

# QTL Analysis for Plant Height with Molecular Markers in Maize

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**Abstract :** Plant height has become one of important agronomic traits with the increase of planting density recently and the rapid developments of molecular markers have provided powerful tools to localize important agronomic QIL at the genomic level. The purposes of this investigation are to map plant height QIL with molecular markers and to analyze their genetic effects in maize. An F<sub>2</sub> 3 population from an elite combination (Zong3 ×87-1) was utilized for evaluating plant height in two locations, Wuhan and Xiangfan, with a randomized complete block design. The mapping population included 266 F<sub>2</sub> 3 family lines. A genetic linkage map, containing 150 SSR and 24 RFLP markers, was constructed, spanning a total of 2 531.6 cm with an average interval of 14.5 cm. Totally 10 QIL affecting plant height were mapped on six different chromosomes with the composite interval mapping. Seven of 10 QIL were detected in two locations. The contributions to phenotypic variations for the single QIL varied between 5.3 and 17.1 %. Additive, partial dominance, dominance, and overdominance actions existed among all detected QIL affecting plant heights. A large number of digenic interactions for plant height were detected by two-way analyses of variance. 107 and 98 two-locus combinations were found to be significant at a 0.01 probability level in two locations respectively. 23 of them were simultaneously detected in both locations. They accounted for phenotypic variations of 4.5 - 11 %. It was noticed that a locus, umc1122, had digenic interactive effects with other four different loci for plant height, which distributed on three chromosomes. A few of plant height QIL was involved in significant digenic interactions, but most significant interactions occurred between markers that are not adjacent to mapped QIL. These results demonstrated that epistatic interactions might play an equal importance role as the single-locus effects in determining plant height of maize.

**Key words :** Digenic interaction, Plant height, Maize, Molecular marker

Molecular markers as a powerful tool have been widely used for localizing and cloning genes, and performing marker assistant selection. This work has attracted great attention with the development of PCR-based markers, such as SSR<sup>[1]</sup>, which is known for the advantages of simple, accurate and co-dominant segregation. Since the 1990's of last century, molecular markers have been extensively employed to investigate genetics of plant height in maize because the plant height is one of the important agronomic traits for the plant breeding. Until now, a number of plant

height QTL or genes were localized in maize<sup>[2, 3]</sup>, and some important plant height genes were also cloned<sup>[4, 5]</sup>. Compared with these results, it was noticed that plant height QTL almost scattered on all chromosomes although there were varying numbers of QTL in different research groups<sup>[6]</sup>.

Recently, epistatic interactions for different traits were observed in different species. Especially in rice, several reports indicated that epistatic interaction might play an important role in the genetic bases

of heterosis<sup>[7-9]</sup>. In addition, there were proofs that interactions between co-adapted parental species genes were the important genetic foundation for the evolution and adaptation of species<sup>[10]</sup>. Doebley *et al.*<sup>[11]</sup> analyzed two different QTL for plant height and inflorescence architecture in a maize-teosinte F<sub>2</sub> population. They found that there were epistatic interactions among these. However, there are few cases in which epistatic interference has been investigated in larger scale loci for the quantitative traits of maize. The purposes of this investigation are to map plant height QTL with molecular markers and to analyze their genetic effects focusing on epistatic interactions with an elite maize combination in China.

## 1 Materials and Methods

### 1.1 Materials

266 F<sub>2-3</sub> families derived from F<sub>2</sub> individuals were used as a mapping population coming from an elite cross between Zong3 and 87-1, which is a widely extended hybrid in China. Young leaves of F<sub>2</sub> individuals were collected to be stored at -70 °C refrigerator for next use.

### 1.2 Field experiments

F<sub>2-3</sub> families, as well as two parents and F<sub>1</sub>, were transplanted into the field on the agronomy farm at Huazhong Agricultural University, Wuhan, China, and in the experimental station of Chia-Tai Agricultural Development Company, Xiangfan, China. A randomized complete block design was employed with three replications. Each field plot included 20 plants grown in a single row of 5-m long with 0.70-m width between rows. The normal agricultural practices were followed in the field management. At maturity, only 15 plants from the 3rd to 17th of each row were used to measure plant height. The average value was designated as plant height for each block. The plant height means the distance from the ground to the top of tassels.

### 1.3 Construction of linkage map

The total DNA of plants was extracted as Saghai-Maroo *et al.* described<sup>[12]</sup>. SSR analysis was followed by a method of Senior *et al.*<sup>[11]</sup>, while RFLP analyses were accomplished according to Helentjaris *et al.*<sup>[13]</sup>. 459 mark-

ers, including SSR and RFLP, were selected for screening polymorphism between two parents. A molecular linkage map was constructed using Mapmarker 3.0<sup>[14]</sup>.

### 1.4 Data analysis

Plant height QTL were mapped with composite interval mapping<sup>[15]</sup>. According to Yu *et al.*<sup>[7]</sup>, the entire genome was searched for digenic interactions in plant height with two-way analyses of variance (ANOVA) through all possible two-locus combinations of marker genotypes. Statistical significance for each term was assessed using an orthogonal contrast test provided by the statistical package Statistica<sup>[16]</sup>.

## 2 Results and Discussion

### 2.1 Performance of plant height

According to field tests, even though no obvious differences for plant height were observed between parents, F<sub>1</sub> displayed significant heterosis in plant height, being 50.10 and 36.15% over two parents in Wuhan and Xiangfan respectively. There were widely variations among F<sub>2-3</sub> families in plant height, Wuhan and Xiangfan, ranging from 129.36 to 235.13 cm and 138.67 to 226.36 cm respectively. There were normal distributions for plant height among 266 F<sub>2-3</sub> family lines in two locations, 90% of which were over-parents approximately. The broad heritability for the plant height was considerably high in both locations, reaching 80.74 and 81.94%, respectively.

### 2.2 QTL for plant height

150 SSR and 24 RFLP markers, showing co-dominant segregation clearly, were selected for constructing a linkage map. Polymorphism markers were classified into 12 groups, covering 10 chromosomes of maize with a total length of 2531.6 cm and an average interval of 14.5 cm (Fig. 1). Compared with other maize linkage maps in Maize DB, marker alignments and intervals on this linkage map were consistent with them. 25 markers displayed the deviation of a ratio of 1:2:1 by  $\chi^2$  test, which distributed on 10 chromosomes.

Eight plant height QTL were detected at LOD value > 2.5 in Wuhan (Fig. 1). These QTL located on 1, 3, 4, 5, 8 and 9 chromosomes were named as Ph1-2, Ph3, Ph4, Ph5-1, Ph5-2, Ph5-3, Ph8 and

Ph9 respectively (Table 1). They could explain 5.87 - 16.56 % of total phenotypic variations. In Xiangfan ,nine plant height QTL were mapped on chromosomes 1, 3, 4, 5 and 9 (Fig. 1), called as Ph1-1, Ph1-2, Ph1-3, Ph3, Ph4, Ph5-1, Ph5-2, Ph5-3 and Ph9 respectively (Table 1). They could account for 5.29 - 17.12 % of phenotypic variations. It should be noted that in both locations, Wuhan and Xiangfan, seven QTL were mapped in the same regions of chromosomes, all of which showed the same direction to

increase plant height. The locations of some QTL mapped in this study, Ph9, Ph1-3, Ph5-2, Ph1-2, are very similar to those of dwarf genes, *d3*(9.03), *d8*(1.10), *d9*(5.02) and *an1*(1.08)<sup>[5]</sup>. Seven detected QTL, Ph1-1, Ph1-2, Ph3, Ph4, Ph5-1, Ph8, Ph9, were corresponded with those detected by Edwards et al<sup>[17]</sup>. The other 3 QTL, Ph1-3, Ph5-2, Ph5-3, were also consistent with those observed by Beavis et al<sup>[18]</sup>.

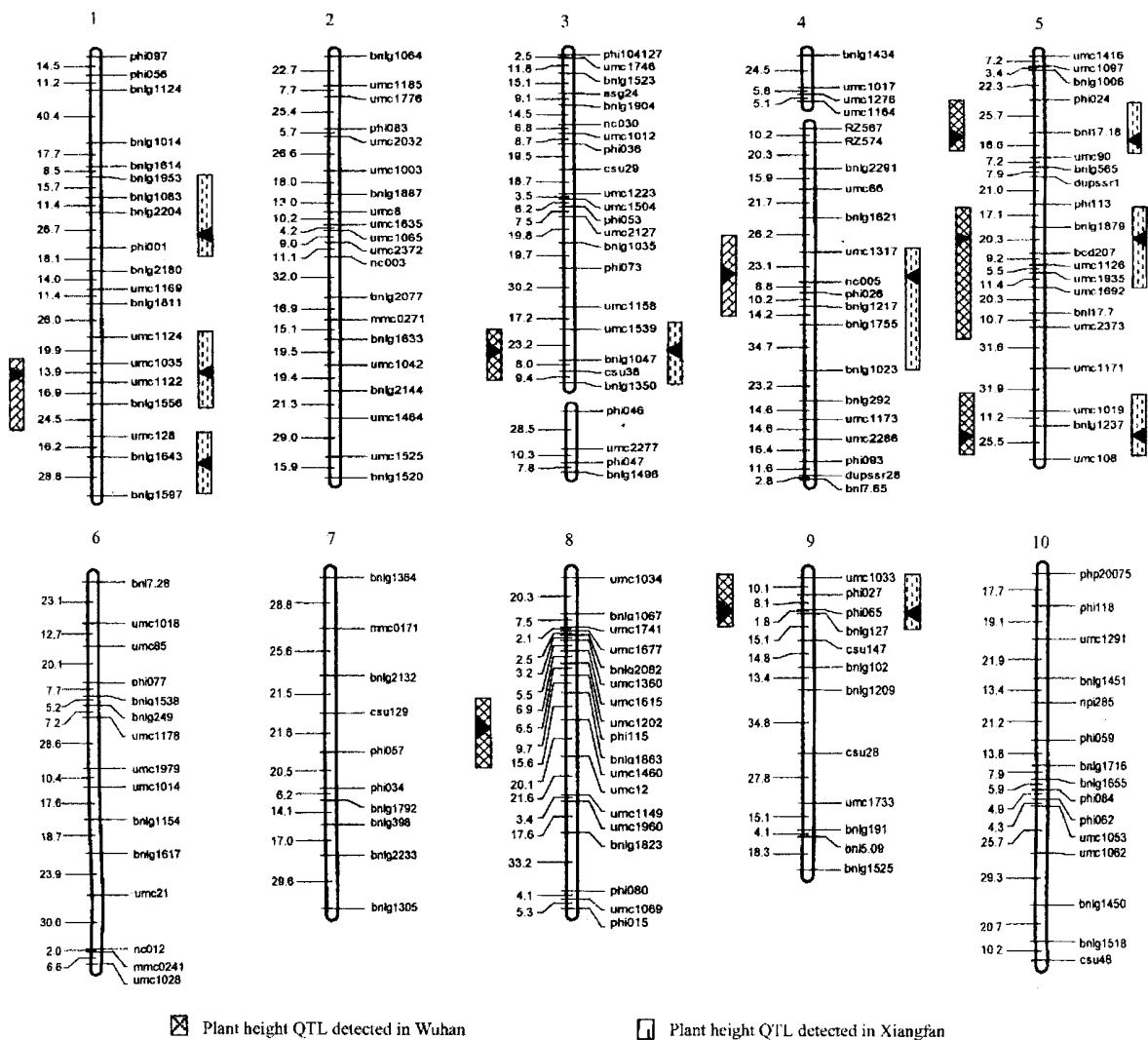


Fig. 1 Genomic position for QTL affecting plant height in two locations

The phenotypic variations explained by single QTL were small, varying between 5.29 and 17.12 % with an average of 8%. The alleles from 87 to 1 at

six of ten QTL had the positive effects to increase plant height while the alleles from 87 to 1 at the other four QTL decreased plant height. That means that

the alleles increasing plant height came from two parents synchronously. As Table 1 showed, all kinds of gene actions existed among all detected QTL. 17.6% (3/17)

was additive, 47.1% (8/17) was partial dominance, 11.8% (2/17) was dominance, and the other 23.5% (4/17) was overdominance.

**Table 1 Putative QIL affecting plant height in both locations detected with LOD 2.5 in E<sub>3</sub> population**

QTL <sup>1)</sup>	Flanking markers		LOD <sup>2)</sup>		A <sup>3)</sup>		D <sup>4)</sup>		R <sup>2</sup> (%) <sup>5)</sup>	
	Wuhan	Xiangfan	Wuhan	Xiangfan	Wuhan	Xiangfan	Wuhan	Xiangfan	Wuhan	Xiangfan
ph1-1		bnlg2204+phi001		3.70		-6.36		5.77		6.8
ph1-2	umc1035-umc1122	umc1035-umc1122	6.03	12.02	-10.83	-13.49	4.95	1.34	9.1	17.1
ph1-3		bnlg1643+bnlg1597		2.78		-6.06		7.39		8.2
ph3	umc1539+bnlg1047	umc1539+bnlg1047	5.44	5.57	4.92	3.47	4.24	5.04	8.8	8.4
ph4	umc1317+nc005	umc1317+nc005	4.64	4.00	2.04	1.40	7.87	6.60	7.1	6.9
ph5-1	bnl17.18+umc90	bnl17.18+umc90	10.97	9.13	-9.71	-7.68	-2.10	-1.10	16.6	13.6
ph5-2	bnlg1879+bcd207	bnlg1879+bcd207	4.08	3.27	-7.62	-6.90	2.53	1.49	5.9	5.4
ph5-3	bnlg1237+umc108	bnlg1237+umc108	4.08	3.58	-7.01	-5.19	3.10	0.89	6.1	5.3
ph8	bnlg1863+umc1460		5.22		5.79		2.56		8.3	
ph9	phi027+phi065	phi027+phi065	7.35	6.25	6.14	5.86	4.32	2.72	9.1	8.3

<sup>1)</sup>Numbers following the two letters represent the chromosome locations of the QTL;

<sup>2)</sup>Log-likelihood value calculated by composite interval mapping;

<sup>3)</sup>Additive effect; positive values of the additive effect indicate that the Zong3 alleles are in the direction of increasing the plant height;

<sup>4)</sup>Dominance effect; positive values of the dominance effect indicate that the heterozygotes have higher phenotypic values than the respective means of two homozygotes;

<sup>5)</sup>Variation explained by each QTL

### 2.3 Digenic interactions for plant height

Through digenic interactive analysis among 12 603 possible combinations, 107 and 98 two-locus combinations for plant height were found to be significant at the 0.01 probability level in two locations respectively. However, only 23 combinations were simultaneously detected in both locations, reaching 0.01% of significant level (Table 2). Therefore, it is reasonable to infer there are digenic interactions rather than by chance. All four types of interactions, AA, AD, DA, DD, were detected. For the single digenic combination, phenotypic variations of 4.5%–11% could be explained with an average of 6.5%. Regarding to different interactive types, phenotypic variations of 2.5%–7.9% could be explained (Table 2).

From Table 2, it should be notable that eight markers involved in interactions were adjacent to mapped QTL, such as umc1122 and bnl17.18, closely linking to Ph1-2 and Ph5-1, respectively. The bnl17.18 seemed to be more effect than umc1122 on

increasing plant height in two locations. The effects of their interactions were more considerable low than those with bnl17.18, but were similar with umc1122. It was interesting to notice umc1122 interacted with four different loci distributed on three chromosomes. The relationship of umc1811 with bnlg1556 was another type interaction. The former was not adjacent to any mapped QTL while the latter was a marker closely linking to QTL Ph1-2 comes. Those combinations could be considered as QTL and non-QTL interaction. The positive effects to increase plant height from bnlg1556 depended on umc1811 genotype (Fig. 2). If umc1811 was heterozygotes (12), the positive effect of bnlg1556 was obviously detected. However, among 23 digenic interactive combinations detected simultaneously in both locations, 13 pairs occurred between markers that are not adjacent to any mapped QTL. They could be called as non-QTL interactions. For the interaction of bnlg1538 with umc1733 (Fig. 2), when combined with different umc1733 genotypes,

**Table 2 Digenic interactions for plant height detected simultaneously in both locations by two-way ANOVA**

Locus A	Chr. locus B	Chr.	Wuhan										Xiangfan									
			P <sub>A</sub>	% <sup>1)</sup>	P <sub>B</sub>	% <sup>2)</sup>	P <sub>AB</sub>	% <sup>3)</sup>	Type <sup>4)</sup>	P	% <sup>5)</sup>	P <sub>A</sub>	% <sup>1)</sup>	P <sub>B</sub>	% <sup>2)</sup>	P <sub>AB</sub>	% <sup>3)</sup>	Type <sup>4)</sup>	P	% <sup>5)</sup>		
umc1169	1 bnlgl154	6					0.001	6.7	AA	0.000	6.2					0.002	6.1	AA	0.000	5.5		
umc1122 * <sup>6)</sup>	1 phi034	7			0.001	4.7	0.001	6.8	AA	0.000	5.7	0.000	6.6	0.000	5.5	0.001	6.5	AA	0.000	6.0		
									DA	0.008	2.8							DA	0.002	3.9		
umc1122 *	1 bnlgl792	7	0.017	2.9			0.001	7.0	AA	0.000	6.9	0.000	7.8			0.011	4.5	AA	0.001	4.7		
									DA	0.009	2.7											
umc1122 *	1 bnlgl823 *	8	0.004	3.7	0.000	7.0	0.001	6.3	AA	0.001	4.2	0.000	10.0	0.000	5.1	0.002	5.6	AA	0.000	5.5		
umc1185	2 umc1173	9					0.009	5.1	AA	0.005	3.0					0.010	5.0	AA	0.010	2.6		
mmc0271	2 phi118	10					0.009	11.0	AA	0.007	6.5					0.011	11.0	AA	0.005	7.0		
umc1042	2 umc1018	6					0.006	6.2	AA	0.010	3.0					0.007	6.2	AA	0.004	3.7		
umc1746	3 np1285	10					0.001	8.5	AA	0.000	6.5					0.000	10.5	AA	0.000	7.9		
phi036	3 bnlgl879 *	5			0.000	5.8	0.003	5.9	AA	0.002	3.7	0.002	4.5	0.009	5.0	AA	0.013	2.5				
bnlgl217	4 bnlgl518	10					0.003	8.2	AA	0.005	4.4					0.001	10.0	AA	0.004	4.6		
																		DA	0.004	4.7		
bnlgl023	4 bnlgl518	10					0.008	7.4	AA	0.001	6.0					0.002	9.0	AA	0.000	7.2		
bnlgl538	6 bnlgl209	9					0.008	6.3	AA	0.014	2.9					0.013	5.9	AA	0.002	4.4		
bnlgl539	7 umc1733	9					0.001	7.2	AA	0.002	3.6					0.003	6.0	AA	0.001	4.2		
umc1178	6 umc1733	9					0.001	6.8	AA	0.001	4.0					0.002	6.2	AA	0.005	3.2		
bnlgl953	1 umc1464	2					0.013	5.3	AD	0.002	4.0					0.004	6.5	AD	0.000	6.0		
umc1223	3 dupssr1 *	5			0.000	12.8	0.001	7.1	AD	0.008	3.3	0.000	10.7	0.003	6.3	DA	0.014	2.8				
bnlgl621	4 phi034	7					0.016	4.7	AD	0.004	3.3					0.010	5.1	AD	0.002	4.0		
umc1122 *	1 bnl17.18 *	5	0.001	4.5	0.000	15.6	0.006	4.5	DA	0.001	4.4	0.000	9.5	0.000	14.8	0.003	4.7	DA	0.003	3.6		
umc1123	2 umc1416 *	5	0.019	7.7	0.000	11.2	0.007	4.7	DA	0.001	4.0	0.000	8.1	0.000	12.8	0.004	4.7	DA	0.000	4.9		
umc1042	2 umc108 *	5					0.012	4.9	DA	0.002	3.8	0.017	3.1	0.010	5.1	DA	0.008	2.9				
umc1464	2 phi073	3	0.015	3.7			0.003	7.1	DA	0.001	5.3					0.007	6.4	DA	0.003	4.2		
phi073	3 php20075	10			0.007	4.3	0.009	5.9	DA	0.003	4.1	0.000	8.3	0.012	5.4	DA	0.002	4.4				
bnlgl811	1 bnlgl556 *	1			0.001	5.0	0.001	7.3	DD	0.001	4.3	0.000	8.8	0.000	7.1	DD	0.001	4.6				

<sup>1)</sup> Phenotypic variation explained by locus A ;

<sup>2)</sup> Phenotypic variation explained by locus B ;

<sup>3)</sup> Phenotypic variation explained by the interactions ;

<sup>4)</sup> AA: additive by additive ; AD: additive by dominance ; DA: dominance by additive ; DD: dominance by dominance ;

<sup>5)</sup> Phenotypic variation explained by special interaction type ;

<sup>6)</sup> Markers with \*are adjacent to mapped QTL

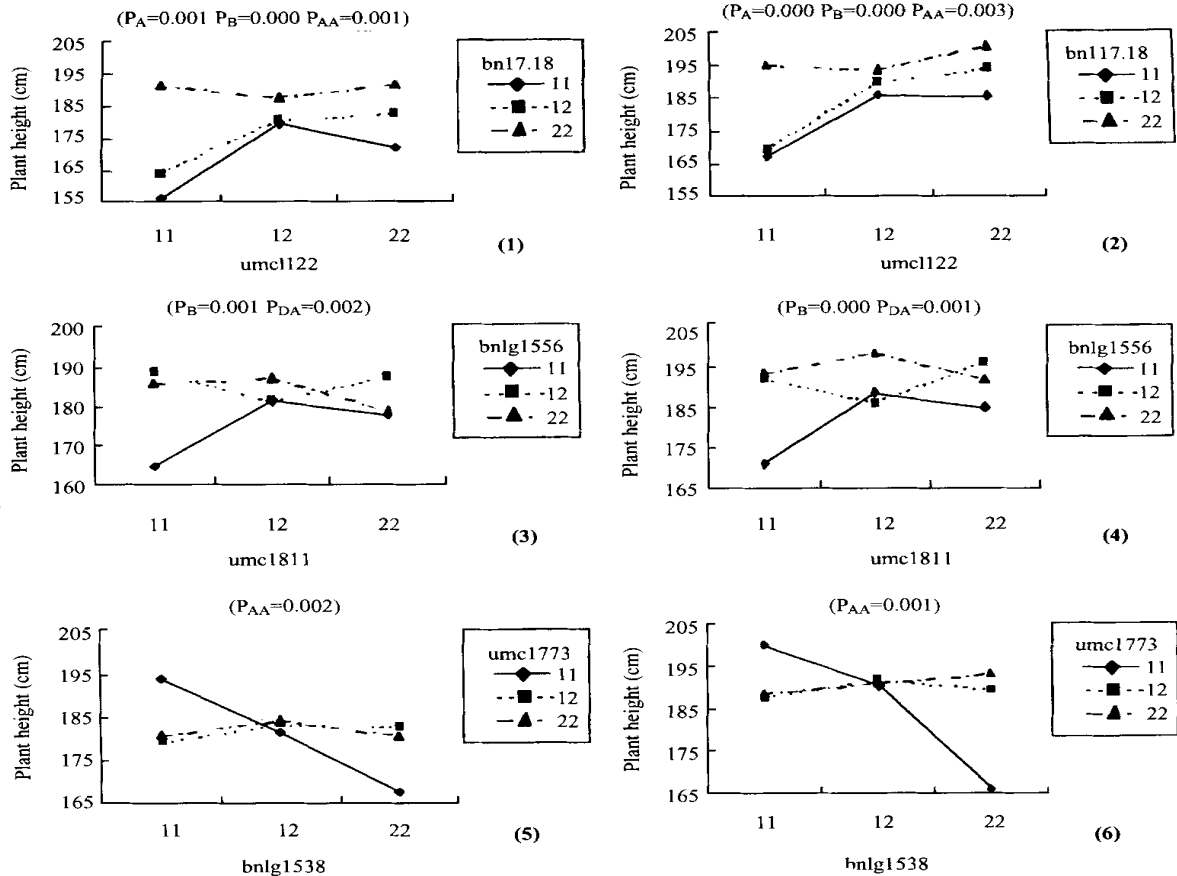
bnlgl538 showed obviously different effects. In this study, bnlgl538 (11) and umc1733 (11) seemed to be the most favorable combination for increasing plant heights.

In this study, a quite number of two-locus epistatic interactions were observed. That indicated the genetic foundation for plant height in maize may be much more complex than either hypothesis of over-

dominance or dominance based at the single locus. Thus, apart from the effect of single locus, the interactive effects should be considered in molecular marker assisted selection for plant height and other relative investigations. Although it is difficult to accurately evaluate the total values of genetic variation accounted by single QTL and two-locus interaction respectively with the same statistical model in this study, the

number of detected two-locus interaction is more than that of the single QTL. In addition, the single two-locus interaction could account for phenotypic variation between 4.5 and 11%, which could be comparable with phenotypic variation between 5.3 and 17.1% explained

by the single QTL. Therefore, it should be reasonable to believe that epistatic interactions between two loci may perform as an equal importance role as the single-locus effects for plant height in maize.



(1), (3) and (5) are the result in Wuhan; (2), (4) and (6) are the result in Xiangfan. 11, 12, 22 represent the three genotypes of each locus: 11 homozygote for Zong3 allele; 22 homozygote for 87-1 allele; 12 heterozygote

Fig. 2 Examples of digenic interaction for plant height

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