNA was transformed into *E. coli* strain DH5α (BRL) by a calcium chloride transformation procedure (Maniatis et al. 1982). Transformed cells were selected on ampicillin-containing medium. Plasmid DNA was prepared from 1.5 ml LB cultures based on the alkaline-lysis procedure of Birnboim (1983). Mini-preparations of plasmid DNA were digested with *Pst* I for 2 h according to the manufacturer's instructions. One microgram of digested, plasmid DNA was loaded into a 1% (w/v) LMT (low-melting temperature) agarose gel (7.5 cm × 15 cm) in 1 × TAE buffer and electrophoresed for 2–3 h at 75 V in a cold room (4 °C). The gel was stained with ethidium bromide to permit visualization of the DNA. Genomic inserts were excised from the gel and placed in 1.5-ml microfuge tubes to which 400 μl dH_2O was added. The tubes were first heated to

65 °C for 10 min to melt the agarose, and then they were vortexed and extracted twice with 500 µl phenol and once with 500 µl chloroform:octanol (24:1 v/v). The DNA was precipitated with EtOH in the presence of 0.1 *M* NaCl and redissolved in 25 µl dH_2O.

Purified DNA inserts (50–250 ng) were labeled using either the random primer labeling procedure of Feinberg and Vogelstein (1983) or by nick-translation (Rigby et al. 1977) using 3000 Ci mmol^{-1} (^{32}P)dCTP and 3000 Ci mmol^{-1} (^{32}P)dGTP. The probe specific activity ranged from $1-4\times10^9$ dpm µg^{-1}. The labeled probes were purified using G-50 spin columns (Maniatis et al. 1982) to remove unincorporated deoxynucleotides.

All hybridizations were carried out at 65 °C in the presence of 1.5 × SSPE (Maniatis et al. 1982), 1% (w/v) SDS, 0.5% (w/v) Blotto (Bovine Lacto Transfer Technique Optimizer i.e., Carnation nonfat dry milk, Johnson et al. 1984), 5% (w/v) dextran sulfate, and 100 µg ml^{-1} denatured salmon sperm DNA. Typically, five membranes (10 cm × 20 cm) were placed in a tray (11 cm by 21 cm, Rubbermaid #5) with 50 ml of the hybridization solution and prehybridized for 4 h at 100 rpm in a forced-air shaker. The entire purified labeling reaction was added to the tray ($5-20\times10^6$ cpm ml^{-1}), and membranes were hybridized for 16–20 h.

The hybridization solution was removed and the membranes were washed twice with 250 ml 2 × SSC and 0.5% (w/v) SDS for 5 min each at RT. The membranes were then washed with 0.1 × SSC and 0.1% (w/v) SDS for 10 min at RT. Finally, the membranes were washed 1 to 2 times with 500 ml 0.1 × SSC and 0.1% (w/v) SDS for 30 min at 65 °C. The membranes were wrapped in Saran Wrap and exposed to X-ray film (Kodak, X-OMAT AR) with one intensifying screen at −70 °C for 1–4 days.

Linkage analysis and QTL mapping

A set of 93 clones was selected that identified a polymorphism at an average interval of 20–25 c*M* along the ten maize chromosomes (D. Hoisington, personal communication). These clones were tested for their ability to identify a codominant RFLP between the inbred parent lines when the parental DNA was digested with *Eco*R I, *Eco*R V, or *Hin*d III. A total of 77 RFLPs were identified with 72 of the clones tested. The 77 markers (polymorphic regions) were mapped using a version of the Mapmaker program developed by Lander et al. (1987). Briefly, all pairwise comparisons were made between markers, and recombination fractions were calculated. The markers were next divided into linkage groups based on pairwise associations. Finally, three-point and n-point analyses were performed to determine the most likely order for markers. Map distances (c*M*) were then estimated using recombination distances and Kosambi's mapping function between ordered marker loci.

The mean performance of the 90 F_3 families was calculated for total dry weight, shoot dry weight, and root dry weight. Individual marker loci were tested for linkage to QTLs affecting total, shoot, and root dry weight by contrasting mean performance of genotypic classes represented by each marker locus (Edwards et al. 1987). Two orthogonal contrasts at each locus were tested: the first compared mean performance of homozygous marker classes (additive effect), and the second compared the heterozygous marker class to the average of the two homozygous marker classes (dominance effect). Marker loci were considered to be significantly associated with a QTL when the F-test for a contrast exceeded an F-value with the probability level equal to 0.01. All possible digenic epistatic interactions were tested between pairs of significant marker loci. Significant interactions were partitioned into four single-degree-of-freedom contrasts testing the linear by linear (additive by additive), linear by quadratic (additive by dominant), quadratic by linear (dominant by additive), and quadratic by quadratic (dominant by dominant) interactions. Multiple linear regression models were developed for the traits studied by using a forward and backward elimination, stepwise regression procedure (SAS Institute 1985). Significant marker loci and significant marker locus interactions were entered into models in a stepwise fashion and only those loci that were significant ($P<0.05$) remained in the model. To localize further the QTL's, intervals between marker loci were tested by contrasting mean performance of marker class intervals in which no recombination had occurred (assuming no double crossovers).

Results

Phenotypic response to low-P stress

Significant differences for total dry weight, shoot dry weight, and root dry weight were found between the two repetitions of the experiment and among F_3 families. One F_3 family was not included in the analysis because of poor germination and poor seedling vigor unrelated to low-P stress. Shoot and root dry weights were up to 10% and 20% greater, respectively, in the first repetition than the second repetition. The interaction between experimental repetitions and families was not significant, thus individual families responded similarly in both repetitions. Dry weights for 15 families fell below the intolerant parent line, H99, and 17 families were above the tolerant parent line, NY821 (Fig. 1). The dry weight of the F_1 hybrid exceeded that of both parents as well as that of the derived F_3 families.

Segregation and linkage of RFLP marker loci

The majority of the RFLP loci adequately fit their expected 1:2:1 segregation ratio. Five marker loci exhibited

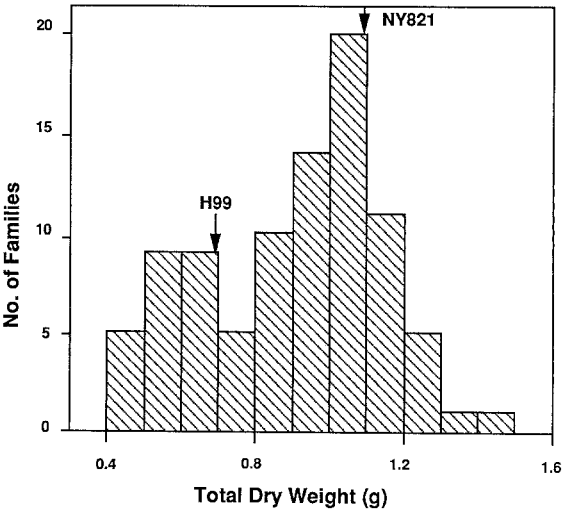

Fig. 1. Histogram of mean total dry weight of the F_3 families. *Arrows* indicate mean total dry weight of the tolerant parent (NY821) and the intolerant parent (H99). The F_1 hybrid is not shown (F_1 mean total dry weight = 1.7507 g)

significant deviations ($P<0.05$) from the expected segregation pattern. Two loci were tightly linked, and the other three loci were on separate chromosomes. Distorted segregation ratios have been observed in other marker-based studies in maize (Edwards et al. 1987; Kahler and Wehrhahn 1986).

The linkage map developed from the segregation of the 77 marker loci was similar to the linkage map developed at the University of Missouri-Columbia (UMC). Five marker loci, identified by five clones, mapped to linkage groups (chromosomes) that were different from the UMC mapping results. Two or more restriction fragments were frequently observed using a single clone. Generally, only a single polymorphism was observed and mapped among the restriction fragments. Observed marker-location differences may be due to alternative mapping of duplicate marker loci.

Identification of genomic regions underlying tolerance to low-P stress

Linear and quadratic contrasts of genotypic classes for the 77 marker loci were examined. Differences between marker class means were presumed to be due to linkage to loci affecting tolerance to low-P stress (QTLs). A probability level of 0.01 was chosen in an attempt to reduce the possibility of falsely declaring a marker locus significant (Type I error). Five marker loci had significant ($P<0.01$) linear contrasts for either total dry weight (Table 1) or root dry weight (data not shown), and four marker loci were significant for shoot dry weight (data not shown). Three of these loci, umc42b, umc46, and umc138, were significant for all three traits. One locus, umc19, was significant only for total and shoot dry weight. The other two remaining loci were significant either for either total dry weight (umc59) or root dry weight (umc117) only. Several flanking marker loci that were linked to the major marker loci were significant at the 0.05 probability level. Intervals between ordered pairs of marker loci were tested for significance by using only those families in which no recombination had occurred between the two marker loci. The same genomic regions were identified using marker intervals as were identified previously using individual marker loci. Marker loci with significant effects were located on four chromosomes (Fig. 2). Two of these marker loci were linked (20 c*M*) and may represent one or more QTLs. Individual marker loci explained as little as 7% and as much as 25% of the total phenotypic variation for total dry weight (Fig. 2).

For four of five significant marker loci, the genotypic class of the tolerant parent, NY821, was superior to other marker classes (Table 2). The opposite was true for genotypic classes represented by marker locus umc59. Families identified by the intolerant genotype, at marker locus umc59, were superior to other genotypic classes (Table 2).

Types of gene action

Dominance and overdominant types of gene action were not detected for the expression of tolerance to low-P stress. Only 1 of the 77 quadratic (dominance) contrasts was significant at the 0.05 probability level (umc66, chromosome 4), and none were highly significant. Although there appears to be consistent directional dominance for tolerance at all significant marker loci, the level of dominance failed to achieve significance for individual marker loci (Table 2). Overdominance was absent completely.

When digenic epistatic interactions were tested, only the interaction between marker loci umc42b and umc138 was significant, and only additive by additive gene action was important (Table 1). Quantitative trait loci linked to markers umc42b and umc138 individually exhibited additive gene action. Improved performance was predicted

Table 1. Mean squares from analysis of variance for total dry weight. Only the five significant marker loci and the significant interaction are shown

Source	*df*	RFLP loci				
		umc19	umc46	umc42b	umc138	umc59
Genotypes	2					
Linear (Add.)	1	0.4232**	0.3938**	0.5543**	0.9107**	0.3640**
Quadratic (Dom.)	1	0.0571	0.0072	0.0465	0.1364	0.0274
Interaction						
(42b by 138)	4					
Add. by Dom.	1			0.3916**		
Add. by Dom.	1			0.0319		
Dom. by Add.	1			0.0103		
Dom. by Dom.	1			0.0032		
Error[a]	82–86	0.0510	0.0530	0.0502	0.0423	0.0512

** $P \leq 0.01$

[a] Error degrees-of-freedom were dependent upon the number of families that were genotyped successfully for a given marker locus

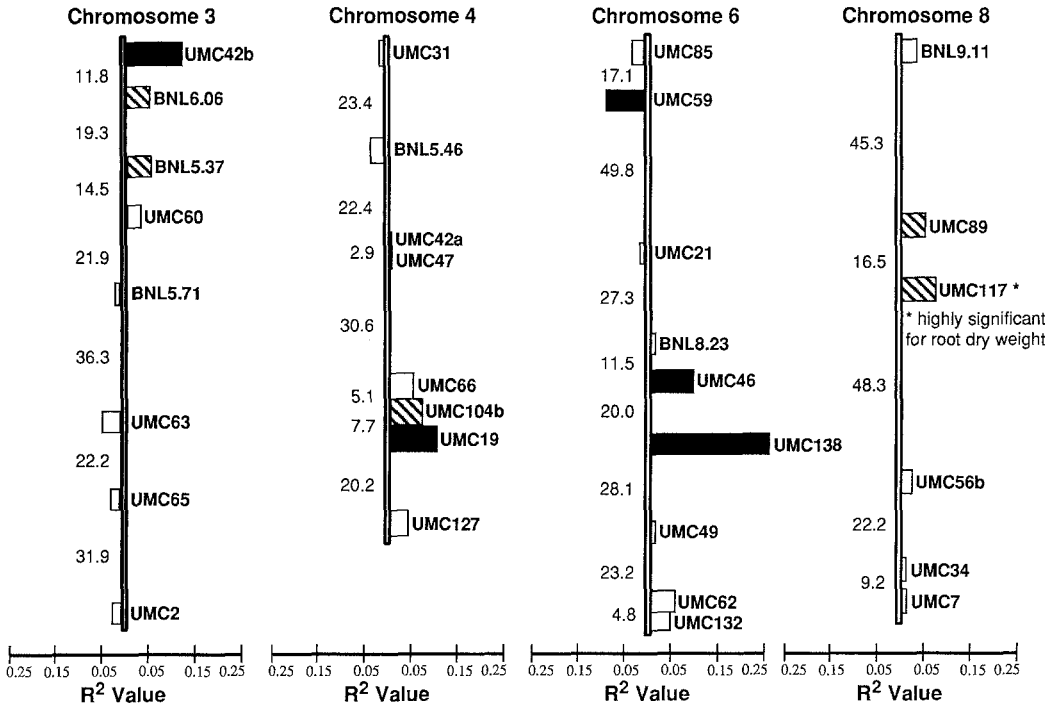

Fig. 2. The location and R^2 values for individual marker loci associated with total dry weight. Highly significant marker loci ($P<0.01$) are represented by *solid bars*, flanking marker loci ($P<0.05$) by *hatched bars*, and non-significant marker loci ($P\leq 0.05$) by *open bars*. Distances, in cM, between marker loci are indicated on the *left*. *Bars* lying to the *right* indicate the alleles with positive effects came from the tolerant parent, NY821. *Bars* lying to the *left* indicate the alleles with positive effects came from the intolerant parent, H99

Table 2. Genotypic class means for total dry weight. Each value represents the total dry weight mean of all F_3 families scored for a particular genotype. Only significant marker loci are shown

Genotype	Class means of RFLP loci				
	umc19	umc46	umc42b	umc138	umc59
NY/NY	0.976	1.017	0.992	1.030	0.814
NY/H99	0.934	0.903	0.928	0.956	0.934
H99/H99	0.790	0.825	0.772	0.720	0.980
LSD (0.05)	0.151	0.154	0.150	0.138	0.151

when both of these markers were represented by alleles from the tolerant parent line NY821.

Cumulative prediction of phenotypic variation by marker loci

The five significant marker loci were used to develop predictive models for performance under low-P stress. Individual marker loci and the interaction between marker loci umc42b and umc138 were entered into a multiple linear regression model in a stepwise fashion using a forward and backward elimination procedure (SAS Stepwise Procedure 1985). Marker loci remained in

Table 3. Multiple regression models for prediction of total, shoot, and root dry weight. Each model was developed by using a stepwise regression procedure with all significant marker loci and significant interactions. Only marker loci and interactions which were significant ($P<0.05$) after being added to the models remained in the models

Parameter	Effect	Estimate	Standard error
Total dry weight ($R^2=0.46$):			
Intercept		0.8834	0.0240
umc19	additive	0.1519	0.0550
umc42b	additive	0.2284	0.0629
umc138	additive	0.2424	0.0651
umc42b by umc138	add. by add.	0.3917	0.1714
Shoot dry weight ($R^2=0.47$):			
Intercept		0.5256	0.0135
umc19	additive	0.1001	0.0310
umc42b	additive	0.1366	0.0354
umc138	additive	0.1269	0.0367
umc42b by umc138	add. by add.	0.2540	0.0965
Root dry weight ($R^2=0.45$):			
Intercept		0.3546	0.0114
umc117	additive	0.0624	0.0291
umc42b	additive	0.0766	0.0300
umc138	additive	0.1089	0.0303
umc42b by umc138	add. by add.	0.1230	0.0798

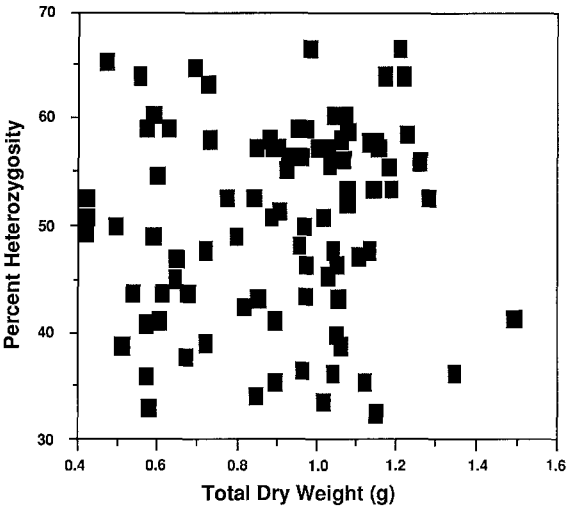

Fig. 3. Plot of percent loci of 77 marker loci which were heterozygous within each F_3 family versus mean total dry weight of each F_3 family

a model if the locus was significant at a probability level equal to 0.05. Collectively, three marker loci, umc19, umc42b, and umc138 were able to explain 46% of the total phenotypic variation for total dry weight (Table 3). The addition of remaining marker loci to the model resulted in a small improvement only ($R^2 = 0.50$). Similarly, a three-locus model was used to predict shoot and root dry weight ($R^2 = 0.47$ and $R^2 = 0.45$ respectively) (Table 3). Marker locus umc117 was added to the multi-locus model replacing umc19 in a three-locus model for root dry weight. The additive by additive interaction between umc42b and umc138 was important for the prediction of all three traits.

Effect of heterozygosity on family performance

To test whether heterozygosity *per se* was predictive of family performance, percent heterozygosity for all 77 marker loci was regressed against family performance for total dry weight (Fig. 3), shoot, and root dry weight (data not shown). Heterozygosity failed to predict family performance for the traits examined ($R^2 < 0.01$ for all traits).

Discussion

In this maize cross, a minimum of three QTLs were identified that affect tolerance to low-P stress. These three loci accounted for nearly half of the observed total phenotypic variation. One or more QTLs may be present in each genomic region linked to significant marker loci. Additional significant marker loci were identified but not included in the multi-locus models. These marker loci may reflect either false-positives (Type I error) or QTLs with either phenotypic effects similar to or correlated with other identified loci having larger predicted effects.

The power of the experiment was limited to the detection of marker loci, each of which explained greater than 7% of the total phenotypic variation (at a probability level of 0.01); therefore, only marker loci linked to QTLs with large effects were identified. Recombination between marker loci and QTLs would result in the underestimation of the magnitude of effect by QTLs. The remaining variation may be represented by a number of loci with smaller phenotypic effects. Alternatively, loci with large phenotypic effects may be located in regions of the genome that were not well covered with marker loci and thus may have escaped detection.

Significant loci exhibited additive gene action. Although dominance tests were not significant in this study, partial dominance may be important. The heterozygous class performance exceeded the mid-parent value for all important marker loci (Table 2). A significant additive by additive epistatic interaction was detected between two loci, umc42b and umc138. Previous biometric studies in bean have also identified epistasis as being important for tolerance to low-P stress (Fawole et al. 1982b; Whitaker et al. 1976).

The QTLs were distributed on four chromosomes. One locus, linked to umc117, strongly influenced root growth, while a second locus, linked to umc19, strongly influenced shoot growth. Remaining QTLs affected root growth and shoot growth equally. Because root and shoot growth tend to be correlated, it was interesting that QTLs were identified that had differential effects on the traits studied. In common bean, shoot dry weight and root dry weight under low-P stress appear to be under separate genetic control (Fawole et al. 1982a).

F_3 families were evaluated only under low-P stress and were not evaluated under adequate-P conditions; thus, detected QTLs may be important for vegetative vigor and may not be unique to low-P tolerance. However, parental lines exhibit equivalent growth under non-stress conditions; thus, allelic differences between parental lines do not result in differential growth under adequate P.

The F_1 hybrid displayed significant heterosis and exceeded the performance of the best F_3 families by 20%. This difference in performance between the F_1 hybrid and F_3 families may have a number of causes. Individual plants within the best F_3 families were equal to the F_1 hybrid and reduced performance by families may simply reflect the segregation of alleles at important loci among individuals in a family, which results in reduced mean family performance. The small population size (90 families) may not have permitted the sampling of some superior genotypes. This may be seen in the genotypic make-up of superior families. No family was homozygous for favorable alleles at all significant marker loci. Finally, the reduced performance of F_3 families may be due to the fixation of several deleterious alleles at loci with small

individual effects. Such loci may have a significant cumulative effect; however, individually they would be undetectable in this study.

The summed effects of the three principle loci (Table 3) is greater than the mean difference between parental lines. This may indicate the presence of additional epistasis. Since no pairwise or higher order epistatic interactions were tested with non-significant marker loci, interactions between identified QTLs and loci in non-significant regions would not be detected. If such interactions exist however, they would result in overestimation by individual QTLs.

The identified QTLs that underlie tolerance to low-P stress may be unique to the population studied and to the test environment. Further investigation will be required to establish the importance of the identified genomic regions in other genetic backgrounds. Field evaluation is required also to establish the effectiveness of the low-P screening system in modeling low-P stress response and to evaluate the stability of QTLs across environments.

Further experimentation is needed to confirm the QTLs identified in this study. The loci may be confirmed using near-isogenic lines derived from this population. Ideally, lines containing the identified genomic segments both individually and in combination with other loci should be developed. Both parental lines should also be mated to additional maize lines of known tolerance to low-P stress. Additional populations may then be developed and tested with appropriate marker loci. Population sizes should be expanded in future studies, and recombinant inbred lines may be used to identify QTLs with smaller phenotypic effects.

The identification of marker loci linked to QTLs involved in low-P tolerance is an important step in the genotypic evaluation of maize germ plasm. Phenotypic selection for low-P tolerance may not be difficult, but identified marker loci may be useful in multiple-trait selection where low-P tolerance is one of many traits of interest. Identified marker loci may also become important starting points in the cloning of QTLs using chromosome walking strategies.

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