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Analysis of the epistatic and QTL×environments interaction effects of plant height in maize (*Zea mays* L.)

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Abstract

A genetic map containing 103 microsatellite loci and 200 F_2 plants derived from the cross R15 × Ye478 were used for mapping of quantitative trait loci (QTL) in maize (*Zea mays* L.). QTLs were characterized in a population of 200 $F_{2:4}$ lines, derived from selfing the F_2 plants, and were evaluated with two replications in two environments. QTL mapping analysis of plant height was performed by using QTLMapper 2.0 software. Three main-effect QTLs and seven pairs of distinctly epistatic loci included a total of fourteen QTLs were located on seven chromosomes. QTL main effects of additive, dominance, and additive × additive, additive × dominance, and dominance×dominance interactions were estimated. Interaction effects between QTL main effects and QTL×environments (*QE*) were also predicted. Less than 30% of single effects for identified QTLs were significant at 5% level. The information about epistatic QTLs and *QE* interaction will facilitate marker assisted selection for plant height breeding programs in maize.

***Keywords*:** Maize; QTL; Plant height; QE.

Introduction

Mapping of quantitative trait loci is of growing interest to both breeders and geneticists (Kao et al., 1999). QTL mapping procedures such as interval mapping (Lander and Botstein, 1989), composite interval mapping (Zeng, 1993; 1994) and mixture interval mapping (MIM; Zhu 1999; Wang et al., 1999) involve tests of the null hypothesis that a QTL is absent. These methods have been instrumental in the identification of QTL responsible for various quantitative traits of agricultural, biological, or biomedical value. Quantitative traits are also influenced by the environment and tend to show varied degrees of genotype × environment (*GE*) interactions. *GE* interaction is another important component of genetic basis. Understanding the genetic principle of *GE* interaction is of great importance to genetic breeding. Wang et al. (1999) proposed a QTL mapping strategy that can estimate additive epistatic effects of QTL and predict their interaction with environments by software QTLMapper version 1.0. However, so far estimations of dominance effects, epistatic effects related to dominance, and predictions of their interaction with environments are lacking. Recently, Gao and Zhu (submitted) extended the additive and additive × additive model by adding dominance effects, epistatic effects of additive × dominance and dominance × dominance as well as their interaction with environments, and updated the software QTLMapper to version 2.0.

Plant height is one of the important agronomic traits in maize breeding, which is the major factor to affect the plant growth status and yield potential. In the past few years, to increase the planting density and prevent plants from lodging, studies on the genetic mechanism of plant height were given great attention to. Since the 1990s, molecular markers have provided a powerful tool to study the trait of plant height at the molecular level (Lin et al., 1995; Vlduu et al., 1999). Until now, a number of plant height QTLs or genes were localized in maize (Coe and Polacco, 1995).

In our research, we used the QTLMapper version 2.0 to map the main effect QTLs and epistatic effect of the plant height according to a SSR linkage map. This manuscript describes a large study of QTL × environment interaction in two environments for the genetic control of plant height in a $F_{2:4}$ maize populations. This information will help us understand the genetic control of plant height and its components under two environments. The results may also be directly applicable in improving plant height breeding in maize.

Materials and methods

Plant material

Two hundreds of $F_{2:4}$ families derived from F_2 individuals were used as a mapping population coming from an elite cross between R15 and 478, which is a widely extended hybrid in China. The mapping population used to obtain the genetic map, made up of 200 F_2 plants, was used to mapping QTLs. So as to increase the number of available seeds and allow the installation of experiments with increased experimental precision, 200 $F_{2:4}$ lines were obtained, by selfing these individual F_2 plants, and the $F_{2:4}$ lines were sib-mated by using 14 plants of each line.

Field trials

Field trials were conducted at the Research Farms of the Maize Research Institute of Sichuan Agriculture University in Ya’an in 2003. The $F_{2:4}$ lines were evaluated in field experiments in two environments: Ya’an and Experimental area of the Department of Chongqing. Each site combination was treated as an environment in subsequent statistical analysis. The 200 $F_{2:4}$ lines were grown in two 10×10 lattice designs with two replications each. In order to cover the differences in the field area, the position of each one of the lattice replications was randomized. Thus, this disposal did not require that the replication of each lattice be placed side by side. Plots consisted of single rows, 0.8 m apart and 3 m long. Plots were overplanted and later thinned to a final plant density of 10 plants m^2 with a total of 14 plants per row. At each location, plot size and management were in accordance with local practice. Shortly after flowering stage, plant height (PH) was measured on ten plants per plot. Plant height was estimated from the soil line to the tip of the tassel. The average value was designated as observation of plant height.

Field data analysis

For the maize plant height traits, means, range, coefficient of variation, kurtosis and skewness of trait distribution were calculated. Frequency distribution of phenotypic values of the plant height in two environments was also detected. Simple analysis of variance was presented to indicate whether genotypes, environments, or *GE* interaction were significant sources of variance or not. All these analyses were performed using the SPSS software (2000).

Construction of linkage map

DNA samples of parents and F_2 individuals were extracted as described by Saghai et al. (1984). Four hundreds and fifty SSR markers were selected for screening polymorphism between the two parents. SSR analysis followed the method described by Senior *et al.* (1993). The genetic linkage map was constructed using the software MAPMAKER/EXP VER.3.0 (Lander *et al.* 1989; Lincoln et al. 1992). Linkage groups were created with a log-10 likelihood ratio (LOD) score of 3.0 and a recombination fraction of 0.4 using the "group" command. The "three point" and "ripple" command was used to verify the order of markers on each chromosome. Data quality was checked using the ''error detection'' command, and unlikely double crossovers, due to possible genotyping errors, were corrected by rechecking the data. The map distance (cM) was derived based on the Kosambi function.

QTL mapping

QTL analysis, including main-effect QTL, epistatic QTL, and *QE* interactions, was carried out using mixed linear model approaches conducted with QTL mapping software QTLMapper version 2.0. A threshold probability of $P \leq 0.05$ was used for the main-effect and epistatic QTL. The additive effect, epistatic effect, and *QE* interaction effect of QTL were also estimated. The QTL were denoted according to the method introduced by McCouch et al. (1997).

Results

Statistics of phenotypic variation

The statistical analysis showed that the value of plant height at the two environments was not significantly different. The skewness and kurtosis were near zero at both environments (Table 1), indicating the phenotypic values were normally distributed and segregated continuously, which indicates that the population was suitable for QTL mapping. Frequency distributions of the value of plant height were shown in Fig. 1 and Fig. 2. Simple analysis of variance was shown in Table 2 indicated that environment difference was not significant but genotype and *GE* interaction were significant sources of variance. This means that genotype and *GE* interaction was real affected the phenotype variance.

Table 1. Summary statistics for phenotypic values of the plant height.

Site	Mean[a]	Range(cm)	Coefficient of Variation (%)	Kurtosis	Skewness
Ya'an	171.8±1.14	132.9~230.1	9.39	0.35	0.38
Chongqing	174.9±1.36	125.5~239.4	11.01	0.25	0.19

[a] With standard errors.

Table 2. Simple analysis of variance for phenotypic values of the plant height.

Source	SS	d.f.	Mean Square	F	P
Environment(E)	13.03	1	13.03	0.090	NS
Genotype(G)	342012.5	199	1718.7	11.889	****
G × E	41638.6	199	144.6	1.447	***
Error	115645.9	800	144.56		

NS, $P > 0.05$; *** $0.0001<P < 0.005$; **** $P < 0.0001$.

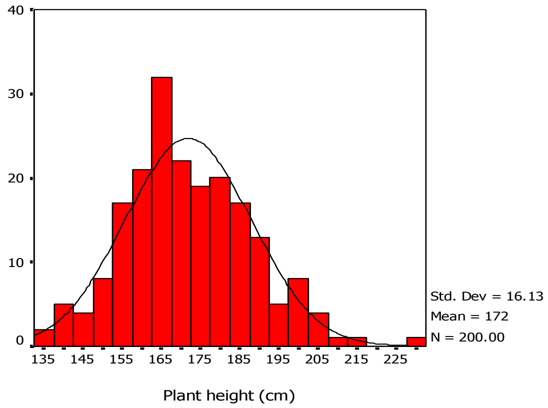

Figure 1. Frequency distribution of phenotypic values of the plant height in Ya'an environment.

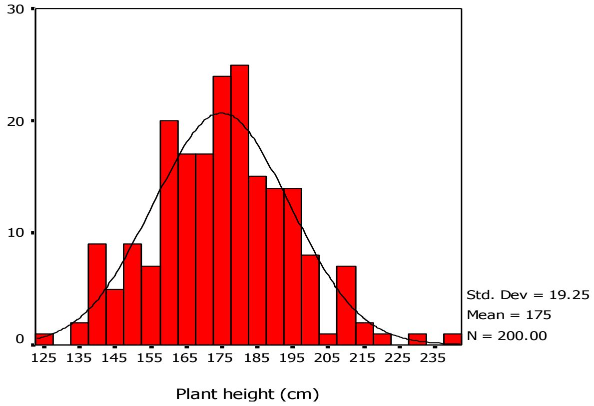

Figure 2. Frequency distribution of phenotypic values of the plant height in Chongqing environment.

Map construction

A total of 450 SSR markers covering all 10 linkage groups in maize were tested for polymorphism between R15 and 478. One hundred and forty nine co-dominant SSR markers were polymorphic between the two parents and scored in the $F_{2:4}$ population. One hundred and three markers were found to be linked, representing 10 known linkage groups with the method of “three point” and “ripple” command by MAPMAKER software. On average 10 markers were placed on each linkage group. The actual number of markers ranged from 5 for linkage groups on chromosome 10 to 15 on chromosome 4. The total map encompassed 1163.9 cM with an average of 11.3 cM between loci with approximately 83% of the genome within 20 cM of the nearest marker (partly shown in Figure 3).

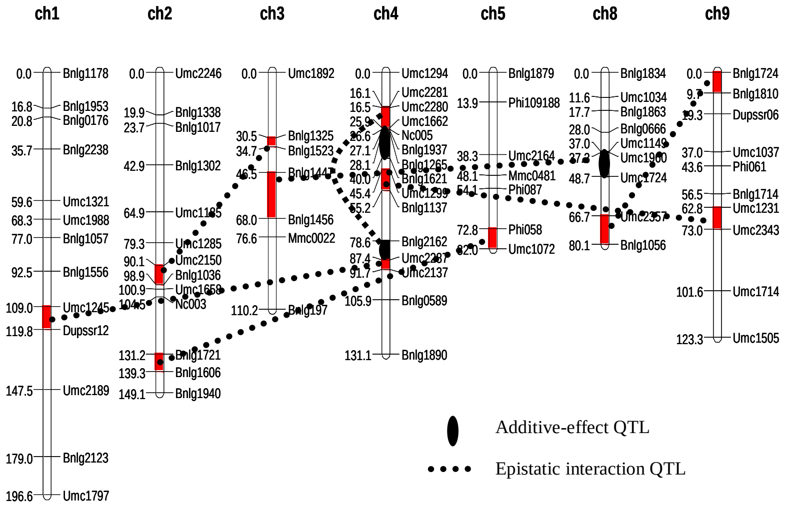

Figure 3. Map positions for additive-effect and epistatic interaction QTL controlling plant.

Main-effect QTLs

The positions and designations for the main-effect QTLs were summarized in Table 3. In total, three main-effect QTLs were detected and mapped to chromosomes 4 and 8. All These three putative QTLs were detected with significant additive effects. The additive effects were 2.02, 7.71 and 8.04 respectively. The range of the phenotypic variation was explained from 1.62% to 12.66%. Collectively, these QTLs explained 25.92% of the phenotypic variation. The favorable alleles for increased plant height were all came from the parent R15. Only one QTL showed dominance effects, which effect was 3.96.

Table 3. Estimation of genetic main-effect QTL controlling plant height.

QTLs	Chr.	Marker interval	Site (M)[a]	LOD	a	d	*Var* (%)[b]
qPH4-1	4	Bnlg1265-Bnlg1621	0.281	5.18	2.02*	3.96*	1.62
qPH4-2	4	Bnlg2162-Umc2287	0.846	5.93	7.71***	2.36	11.64
qPH8-1	8	Umc1960-Umc1724	0.373	5.41	8.04***	-0.01	12.66

*, **, *** is significance level at 0.05, 0.01 and 0.005, respectively

[a] The Site(M) column is the genetic distance (in Morgan) of the testing points from the left end of the chromosomes on which the testing points are set.

[b] Variance explained by the QTL

Digenic interactions, QTL identification and location

LOD values of seven pairs of epistatic interaction QTLs were beyond the significance threshold, suggesting that these loci might be QTLs controlling plant height in maize. The positions and designations for these QTLs were summarized and shown in Table 4 and Fig. 3. A total of fourteen QTLs among four epistatic loci were distributed on chromosomes 1, 2, 3, 4, 5, 8 and 9. Four QTLs were detected on chromosome 4, but one QTL of *qPH4-2* was detected by main-effect QTL command. The same as the QTL (*qPH8-1*) were detected on chromosome 8. However, these QTLs need to be confirmed according to the significance test for their QTL main effects and *QE* interaction effects based on the null hypothesis for the genetic model of QTL mapping.

Estimations of genetic main effects of QTLs

Among fourteen QTL main effects of seven pairs of epistatic loci, less than 30 percent were significantly different from zero, and most of these had additive effects and additive×additive epistatic interaction effects (Table 5, Figure 3). The additive effects of QTL with the genotype of R15 were all positive, which implied that QTL controlling plant height were all came from the higher parent. The positive (negative) epistatic effects of additive × additive interaction suggested that the two epistatic loci with homozygous alleles from the same parent could increase (decrease) the plant height. The negative additive×dominance epistatic effects could decrease the plant height. In the present study, *qPH8-1* had the largest positive additive effect (9.90); at the same time, its additive×additive, and dominance×dominance epistatic effects were 5.26 and -5.68 with *qPH3-2*, respectively. The effects of all dominance QTLs were found positive; however, all significant dominance×dominance epistatic effects were negative. For two pairs of epistatic loci of *qPH2-1* and *qPH3-1*, *qPH4-5* and *qPH9-1* were not found any significant effects.

Table 4. Digenic interaction QTL controlling plant height.

	QTL_I [a]				QTL_j		
Chr.	Marker interval	Site1(M)[b]	QTL designation	Chr.	Marker interval	Site2(M)	QTL designation
1	Umc1245-Dupssr12	0.060	*qPH1-1*	4	Umc2287-Umc2137	0.000	*qPH4-3*
2	Umc2150-Bnlg1036	0.000	*qPH2-1*	3	Bnlg1325-Bnlg1523	0.020	*qPH3-1*
2	Bnlg1721-Bnlg1606	0.060	*qPH2-2*	5	Phi058-Umc1072	0.060	*qPH5-1*
3	Bnlg1447-Bnlg1456	0.020	*qPH3-2*	8	Umc1960-Umc1724	0.000	*qPH8-1*
4	Umc2280-Umc1662	0.000	*qPH4-4*	4	Bnlg2162-Umc2287	0.060	*qPH4-2*
4	Umc1299-Bnlg1137	0.000	*qPH4-5*	9	Umc1231-Umc2343	0.080	*qPH9-1*
8	Umc2357-Bnlg1056	0.000	*qPH8-2*	9	Bnlg1724-Bnlg1810	0.060	*qPH9-2*

[a] QTL_i and QTL_j are a pair of QTL detected by two-dimensional searching,

[b].The Site1(M) column is the genetic distance (in Morgan) of the testing points from the left end of the chromosomes on which the testing points are set. The Site2 (M) column is the genetic distance (in Morgan) of the testing points from the left marker on the interval on which the testing points are set.

Table 5. Estimation of epitasis QTL for plant height.

QTL i	QTL j	LOD	A_i	D_i	A_j	D_j	AA_{ij}	DA_{ij}	DD_{ij}
qPH1-1	*qPH4-3*	22.60			7.33***	6.52**	-3.99**		-8.31**
qPH2-1	*qPH3-1*	11.60							
qPH2-2	*qPH5-1*	11.34	5.47*			5.2*	-7.81***		
qPH3-2	*qPH8-1*	28.28		4.43*	9.90***	3.99*	5.26**		-5.68*
qPH4-4	*qPH4-2*	19.18			8.82***				
qPH4-5	*qPH9-1*	18.05							
qPH8-2	*qPH9-2*	11.49						-3.74*	

[a] a_i and d_i are the additive and dominance effects of QTL $_i$, respectively; a_j and d_j are the additive and dominance effects of QTL $_j$, respectively; aa_{ij}, ad_{ij} and dd_{ij} are the epistatic effects of additive × additive, additive × dominance, and dominance × dominance between QTL $_i$ and QTL $_j$, respectively;

*, ** and *** denote significance level at 0.05, 0.01 and 0.005, respectively.

Table 6. Prediction of QE interaction effects for plant height.

Environment	QTLi	QTLj	e_{Ai1}	e_{Aj1}	e_{Dj1}	e_{AAij1}	e_{ADij1}
Ya'an (h=1)	*qPH1-1*	*qPH4-3*					
	qPH2-1	*qPH3-1*				3.59***	
	qPH2-2	*qPH5-1*				2.56**	
	qPH3-2	*qPH8-1*					
	qPH4-4	*qPH4-2*				-2.83**	
	qPH4-5	*qPH9-1*	-3.59**		-2.81**	-2.05*	
	qPH8-2	*qPH9-2*		2.49*	-2.65*	3.32**	-2.98*
chongqing (h=2)	*qPH1-1*	*qPH4-3*					
	qPH2-1	*qPH3-1*				-3.59***	
	qPH2-2	*qPH5-1*				-2.56**	
	qPH3-2	*qPH8-1*					
	qPH4-4	*qPH4-2*				2.83**	
	qPH4-5	*qPH9-1*	3.59**		2.81**	2.05*	
	qPH8-2	*qPH9-2*		-2.49*	2.65*	-3.33**	2.89*

[a] $e_{AiE\,h}$ and $e_{AjE\,h}$ are additive interactions of QTL*i* or QTL*j* with environment *h*; $e_{DiE\,h}$ are dominance interactions of QTL*i* or QTL*j* with environment *h*; $e_{AAijE\,h}$, $e_{ADijE\,h}$ and $e_{DAijE\,h}$ are the interactions between *AAij*, *ADij* ,*ADji* and environment *h*;

*, **, *** is significance level at 0.05, 0.01 and 0.005, respectively

Prediction of QTL × environment interaction effects

The advantage of QTL mapping approaches using mixed linear models is for simultaneously dealing with complicated epistasis and *QE* interaction, so that it provides a powerful tool for geneticists and breeders to further analyze the interaction between QTLs and environments. Significant *QE* interaction effects are summarized in Table 6, excluding, for simplicity, epistatic loci pairs with no significant *QE* interaction effects. There are few QTLs with significant *QE* interaction effects, suggesting that plant height in maize might not be extremely sensitive to environments. Two pairs of epistatic loci have not any significant *QE* interaction effects in two environments, but the two pairs of loci have significant additive and additive×additive interaction (shown in Table 5). This indicated that these two pairs of loci were stabile in two environments. However, the other five pairs of loci had *QE* interaction effects.

Discussion

Quantitative geneticists have long recognized the importance of genotype by environment interaction and it has been documented for numerous crops and for various traits. Information about additive effects and epistasis related to additive effects will be helpful to traditional breeding. In breeding practice, it is a risk to apply the superior genotype to various environments when it is predicted based on the QTL information obtained only in one environment. If the superior genotypes predicted in different environments differ greatly, their superiority may be dramatically reduced across environments. Hence, in order to develop broadly adaptable cultivars, we need to partition the QTL main effects and *QE* interaction effects**.**

In the present study, digenic epistatic effects and their interaction with environments have been broadly detected for the plant height in maize. The most important result of this study is the statistical characterization of the genetic components that control the expression of the traits, including main effects of the QTL, additive by additive epistatic interactions or epistatic QTL, and QTL by environmental interactions. In this study, three additive QTLs and seven pairs of epistatic QTLs showed *QE* interactions between the two environments. The composition of epistatic interactions was interesting on that two of three QTLs with additive effects and other five non-additive effects QTLs were engaged in digenic epistatic interactions. The results might suggest that the epistatic interactions may be largely due to induction of the loci without detectable QTL additive effect, as signify the importance of keeping the concept in mind that the loci without detectable QTL additive effect can also be putative QTLs when doing QTL analysis. The information of additive effects and epistasis related to additive effects will be helpful to traditional breeding. The present study revealed that the additive effects were all positive, and the ratio of the negative and positive effects of additive × additive epistatic were 2:1, which implied that QTLs underlying plant height were all came from the higher plant height parents and additive × additive epistatic could increase or decrease the plant height. In this research we found that *qPH8-1* had the largest positive additive effect and positive additive×additive epistatic effects, but had no significant *QE* interaction with *qPH3-2*. This indicated that the QTL of *qPH8-1* was stable in two environments and can be used in marker assisted selection. On the basis of our results it was clear that the QTL governing plant height in maize might not be extremely sensitive to environment. Therefore pyramiding and manipulation of genes in selection programs should consider not only the additive effects of genes or QTLs, but also the additive × additive epistatic effects among these genes and others.

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