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Genetic dissection of plant height by molecular markers using a population of recombinant inbred lines in maize

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Abstract Plant height is an important trait for maize breeding because it is related to planting density and lodging resistance. It is influenced by many qualitative genes and quantitative trait loci (QTL). In this study, the genetic basis of plant height and its related traits were dissected, using simple sequence repeat (SSR) markers with a maize population of 294 recombinant inbred lines (RIL). Correlation results showed that plant height had a significant positive correlation with leaf number, average internode length and internode number. Increased plant height was affected most by average internode length. Six QTL for plant height were detected, which were consistent with those reported in previous studies. Moreover, eight QTL for leaf number, seven for internode number and six for average internode length were identified. Four of six QTL detected for average internode length were located on the same chromosomal region as the QTL affecting plant height and shared common molecular markers. This latter result strongly suggests that average internode length was the main contributor to plant height in maize.

Keywords Plant height · Average internode length · QTL · Maize (*Zea mays* L.)

Abbreviations

QTL	quantitative trait loci
RIL	recombinant inbred line
SSR	simple sequence repeats
LOD	logarithm of odds
PH	plant height
LN	leaf number
IN	internode number
AIL	average internode length

Introduction

Plant height is an important trait for maize breeding. It is significantly correlated to grain yield, and is affected by many qualitative genes and quantitative trait loci (QTL; Beavis et al. 1991). In order to increase grain yield, the planting density of maize is increasing gradually in the major areas of maize production throughout the world, but some hybrids with tall plants

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are often susceptible to lodging, which is always accompanied by reduction of grain output. To avoid plant lodging, the main breeding strategy is to decrease plant height, enhance stalk strength, and/or increase brace roots.

Biologically, plant height is comprised of two main components: internode number and average internode length. With the development of molecular genetics, it was widely used as a model trait for dissecting the genetic basis of QTL. Beavis et al. (1991) reported that most QTL detected for plant height were in close proximity to mapped qualitative genetic loci for the trait. Plant height was associated with 11 chromosomal regions in the study by Berke and Rocheford (1995). To date, numerous QTL for plant height were identified in a number of studies (Schön et al. 1993; Koester et al. 1993; Veldboom and Lee 1994, 1996; Lance and Lee 1996; Kozumplik et al. 1996; Lübberstedt et al. 1997; Austin et al. 2001, Yan et al. 2003). In these reports, only the total plant height in the mature stage of development was used as criteria for genetic analysis while few reports have analyzed the components contributing to plant height in maize.

In this study, the main objectives were to characterize QTL for plant height, and its components with a population of recombinant inbred lines (RIL) using molecular markers, and to analyze the relationship of plant height to its components.

Materials and methods

Materials

A population of 294 inbred lines (RIL) was selected for analysis. It was derived from a cross between two inbred lines, Z3 and 871, using a single seed descent method until the F8 generation. The cross was an elite hybrid cultivar name Yuyu22, which was planted in about 1.7 million hectares per year during 2002–2004 in China. The inbred line Z3 (P_1) is dent, selected from a synthetic population with Chinese domestic germplasm; the other parental line, 871 (P_2), is flint, selected from an exotic germplasm.

Field evaluation

The RIL population, as well as the two parents, was evaluated on the agronomy farm at China Agricultural University (Beijing, China) over 3 years (2002–2004). The field experiment followed a randomized complete block design with three replications in each year. Each plot included one row that was 4-m long and 0.67-m wide with a density of 45,000 plants per hectare.

Fifteen plants were planted in each plot, among which, ten consecutive plants starting from the third plant (i.e., plants 3–12 in the row) were used for trait measurements. In seedling stage, the fifth and tenth leaf of sampled plants were designated as markers for evaluating leaf number in the field. After pollen shedding, the same ten plants, which were used to measure leaf numbers, were evaluated for plant height, internode numbers and average internode length. Plant height was measured from the ground to the top of tassels. The internode number was defined as the number of elongated above-ground internodes. The average internode length was characterized as average length of the above-ground internodes, and was calculated as plant height divided by internode numbers. The average performance data over 3 years was employed as input data for further analyses. Estimates of means and variances for the measured traits were conducted using SAS software (SAS Institute 1996). The broad-sense heritabilities and confidence intervals of measured traits were computed according to Knapp et al. (1985), the estimates of σ_g^2, $\sigma_{g\times y}^2$, and σ_e^2 were obtained from the linear model in a two-way analysis of variance (ANOVA).

Construction of molecular linkage maps and QTL analysis

A total of 846 pairs of simple sequence repeat (SSR) markers were selected from the maize genome database (www.maizegdb.org) in order to screen polymorphisms between two parents. Of these, 285 SSR markers had distinct polymorphisms between two parents and were used to amplify the DNA of 294 RILs. Molecular linkage maps were constructed using Mapmakers 3.0

(Lander et al. 1987) at a LOD threshold greater than 3.0.

The composite interval mapping method, Mapmaker 3.0 (Zeng 1994) was employed for mapping QTL of measured traits using the average values of 3 years by the software QTL Cartographer (Wang et al. 1994). Model 6 of the Zmapqtl module was used to identify QTL, scanning intervals of 2 cM between markers and putative QTL with a window of 10 cM. The number of marker cofactors for background control was set by forward–backward stepwise regression with five controlling markers. A genome-wide critical threshold value for the experiment wise type I error rate ($\alpha = 0.05$) was set for each trait independently by randomly permuting 1,000 times. For main QTL effects, positive and negative signs of the estimates indicated that Z3 and 871 contributed toward higher value alleles for the traits.

Results

Performance of plant height and its related traits

Among the four traits measured, some differences between the two parents were identified (Table 1). Parent Z3 (P_1) had fewer internodes than parent 871 (P_2) and P_1 was 9.6 cm shorter than P_2. The values of the measured traits among the RIL population varied widely: ranges for plant height were 128–242 cm, for leaf number 16–23, for internode numbers 10.9–17.1 and for average internode length 9–19 cm. The averages for each of these traits were 19.8, 14.3, 13.4, and 190.7, respectively (Fig. 1; Table 1). The broad heritability for leaf numbers, internode numbers, average internode length, plant height reached 94, 92.8, 92.9, and 92.8%, respectively (Table 1). The interaction between genotype and year for the measured traits was not significant (data not shown).

The four measured traits were phenotypically correlated with each other (Table 2). Plant height had the strongest positive correlation to average internode length ($r = 0.77$) and was weakly correlated to internode ($r = 0.38$) and leaf ($r = 0.22$) number ($p < 0.01$ for each). Leaf number had a very strong positive correlation to internode number ($r = 0.92$) and a weak one with average internode length ($r = -0.39$; $p < 0.01$ for each). Internode number and average internode length ($r = -0.28$) were weakly correlated ($p < 0.01$).

QTL analysis

A genetic linkage map was constructed using 263 SSR markers that covered the whole genome of maize, spanning 2,361 cM length with an average interval of 9 cM between markers. These charac-

Table 1 Plant height and its related traits in the RIL population and its parents

Population	Trait	LN	IN	AIL (cm)	PH (cm)
P_1 [a]		18.0	12.8	14.4	179.5
P_2 [a]		19.2	14.0	13.5	189.1
RIL population	Average	19.8 ± 1.2	14.3 ± 1.1	13.4 ± 1.5	190.7 ± 22.5
	Ranges	15.8–23.1	10.9–17.1	9.4–18.5	127.5–242.2
	σ_g^2 [b]	1.28	0.98	1.94	415.23
	$\sigma_{g\times l}^2$ [b]	0.16	0.13	0.32	80.07
	σ_e^2 [b]	0.25	0.29	0.39	50.90
	H_B^2 [c]	94.00	92.80	92.90	92.80
	Confidence intervals[d]	92.9–94.9	91.4–0.93.8	91.6–94.0	91.4–93.9

LN leaf numbers, *IN* internode numbers, *AIL* average internode length, *PH* plant height

[a] P_1 represents one parent, Z3, and P_2 represents another parent, 871

[b] σ_g^2, the genotypic variance; $\sigma_{g\times l}^2$, the genotype and location interaction variance; σ_e^2, the environments variance

[c] The broad-sense heritability of the four measured traits

[d] The confidence intervals of broad-sense heritability between 5 and 95% significant levels

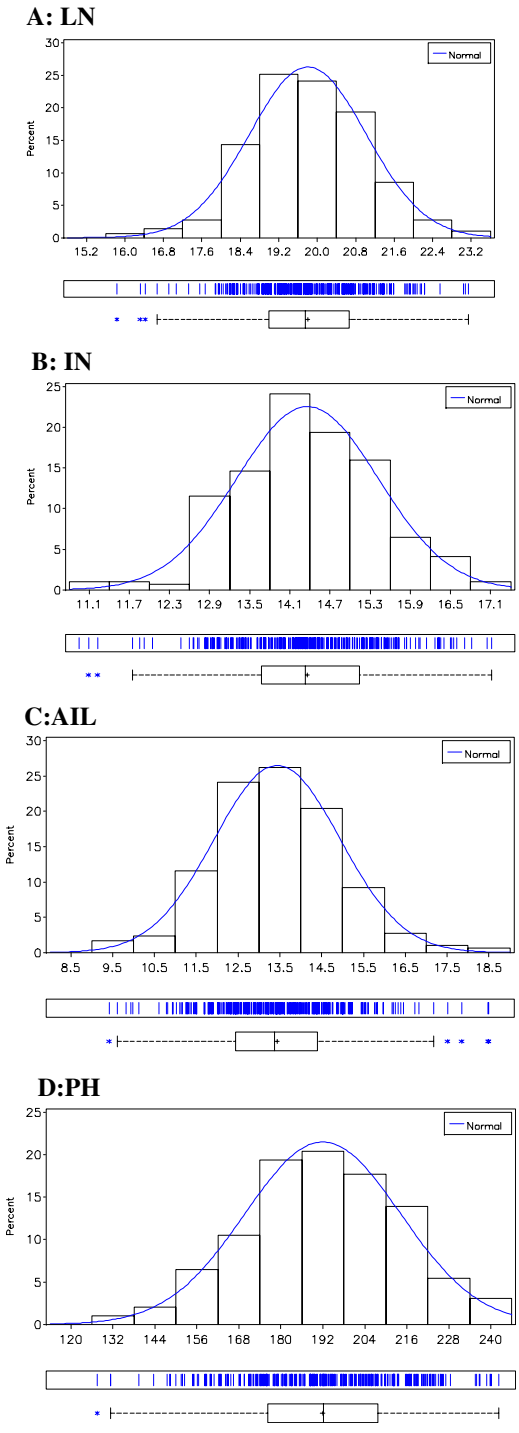

Fig. 1 Frequency distributions of a leaf number, b internode number, c average internode number, and d plant height in RIL population. a *LN* leaf number, b *IN* internode number, c *AIL* average internode length, d *PH* plant height

Table 2 Correlation coefficients between plant height and its related traits in RIL population

Trait	IN	AIL	PH
LN	0.92*	–0.39*	0.22*
IN		–0.28*	0.38*
AIL			0.77*

LN leaf number, *IN* internode number, *AIL* average internode length, *PH* plant height

*$p < 0.01$

teristics were consistent with linkage maps published in the maize genome database (www.maizegdb.org).

As shown in Table 3, 27 QTL were detected for the four measured traits using the composite interval mapping method. For plant height, six QTL were detected, located on chromosomes 1, 2, 3, and 5. The QTL, *qPH1a*, located on chromosome 1, between markers *umc2151-bnlg1556*, had the highest additive effect with a value of 11.34 cm. It accounted for 24.53% of the phenotypic variance. Another QTL, *qPH2*, explained 16% of the phenotypic variance. The inbred line of 871 alleles at QTL *qPH1a*, *qPH1b*, *qPH2, qPH5a, qPH5b* and the alleles from Z3 at QTL *qPH3* were associated with increased plant height. The total QTL effects detected for plant height accounted for 71% of the phenotypic variance.

Eight QTL were detected for leaf number, and were located on chromosomes 1, 3, 4, and 9. The QTL, *qLN9a* and *qLN9b*, accounted for 14 and 11% of the phenotypic variance, respectively. Among the mapped QTL, six alleles derived from inbred line 871 were associated with increased leaf number. Only two alleles from Z3 were associated with increased leaf number. The total QTL effects detected for leaf number explained 69% of the phenotypic variance.

For internode numbers, seven QTL were detected, five of which were located on chromosome 1 and the others on chromosomes 6 and 7. The QTL, *qIN1e* and *qIN7* had relatively large contribution rates (R^2), accounting for 11 and 10% of the phenotypic variance, respectively. Six alleles from the line 871 were associated with increased internode numbers and only one allele derived from Z3 was related to increased internode numbers. In all, 53% of the phenotypic

Table 3 Codes, locations, genetic effects, and phenotypic variation (R^2) of putative QTL for plant height and its related traits

Traits	QTL	Flanking markers	Bin	Logarithm rate (LOD)	A[a]	R^2 [b]	$LOD^c_{0.05}$
Plant height	*qPH1a*	umc2151-bnlg1556	1.07	13.52	–11.3	24.53	3.3
	qPH1b	umc2029-phi011	1.08	3.35	–6.06	7.17	
	qPH2	umc2372-umc1497	2.07	5.11	–9.11	16.2	
	qPH3	umc2166-umc1311	3.05	5.72	6.41	8.11	
	qPH5a	umc1692-umc2373	5.03	5.14	–5.79	6.59	
	qPH5b	umc1155-bnlg1237	5.05	3.94	–6.58	8.56	
Leaf number	*qLN1a*	umc1590-umc1035	1.06	3.82	–0.34	7.44	3.1
	qLN1b	umc1335-umc1122	1.07	4.58	–0.36	8.71	
	qLN1c	bnlg1643-bnlg1597	1.09	3.45	0.31	6.54	
	qLN3a	umc1223-umc1773	3.04	4.9	–0.31	6.73	
	qLN3b	umc2002-bnlg1035	3.04	5.27	–0.31	6.32	
	qLN4	phi213984-umc2082	4.02	4.45	0.34	7.95	
	qLN9a	bnlg127-bnlg1208	9.03	6.89	–0.46	14.29	
	qLN9b	bnlg1208-umc1771	9.04	7.6	–0.41	11.25	
Internode number	*qIN1a*	phi427913-phi001	1.02	4.36	–0.27	6.25	3.1
	qIN1b	phi001-bnlg1484	1.03	4.72	–0.32	8.76	
	qIN1c	umc2112-umc1689	1.05	3.31	–0.23	4.11	
	qIN1d	umc1590-umc1035	1.06	3.57	–0.32	7.45	
	qIN1e	umc2151-umc1122	1.07	5.06	–0.37	10.82	
	qIN6	umc1020-phi299852	6.07	4.47	0.26	6.13	
	qIN7	bnlg1792-mmc0411	7.02	4.97	–0.34	9.91	
Average internode length	*qAIL1*	umc2029-phi011	1.09	6.95	–0.62	15.96	3.2
	qAIL3a	umc1773-bnlg1035	3.04	5.7	0.39	6.5	
	qAIL3b	umc2127-umc2166	3.05	6.4	0.51	11.29	
	qAIL5a	umc1692-umc2373	5.03	3.77	–0.33	4.31	
	qAIL5b	umc1155-umc1019	5.05	4.91	–0.49	10.4	
	qAIL9	bnlg1209-umc1771	9.04	4.45	0.38	6.05	

[a] Additive effect: positive values indicate that the Z3 alleles are increasing the traits and vice versa for negative values

[b] R^2 contribution rate

[c] $LOD_{0.05}$ logarithm of odds at $p < 0.05$

variance for the internode number accounted was explained by detected QTL.

Six QTL were detected for average internode length and were located on chromosomes 1, 3, 5, and 9. The QTL *qAIL1*, *qAIL3b*, and *qAIL5b* had relatively large contribution rates (R^2), accounting for 16, 11, and 10% of the phenotypic variance, respectively. The alleles from the line 871 at QTL *qAIL1*, *qAIL5a*, and *qAIL5b* were associated with increased average internode length. The total QTL effects detected for average internode length explained 55% of the phenotypic variance.

Discussion

Compared with previous reports (Table 4), all six QTL for plant height detected in this study seemed to have similar, or the same, chromosomal locations with different mapping populations. Lübberstedt et al. (1997) identified 20 QTL for plant height using two testcross population of F_3 lines, including the six QTL detected in this study. Four QTL detected by Melchinger et al. (1998) were situated at the same chromosomal loci as the QTL reported in this study. One QTL, *qPH5a*, is located in the same chromosomal region as many QTL for plant height identified in other independent studies with at least seven different mapping populations in maize (Beavis et al. 1991; Ajmone-Marsan et al. 1994; Schŏn et al. 1994; Berke and Rocheford 1995; Kozumplik et al. 1996; Lübberstedt et al. 1997; Melchinger et al. 1998). In other studies, *qPH1a* and *qPH1b* (Schŏn et al. 1994), and *qPH3* and *qPH5b* (Abler et al. 1991; Ajmone-Marsan et al. 1994) were also

Table 4 Comparison of QTL locations for plant height between this study and previous studies

QTL[a]	Chromosomal bin[a]	Linked markers[b]	Reference
qPH1a	1.07	umc23a-umc58	Schŏn et al. (1994) Lübberstedt et al. (1997) Melchinger et al. (1998)
qPH1b	1.08	umc83a bnl15.18-umc37a isu18-umc37a an1 anther ear1 an1 anther ear1	Beavis et al. (1991) Schŏn et al. (1994) Veldboom and Lee (1994) Veldboom and Lee (1996) Veldboom and Lee (1996) Lübberstedt et al. (1997)
qPH2	2.07	php20005-umc131	Beavis et al. (1994) Lübberstedt et al. (1997) Melchinger et al. (1998)
qPH3	3.05	bnl5.37a-csu154b(eif5A) umc26a-umc42b	Abler et al. (1991) Ajmone-Marsan et al. (1994) Kozumplik et al. (1996) Lübberstedt et al. (1997) Melchinger et al. (1998)
qPH5a	5.03	bnl7.56 bnl5.71a-umc1 umc27a-umc90	Beavis et al. (1991) Ajmone-Marsan et al. (1994) Schŏn et al. (1994) Berke and Rocheford (1995) Kozumplik et al. (1996) Lübberstedt et al. (1997) Melchinger et al. (1998)
qPH5b	5.05	bnl5.71a-umc1	Ajmone-Marsan et al. (1994) Lübberstedt et al. (1997)

[a] QTL for plant height and their chromosomal locations detected in this study

[b] QTL for plant height and their flanking markers identified in previous reports, which has the same chromosomal regions with detected QTL in this paper

identified. These demonstrate that a large number of QTL influence plant height in maize and some of them may have a common origin.

Based on correlation results (Table 2), plant height increases significantly as the average internode length increases. It also has a weak correlation with increased number of leaves, and internodes. Thus, the above-ground internode length contributes largely to taller plants while the numbers of leaves and internodes have a lesser influence. Interestingly, four of six QTL detected for plant height had the same chromosomal regions as the QTL for average internode length, sharing common molecular markers, such as *qPH1b* versus *qAIL1*; *qPH3* versus *qAIL3b*; *qPH5a* versus *qAIL5a*, and *qPH15b* versus *qAIL5b*. In contrast, only one QTL detected for plant height was located on the same chromosomal regions as the QTL for internode number. These results suggest that average internode length was the main contributor to plant height genetically. Therefore, in order to understand the molecular mechanisms contributing to plant height, average internode length should be the primary focus of further investigations.

To date, a number of qualitative mutant genes controlling plant height have been mapped (Coe Jr 1980; Touzet et al. 1995; Helentjaris et al. 1993; Rhoades and Dempsey 1954; Robertson 1974), and a few of these genes were cloned recently, such as *D8* (Ikeda et al. 2001) and *D3* (Winkler and Helentjaris 1995). In a previous study (Beavis et al. 1991), QTL detected for plant height were compared in four populations, and nine QTL were identified in the chromosomal regions known to have qualitative genetic loci (*D8*, *D3*, *na2*, *gl17*, *yd2*, etc.) affecting plant height. In this study, four QTL detected for average internode length reside in the same chromosomal regions as five qualitative loci for plant height, such as *qAIL1* versus *D8*, *qAIL9* versus *D3, qAIL5b* versus *na2* and *gl17, qAIL3b* versus *yd2*. The

plant height QTL, *qPH1b, qPH3*, and *qPH5b*, share the same molecular markers as *D8*, *yd2*, *na2*, and *gl17*. Robertson (1985) pointed out that minor allelic variants at qualitative loci could be responsible for quantitative effects. In support of this suggestion, the results of this study imply that the QTL for average internode length may be related to qualitative genes controlling plant height. Qualitative genes may influence plant height by controlling average internode length. Hence, qualitative genes, which are located in the same chromosomal regions as QTL for average internode length, should be considered candidate genes. By the approach of associated analysis, quantitative trait genes for plant height may be isolated in the future.

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