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Original Research

QTL mapping analysis of maize plant type based on SNP molecular marker

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Abstract: In this study, the elite maize inbred line (Zheng683-1) was used as a recurrent parent and the four maize inbred lines (ZPH1388, ZPH5, Dong 237 and Chang 7-2) were used as donor parents. The four F1 hybrids were produced by crossing between them and were continued backcrossing to the recurrent parent to produce four BC3F1s. The BC3F1 were pollinated by selfing four generations to generate the recombinant inbred lines (BC3F5) that showed variation in plant height, ear height, leaf angle. There were 53 lines for Zheng683-1 x ZPH1388, 53 lines for Zheng683-1 x ZPH5, 48 lines for Zheng683-1 x Dong237 and 61 lines for Zheng683-1 x Chang 7-2. The four populations were genotyped by using SNP marker and identified the QTLs of targeted traits by using QTL IciMapping V4.1 software and stepwise regression analysis. The main results are as follows:1. 19 additive QTLs and 2 dominant QTLs about plant height were detected in four introgression lines, and 7, 4, 5, 5 QTLs related plant height in ZPH1388-IL, ZPH5-IL, Dong237-ILand Chang7-2-IL. Among the 21 plant height QTLs, the largest contribution to phenotypic variation was QTLqPHa12 from Dong237-IL population, which evaluated 43.44% of the phenotypic variation of plant height . Followed by qPHa3 from ZPH1388-IL, the phenotypic contribution rate was 20% 2. Sixteen QTLs related ear height were detected in the population of the 4 introgression lines, of which there were 15 additive and 1 dominant QTLs. In addition, 9, 3, and 4 QTLs were detected in ZPH1388-IL, ZPH5-IL and Dong237-IL respectively. Among the 16 ear height QTLs, the largest contribution to phenotypic variation was QTL (qEHa1) from the ZPH1388 IL, which recorded the phenotypic variation of ear height by 26.01%. Next the phenotype contribution rates were 22.05% and 21.46%, respectively for qEHa13 and qEHa15 from Dong237-IL. Fourteen QTLs related leaf angle was detected in the population of the introgression lines, of which there were 5 additive QTLs and 9 dominant QTLs. Moreover, 2, 9, and 3 QTLs were detected in ZPH1388-IL, ZPH5-IL, Dong237-IL, respectively. Among the 14 leaf angle QTLs, the largest contribution to phenotypic variation was QTL (qLAa4) from the Dong237-IL then qLAa3 from ZPH5-IL, detecting the phenotypic variation of leaf angle of 28.56% and 24.08%, respectively.The results showed that the QTL locus was characterized by uneven chromosome distribution. The chromosome 1, 2, 5, and 7 are the regions with the QTL concentrated distribution of plant type traits. The QTL of plant type also showed QTL concentrated distribution in some regions of chromosomes. For example, there are three QTLs about plant height and ear height in the region of chromosome bin1.02, three QTLs about plant and ear heights, as well as leaf angle in the region of chromosome bin 2.02. These areas are QTL enrichment region (or the hot spots) of the pleiotropic gene loci related the plant type and ear traits.

Key words: Ear height; Leaf angle; Plant type traits; Plant height; QTL; SNP.

Introduction

After Australian scholar, Donald first proposed Ideotype of plants, the maize plant type was mainly manifested in the spatial distribution state of the maize plant (1). In general, the leaf shape, leaf angle, light acceptance posture and spatial distribution, plant height and ear height are the main factors that constitute the plant type. The morphological extension of plant type includes the root type, stem type and ear type, etc. (2). Many breeders take the selection of ideal maize plant type as an important breeding objective. In the process of maize breeding, plant traits have important influences on the maize material or inbred lines' disease resistance, stability, adaptability, high yield and lodging resistance. For example, maize plant height and ear height are highly correlated to the lodging resistance (3). Leaf angle is one of the most important traits in plant type characters, which is a comprehensive embodiment of the compact degree of maize plant type. Therefore, studying plant-type traits relating genetic mechanism is of great significance to the improvement of maize's stability, fecundity, lodging resistance and dehydration, high yielding and adaptability. The planting area and total yield of maize in China have exceeded those of wheat and rice. Since 2012, it has become the largest grain crop in China. Therefore, maize production is related to Chinese food security (4-5). At present, there is a pressing need for new varieties of machine-harvested maize in China. In order to meet the production demand, maize breeding has gradually shifted from types with late maturity, large ear type with sparse planting to those with early maturity, rapid dehydration and resistance to lodging and diseases. Due to the increase in planting density, lodging has been a common problem. Therefore, it is difficult and hot in the theory and practice of maize breeding to improve plant type, increase density and resolve lodging resistance (6-7).

In recent years, domestic and foreign scholars have conducted studies of the plant related characteristics successively. Using the RIL population in high nitrogen and low nitrogen conditions, Zheng Zuping, Huang

Table 1. Phenotypes of Zheng683-1-IL population traits.

Trait	Zheng683-1		ZPH1388-IL		ZPH5-IL		Dong237-IL		Chang7-2	
	Mean±SD	**c.v**	**Mean±SD**	**c.v**	**Mean±SD**	**c.v**	**Mean±SD**	**c.v**	**Mean±SD**	**c.v**
Plant Height (PH) (cm)	180.77±7.2404	0.02	189.05±16.54	0.09	186.99±10.68	0.06	188.77±16.21	0.09	183.14±11.32	0.06
Ear Height (EH) (cm)	56.23±4.62	0.05	61.11±5.82	0.1	62.67±6.11	0.1	59.60±7.51	0.13	57.4±6.18	0.11
Leaf Angle (LA) (^{O})	30.91±0.64	0.01	30.27±2.65	0.09	30.33±3.63	0.12	31.4±2.73	0.09	29.42±3.05	0.1

PH, plant height; EH, ear height; LA, leaf angle.

Yubi et al made QTL mapping on related traits of maize plant type, such as plant height, ear leaf length, total leaf number per plant and ear height, ear leaf width and ear leaf area, and finally detected a total of 19 and 14 pairs of additive effects related to plant types (8). One of the additive effects was related to plant height; 3 pairs of epistatic effect QTLs and 3 additive effect QTLs control the ear height. 4 additive effects QTLs and 3 pairs of the epistatic effect QTLs, and 3 pairs of the epistatic effect QTLs and 4 additive effect QTLs affecting the ear leaf length were detected; 3 pairs of the epistatic effect QTL and 2 additive effect QTLs controlled the ear leaf width; 2 pairs of the epistatic effect QTL and 2 additive effect QTLs controlled the ear leaf area. Setting up a maize excellent inbred line Xu 178 as the background, with Zong 3 as a single fragment substitution line group of the donor, Xu Cheng et al conducted QTL mapping analysis of plant type related traits such as height of ear, plant height, leaf area, total leaf number and number of leaves on ear, and detected 72 QTLs of related traits of 5 maize plant types, including 12 ear height QTLs, 23 plant height QTLs, 6 total leaf number QTLs, 16 leaf area QTLs, and 15 ear number QTLs (9). Among them, there were 3 ear height QTL, 10 plant height QTL, 3 total leaf number QTL, 1 leaf area QTL and 2 leaf number QTL on the ear. Ku et al constructed F2 temporary separation mapping population with Yu 82 x Shen 137 as the material and 3 leaf angle QTLs were detected on chromosome 1, 2 and 5 by composite interval mapping (10). In this study, four sets of BC3F5 introgression lines were used as materials to conduct QTLs mapping analysis on the related traits of plant types, such as maize plant height, ear height and leaf angle, providing a scientific basis for developing functional markers, identifying alleles and molecular marker-assisted selection breeding of maize plant types-related primary QTLs.

Materials and Methods

Plant materials and field experiments

This study was performed using four sets of introgression line (IL) populations derived from four recurrent parents ZPH1388-IL, ZPH5-IL, Dong 237-IL, Chang 7-2-IL and the same donor parent Zheng 683-1. The plant and ear heights, leaf Angle of the four BC3F5 were measured including 53,53,48 and 61 individuals, respectively. The ILs and their parents were planted at the Yuanyang, Henan Province (N35.05°, E113.76°), Northwest Agricultural and Forestry University,

Genotyping and QTL mapping

DNA was extracted using the cetyltrimethylammonium bromide (CTAB) procedure (11), 5k SNP markers which were selected from the National Maize Improvement Center of China, China Agricultural University, to genotype the 215 ILs. The QTL analysis was performed through the software of IciMapping V4.1, using Stepwise regression analysis for QTL identification (12-13).

Results

Phenotypic analysis of the plant type traits of 4 introgression maize lines

Based on the filed performance of plant type traits in Yuanyang, Henan Province in 2016 from ZPH1388-IL, ZPH5-IL, Dong 237-IL, Chang 7-2-IL and Zheng 683-1, it can be seen that introgression lines the plant heights and ear heights of ZPH1388-IL, ZPH5-IL, Dong 237-IL and Chang 7-2-IL were higher than those of Zheng 683-1 (Table 1). Among them, the plant heights and ear heights of ZPH1388-IL and ZPH5-IL were significantly higher than those of Zheng 683-1, and the ear heights of ZPH1388-IL and ZPH5-IL were significantly greater than that of Zheng 683-1. The leaf angle of Dong 237-IL was larger than that of Zheng 683-1, but the leaf angle of Chang 7-2-IL was smaller than that of Zheng 683-1. The variation coefficients of the four introgression lines (ZPH1388-IL, ZPH5-IL, Dong 237-IL, Chang 7-2-IL) were larger at the height of ear and the leaf angle, showing that on the traits of ear height and leaf angle, ZPH1388-IL, ZPH5-IL, Dong 237-IL and Chang 7-2-IL provided greater phenotypic variations for Zheng 683-1. ZPH1388-IL and Dong 237-IL showed the greatest variation coefficient on plant height, indicating that ZPH1388-IL and Dong 237-IL provided greater phenotypic variations for Zheng 683-1 than ZPH5-IL and Chang 7-2-IL.

Genetic analysis of plant type traits of 4 introgression lines

The broad-sense heritability of the plant type traits of the 4 introgression lines in 2016 showed that the broad-sense heritability of all plant type traits tested by the 4 groups was higher (Table 2). In ZPH1388-IL population, the ear height and the leaf angle showed higher broad-sense heritability (over 80%) compared to the plant height, which were relatively low (49.5%). Concerning Dong 237-IL, The plant height, ear height and leaf angle showed relatively higher broad-sense heritability, namely 95.9%, 86.6% and 85.0%, respectively. The traits of 3 plants types of ZPH5-IL and Chang 7-2IL also presented higher broad-sense heritability (over 80%). For the introgression lines, the ear height of ZPH1388-IL, leaf angle of ZPH5-IL and leaf angle

Table 2. Broad-sense heritability of plant type traits and correlation analysis between four introgression lines population and Zheng683-1

Material	PH	EH	LA
ZPH1388-IL	49.5%	81.6%	85.5%
ZPH5-IL	88.7%	86.6%	85.5%
Dong237-IL	95.9%	92.9%	85.0%
Chang7-2IL	87.0%	87.1%	86.6%
r1	0.545	0.993**	0.978*
r2	0.58	0.954*	0.999**
r3	0.861	0.969*	0.913
r4	0.429	0.903	0.996**

r1: ZPH1388-IL was correlated to the phenotype of traits of Zheng 683-1; r2: ZPH5-IL was correlated to the phenotype of traits of Zheng; r3: Dong237-IL was correlated to the phenotype of traits of Zheng 683-1; r4: Chang 7-2-IL was correlated to the phenotype of traits of Zheng 683-1; ** and * significant difference at ($P< 0.01$) and ($P<0.05$) level respectively.

of Chang 7-2IL were extremely significantly correlated to Zheng 683-1. Leaf angle of ZPH1388-IL, ear height of ZPH5-IL and ear height of Dong 237-IL were significantly correlated to Zheng 683-1.

Analysis of SNP markers in 4 introgression lines

The total number of SNPs used for the classification of 4 sets of introgression lines ZPH1388-IL, ZPH5-IL, Dong 237-IL and Chang 7-2-IL were 4411, 4406, 4393 and 3065, respectively. After comparison and screening, the SNP number of the four introgression lines with polymorphisms was 1841, 1589, 1673 and 1368, respectively (Figure 1-4). Among them, more than 200 polymorphic SNPs have more polymorphisms on chromosome 1 and 4 of ZPH1388-Il, chromosome 1 of ZPH5-Il, chromosome 1 and chromosome 5 of Dong 237-IL. The numbers were 293, 209, 229, 250 and 252, respectively. However, there were fewer polymorphic SNPs on chromosome 10 of ZPH5-IL and chromosomes 3 and 10 of Chang 7-2-IL, namely 75, 79 and 61, respectively. The numbers of SNPs with polymorphisms in 4 sets of introgression lines with the same chromosomes and different lines are different, showing that although the backgrounds of the introgression lines were the same, different fragments and amounts that the donor parents, ZPH1388-IL, ZPH5-IL, Dong 237-IL and Chang7-2-IL introgressed were diverse from each other. The difference in the number of polymorphic SNPs between different chromosomes in the same introgression line population may be that the number of donor parent fragments introgressed into each chromosome was different. It may also be due to the differences in the number of SNPs it has.

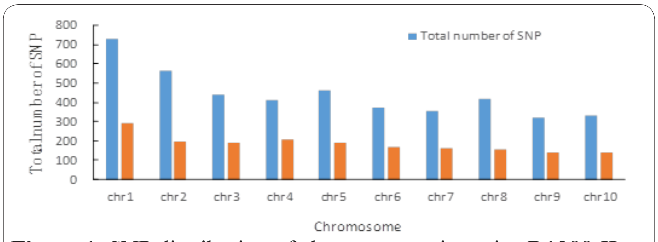

Figure 1. SNP distribution of chromosomes in maize D1388-IL.

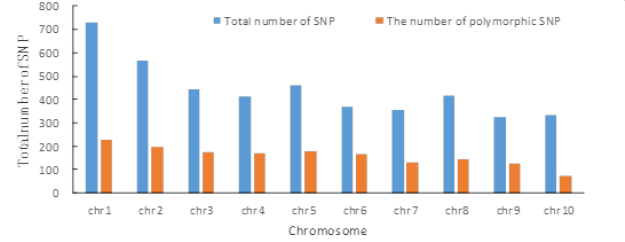

Figure 2. SNP distribution of chromosomes in maize ZPH5-IL.

The additive QTL of plant type traits detected from 4 introgression lines

For the 4 introgression lines, a total of 39 additive QTLs related to maize plant types were detected (Table 3). The results showed that 16, 7, 11, and 5 additive QTLs related to maize plant types were detected respectively in ZPH1388-IL, ZPH5-IL, Dong 237-IL and Chang 7-2-IL. A total of 19 additive QTLs related to plant height were detected in the 4 groups, in which there were 4 on chromosome 1, 3 on chromosome 2, 1on chromosome 3, 1 on chromosome 4, 8 on chromosome 5, 1 on chromosome 7 and 1 on chromosome 9. Fifteen additive QTLs related to ear height were found, comprising 3 on chromosome 1, 2 on chromosome 2, 1 on chromosome 4, 3 on chromosome 5, 2 on chromosome 6, 3 on chromosome 7 and 1 on chromosome 10. Five additive QTLs related to leaf angle were noticed, including 1 on chromosome 1, chromosome 2 and chromosome 4 respectively and 2 on chromosome 3.

The 5 additive QTLs related to plant height were recorded in ZPH1388-IL showing 58.24% of the phenotypic variations of this population. Among them, qPHa3 contributed the most to the plant height explaining 20% of plant height phenotypic variations. qPH1, qPH3 and qPH4 had a synergistic effect, but the introgression of qPH2 and qPH5 reduced the plant height of the introgression line. There were 9 additive QTLs related to ear height in this population, and the contributions were all from ZPH1388. The contribution of qEHa1 was the largest, accounting for 26.01% of the ear height phenotype variations and having a synergistic effect. There were 2 additive QTLs qLAa1 and qLAa2 related to leaf angle in ZPH 1388-IL population, explaining 15.02% and 16.17% of the leaf angle phenotype variations, respectively. The introgression of qLAa1 could lead to the

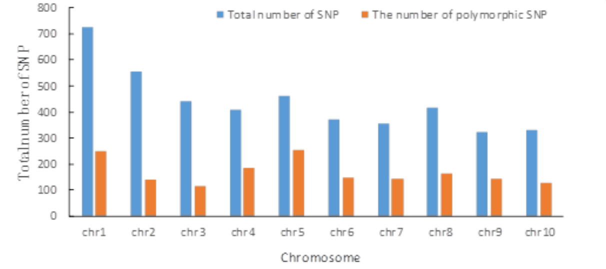

Figure 3. SNP distribution of chromosomes in maize Dong237-IL.

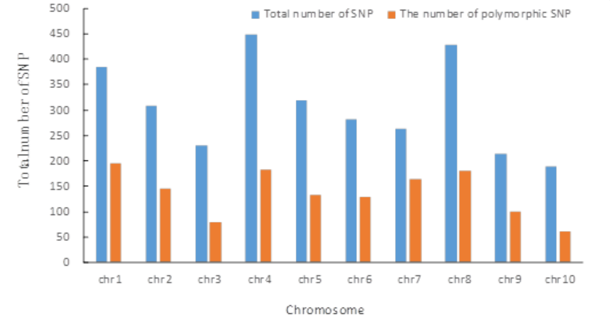

Figure 4. SNP distribution of chromosomes in maize Chang7-2-IL.

Table 3. The additive QTL of plant type traits detected for nine measured traits using four introgression lines.

ZPH1388-IL

Trait	QTL	Chromosome	Marker	LOD	PVP (%)	Additive effect
PH	qPHa1	1	M76	3.35	7.40	12.35
	qPHa2	1	M716	3.98	9.56	-12.06
	qPHa3	1	M724	7.35	20.00	19.56
	qPHa4	5	M2583	6.36	16.55	16.01
	qPHa5	9	M3936	2.20	4.73	-7.74
EH	qEHa1	1	M48	14.07	26.01	10.53
	qEHa2	1	M579	6.68	8.85	3.13
	qEHa3	2	M823	9.05	14.67	4.49
	qEHa4	2	M1104	2.54	2.68	2.39
	qEHa5	4	M2040	4.21	4.76	2.30
	qEHa6	6	M2976	6.09	8.53	3.02
	qEHa7	7	M3220	7.07	9.21	6.26
	qEHa8	7	M3313	5.71	6.97	2.81
	qEHa9	10	M4146	5.06	5.99	3.61
LA	qLAa1	3	M1590	2.13	15.02	2.63
	qLAa2	4	M2096	2.45	16.17	-1.81

ZPH5-IL

Trait	QTL	Chromosome	Marker	LOD	PVP (%)	Additive effect
PH	qPHa6	2	M748	2.12	10.01	5.32
	qPHa7	1	M908	2.52	13.08	-8.27
	qPHa8	2	M1204	2.24	10.58	-5.88
	qPHa9	5	M2271	3.01	15.33	9.03
EH	qEHa10	1	M80	2.04	16.20	3.83
	qEHa11	5	M2271	2.35	18.73	4.71
LA	qLAa3	3	M1572	3.51	24.08	6.31

Dong237-IL

Trait	QTL	Chromosome	Marker	LOD	PVP (%)	Additive effect
PH	qPHa10	5	M2327	9.63	12.59	14.62
	qPHa11	5	M2425	2.17	1.67	-4.29
	qPHa12	5	M2604	17.54	43.44	-22.71
	qPHa13	5	M2609	10.66	17.95	12.86
	qPHa14	7	M3203	6.31	8.02	9.76
EH	qEHa12	5	M2484	2.82	10.42	3.35
	qEHa13	5	M2584	5.14	22.05	-5.63
	qEHa14	6	M2637	2.09	7.70	3.67
	qEHa15	7	M3208	4.66	21.46	5.45
LA	qLAa4	1	M340	3.90	28.56	3.52
	qLAa5	2	M769	2.08	13.06	-2.43

Chang7-2

Trait	QTL	Chromosome	Marker	LOD	PVP (%)	Additive effect
PH	qPHa15	4	PZE-104031870	7.08	15.59	-22.38
	qPHa16	5	PZE-105144035	6.44	13.54	-10.36
	qPHa17	5	PZE-105107675	7.28	18.53	10.12
	qPHa18	3	PZE-103016707	2.05	4.17	-4.76
	qPHa19	2	SYN13011	3.71	7.22	4.19

PH, plant height; EH, ear height; LA, leaf angle.

increased leaf angle of the introgression line, but that of qLAa2 could have the inverse effect.

Four additive QTLs were detected in the introgression line population ZPH5-IL which could explain 49% of the variations of the plant height. Among them, the additive QTLs qPHa6 and qPHa9 had a synergistic effect, while qPHa7 and qPHa8 could reduce the plant height of the introgression line. Among the 4 plant height QTLs, qPHa9 contributed the most to the plant height, explaining 15.33% of the plant height variations in this population. For 2 additive QTLs qEHa10 and qEHa11 related to ear height, both of their contributions to the ear height were from ZPH5 with synergistic effects, which explained respectively 16.2% and 18.73 of ZPH5-IL's ear height phenotype variations. Only one additive QTL qLAa3 related to the leaf angle was detected in this population, which evaluated 24.08% of phenotypic variations and had a synergistic effect.

Five additive QTLs related to plant height were detected in Dong 237-IL population, They could explain 83.67% of the phenotypic variations, among which only qPHa12 could explain 43.44% of the phenotypic variations and reduce the plant height of the introgression line. However, qPHa10, qPHa13 and qPHa14 had a synergistic effect. In the 4 additive QTLs of ear height in this population, qEHa13 and qEHa15 could explain 43.51% of the variations of ear height, and 22.05% and 21.46% of the variations of the phenotype of the population, respectively. qEHa13 had a synergistic effect, while qEHa13 could lower the ear height of the introgression line. Two leaf angle QTLs (qLAa4 and qLAa5) were detected in Dong 237-IL population, contributing 28.56% and 13.036% respectively to the leaf angle of the population. qLAa4 could increase the leaf angle of the introgression line.

Only 5 QTLs related to plant height were detected in the introgression line Chang 7-2 as a donor, and the ones that contributed more to the phenotypic plant height were qPHa15, qPHa16 and qPHa17, which could explain 47.66% of the group's phenotypic variations. Among them, qPHa15, qPHa16 and qPHa18 could reduce the plant height, while qPHa17 had a synergistic effect.

There were no QTLs located to the same locus in the 4 introgression lines. In addition, more QTLs were located in ZPH1388-IL and Dong 237-IL, indicating that the genetic differences between donors ZPH1388, Dong 237 and D683-1 were relatively large. The difference between Chang 7-2 and D683-1 was the smallest, which might be due to the different hereditary bases of 4 donor parents and limited introgression lines.

The dominant QTL of plant type traits detected in 4 introgression lines

12 dominant QTLs related to plant type were detected in the 4 introgression lines (Table 4), The results showed that there were 2, 1 and 9 dominant QTLs related to plant height, ear height and ear angle. Both of the two additive QTLs related to plant height were from ZPH 1388-IL, which could explain 43.18% of the plant height phenotype variations in this population. QTL qEHd1 related to ear height was detected in 4 groups, and qEHd1 could explain 14.2% of ZPH5IL phenotypic variations. Nine QTLs related to the leaf angle was detected. qLAd9 which was from Dong 237-IL and other 8 were all from ZPH5-IL. The number of dominant QTLs detected in 4 introgression lines was significantly lower than that of additive QTLs, which indicated that the phenotypic variations of the related traits of 3 plant types in 4 introgression lines were mainly from additive effect, and the contribution of dominant QTLs to plant type was lower than that of additive QTLs. Additionally, no dominant QTLs were detected in the introgression line of Chang 7-2 as a donor. This was similar to the detection result of additive QTLs in the 4 introgression lines, which might be related to the smallest difference between Chang 7-2 and Zheng 683-1, or the fewer donors due to the limited groups of introgression lines.

QTL concentration region of plant type traits

The QTL controlling the plant type traits can be located in the same or adjacent chromosomal regions. By analyzing the regions of SNP markers on chromosomes (Table 5), the results showed that there were 6 QTL concentration regions in the maize genome. For example, there were two plant height QTLs (qPHa1 and qPHa7) and one ear height QTL (qEHa10) between SNP markers M76 and M80 in the region of bin1.02 of chromosome 1. Similarly, there was 1 plant height QTL, 1 ear height QTL and 1 leaf angle QTL between SNP marker M809 and SYN13011 in the region of bin2.02 on chromosome 2, which were qPHa19, qEHa3 and qLAd2, respectively. Three QTLs related to leaf angle (qLAa1, qLAa3 and qLAZPH5) were detected between SNP markers M1590 and M1595 in the region of bin3.06 on chromosome 3. There was 1 QTL related to ear height and 2 others related to plant height between SNP markers M2271 and M2327 chromosome sections in the region of bin 5.03 on chromosome 5, namely qEHa11, qPHa9 and qPHa10, respectively. One QTL

Table 4. The dominant QTL of plant type traits detected for nine measured traits using four introgression lines.

Trait	QTL	Chromosome	Marker	LOD	PVP (%)	Dominant effect	Introgression lines
PH	qPHd1	3	M1385	3.76	14.2	28.2	ZPH1388-IL
	qPHd2	8	M3700	2.27	11.11	14.98	ZPH1388-IL
EH	qEHd1	5	M2390	2.89	19.27	6.61	ZPH5-IL
LA	qLAd1	1	M642	3.39	8.82	3.84	ZPH5-IL
	qLAd2	2	M809	2.43	6.49	1.45	ZPH5-IL
	qLAd3	2	M1158	3.63	9.93	2.06	ZPH5-IL
	qLAd4	3	M1311	2.97	7.69	-2.54	ZPH5-IL
	qLAd5	3	M1595	4.02	10.74	4.24	ZPH5-IL
	qLAd6	3	M1616	2.07	9.94	1.18	ZPH5-IL
	qLAd7	8	M3616	3.29	9.42	1.99	ZPH5-IL
	qLAd8	10	M4362	4.52	12.38	-2.12	ZPH5-IL
	qLAd9	5	M2224	3.4	19.14	2.29	Dong237-IL

PH, plant height; EH, ear height; LA, leaf angle.

related to plant height and 2 others related to ear height were located between SNP markers M2425 and M2484 in the region of bin 5.05 on chromosome 5, qPHa11, qEHa17 and qEHa12. One plant height QTL (qPHa14) and 2 ear height QTLs (qEHa15 and qEHa7) were detected between SNP markers M3203 and M3220 in the region of bin 7.03 on chromosome 7. These regions were the relative concentration areas of plant type related traits QTLs such as plant height, ear height and leaf angle, or the accumulation areas of pleiotropic gene loci. These QTL concentration regions could be expressed and detected in different introgression lines, indicating that these loci were probably important functional chromosomal regions required for the formation of 4 donor parent maize plant types with different genetic backgrounds.

Phenotypic effect analysis of alleles

Among the 51 plant related traits QTLs identified, 37 were synergistic QTLs, such as plant height QTLs (qPHa4 and qPHd1), ear height (qEHa11 and qEHd1), leaf angle (qLAa3 and qLAa4) (Table 3, 4 and 6, Figure 5-10). Most of the chromosome segments were the same among the four introgression lines, and there are differences on only one or a few donor chromosome segments. Through the phenotypes of plant lines in which QTLs could be detected, the functions of the microgenes and their contributions to phenotypic variations could be understood. Among the 4 introgression line groups, 9 introgression lines containing additive alleles and 6 introgression lines containing dominant alleles were selected for corresponding QTL genotype analysis (Table

Table 5. Position of QTL on chromosome.

QTL	Marker	Chromosome bin	QTL	Marker	Chromosome bin
qEHa1	M48	1.01	qLAa2	M2096	4.09
qPHa1	M76	1.02	qLAd9	M2224	5.01
qPHa7	M908	1.02	qPHa9	M2271	5.03
qEHa10	M80	1.02	qEHa11	M2271	5.03
qLAa4	M340	1.05	qPHa10	M2327	5.03
qEHa2	M579	1.1	qEHd1	M2390	5.04
qLAd1	M642	1.1	qPHa11	M2425	5.05
qPHa2	M716	1.12	qPHa17	PZE-105107675	5.05
qPHa3	M724	1.12	qEHa12	M2484	5.05
qPHa6	M748	2.01	qPHa16	PZE-105144035	5.06
qLAa5	M769	2.01	qPHa4	M2583	5.08
qLAd2	M809	2.02	qEHa13	M2584	5.08
qEHa3	M823	2.02	qPHa12	M2604	5.09
qPHa19	SYN13011	2.02	qPHa13	M2609	5.09
qEHa4	M1104	2.07	qEHa14	M2637	6.01
qLAd3	M1158	2.08	qEHa6	M2976	6.08
qPHa8	M1204	2.08	qPHa14	M3203	7.03
qLAd4	M1311	3.01	qEHa15	M3208	7.03
qPHa18	PZE-103016707	3.03	qEHa7	M3220	7.03
qPHd1	M1385	3.04	qEHa8	M3313	7.05
qLAa1	M1590	3.06	qLAd7	M3616	8.06
qLAa3	M1572	3.06	qPHd2	M3700	8.08
qLAZPH5	M1595	3.06	qPHa5	M3936	9.04
qLAd6	M1616	3.07	qEHa9	M4146	10.01
qPHa15	PZE-104031870	4.05	qLAd8	M4362	10.07
qEHa5	M2040	4.08			

Table 6. Several introgression lines and plant type traits QTL introgressed from donor.

Traits	Additive QTL	Introgression lines	Dominant QTL	Introgression lines
PH	qPHa2	ZPH1388-5	qPHd1	ZPH1388-16
	qPHa4	ZPH1388-34	qPHd2	ZPH1388-26
	qPHa12	Dong237-48		
EH	qEHa3	ZPH1388-51	qEHd1	ZPH5-10
	qEHa11	Dong237-9		
	qEHa13	Dong237-44		
LA	qLAa2	ZPH1388-18	qLAZPH5	ZPH5-48
	qLAa3	ZPH5-16	qLAd8	ZPH5-15
	qLAa4	Dong237-29	qLAd9	Dong237-29

PH, plant height; EH, ear height; LA, leaf angle.

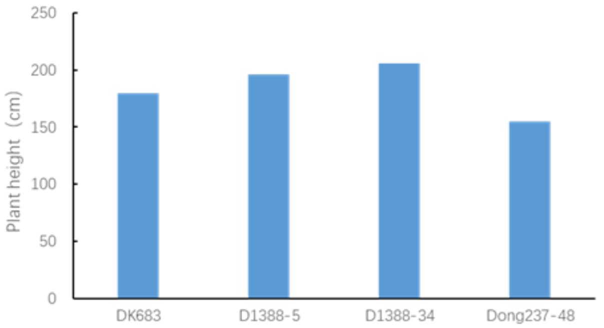

Figure 5. Comparison of the additive phenotypes about plant height of the introgression lines.

5). The phenotypic analysis of these introgression lines showed that the traits corresponding to the introgression lines containing synergistic QTL at the plant height, ear height and leaf angle were all higher than those of the related traits of Zheng 683-1 (Figure 5-10). For example, qPHa2, qPHa4, qPHd1 and qPHd2 contained synergistic QTLs, so their plants were all higher than Zheng 683-1. Conversely, the plant height with the introgression of qPHa12 was lower than Zheng 683-1. The ear heights of the plants with the synergistic QTLs (qEHa3, qEHa11 and qEHd1) were higher than that of Zheng 683-1. The plants carrying qEHa13 were lower than the background parent. The introgression lines ZPH5-16, Dong 237-29 and ZPH5-48 had synergistic QTLs qLAa3, qLAa4 and qLAZPH5, respectively. The leaf angle was larger than that of Zheng 683-1, but those of plants ZPH1388-18 and ZPH5-15 were smaller than Zheng 683-1 because they were introgressed with qLAa2 and qLAd8, respectively. The results indicated that the introgression of QTL could change the plant height, the height of ear and the leaf angle of Zheng 683-1. The introgression line Dong 237-29 was also introgressed with additive and dominant QTL qLAa4 and qLAd9 at the same time. It was found that compared with other single-fragment introgression lines, its leaf angle increased significantly more than Zheng 683-1, indicating that both qLAa4 and qLAd9 contributed to the leaf angle, and there might be an interaction between them.

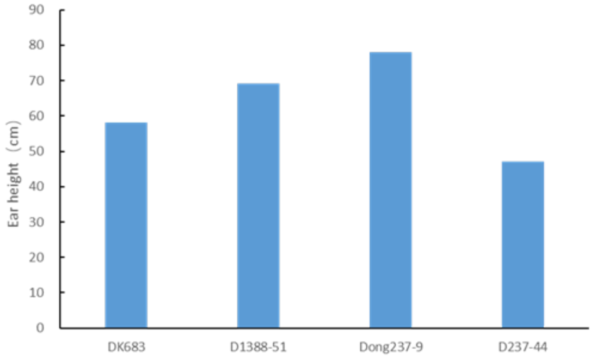

Figure 6. Comparison of the additive phenotypes about ear height of the introgression lines.

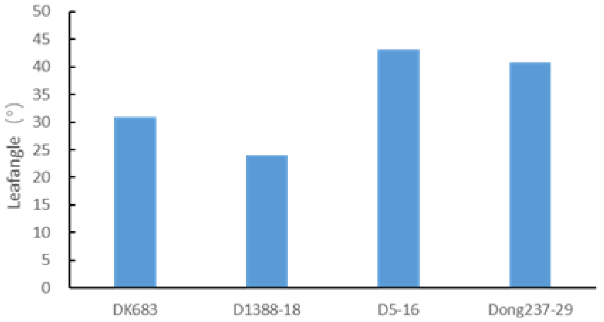

Figure 7. Comparison of the additive phenotypes about leaf angle of the introgression lines.

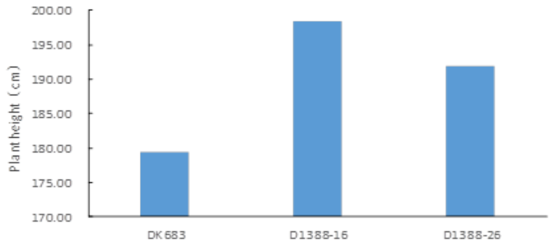

Figure 8. Comparison of the dominant phenotypes about plant height of the introgression lines.

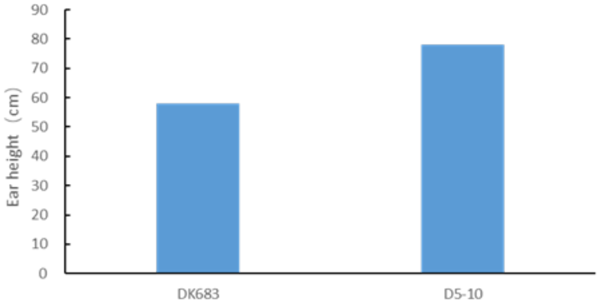

Figure 9. Comparison of the dominant phenotypes about ear height of the introgression lines.

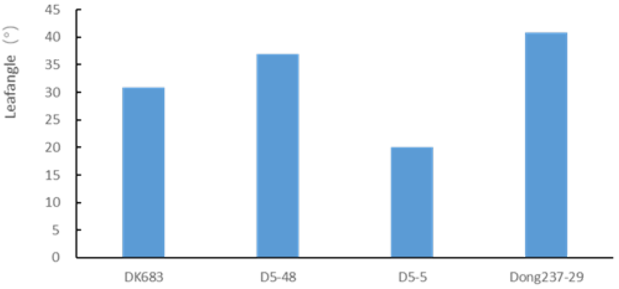

Figure 10. Comparison of the dominant phenotypes about leaf angle of the introgression lines.

Discussion

QTL distribution of maize plant type traits

There have been many reports on the QTL mapping of maize-type related traits (3, 14-16). In this study, 39 additive QTLs and 12 dominant QTLs related to plant type were positioned in 4 introgression lines. The results of QTL mapping showed that the QTL loci had the characteristic of inhomogeneous chromosome distribution. Chromosomes 1, 2, 5 and 7 were the regions where the plant type traits QTL were concentrated. QTLs (8 and 11) were detected on chromosomes 1 and 5, respectively accounting for 15.7% and 21.6% of the total. The QTLs detected on chromosomes 1 and 5 evaluating 37.3% of the total detected. Six QTLs were positioned on chromosome 2, expressing 11.8% of the total amount. Four QTLs were identified on chromosome 7, recording 7.8% of the total. Three QTLs were found on chromosomes 3 and 4, respectively. Moreover, 1, 1 and 0 QTL was positioned on Chromosomes 9, 10 and 8, respectively, which were rare regions of plant type QTL distribution. In addition, QTL of plant type traits also showed the characteristics of centralized distribution in some regions of chromosomes. For example, in the region of bin1.02 on chromosome 1, have 3 plant height and 3 ear height QTLs between SNP markers M76 and M80. In the region of bin2.02 on chromosome 2, there was respectively 1 QTL containing plant height, ear height and leaf angle between SNP marker M809 and SYN13011. Three QTLs associated with the leaf angle were detected between SNP markers M1590 and M1595 in the region of bin3.06 on chromosome 3. There were three QTLs related to ear height and plant height between the SNP markers M2271 and M2327 chromosome in the bin5.03 on chromosome 5. Three QTLs related to the plant and ear heights were positioned between SNP markers M2425 and M2484 in the bin5.05 on chromosome 5 at the same time. These were the areas where the plant type traits QTLs were concentrated.

Most of the QTLs detected in this study were located in the same or adjacent chromosome regions as those of corresponding traits in the MaizeGDB database or reported. For example, multiple QTLs controlling plant height were positioned in the 5.03 region of maize chromosome (17-20). In this study, 2 plant-height related QTLs (qPHa10 and qPHa9) were detected in this region and ear height QTL was positioned in maize chromosome 1.01 region (17). In this study, 1 ear height related QTL (qEHa1) was detected in the region. Leaf angle QTL detected in the regions of 10.07 (21) and 2.02 (22) of maize chromosomes was in the same region as the leaf angle QTL (qLAd8) detected in this study. Under different environmental conditions, these QTLs were verified multiple times with different types of molecular markers in different mapping populations, indicating that the QTLs located in these chromosomal regions may actually exist.

Comparison of plant type mapping results of different mapping groups of introgression lines

In this study, it was found that the numbers of QTLs related to plant type traits of the four introgression lines were significantly different. In total, 18 QTLs were detected in ZPH1388-IL with the largest number, followed by ZPH5-IL with 16 ones. There were 12 QTLs related to plant type traits in Dong 237-IL, and the fewest QTLs were detected in Chang 7-2 with only 5 plant height related additive QTLs. Plant height related QTLs (qPHa1 and qPHa7) were detected in the region of 1.02 in ZPH1388-IL and ZPH5-IL, respectively. In the region of chromosome 3.06, ZPH1388-IL had 1 leaf angle QTL qLAa1, while ZPH5-IL had 2 leaf angle QTLs (qLAa3) and qLAZPH5. In the 5.03 chromosomal region, respective 1 plant height QTL qPHa9 and qPHa10 were detected in ZPH5 and Dong 237. The QTLs of the same traits were detected in the same chromosomal regions in different introgression lines, indicating that these introgression lines had the same genetic effect in these regions.

The number of QTLs detected in different introgression lines of the same plant type traits varied greatly. A total of sixteen QTLs associated with the height of ear were detected in 4 introgression lines populations. Nine QTLs were located in ZPH 1388-IL, accounting for 56.25% of the total number of ear height related QTLs. Among the 14 leaf angle related QTLs, 9 ones were detected in ZPH5-IL population, occupying 64.29% of the total number of leaf angle related QTLs. Among the 21 plant height related QTLs, 7, 4, 5 and 5 QTL sites were detected in ZPH1388-IL, ZPH5-IL, Dong 237-IL and Chang 7-2-IL, respectively, accounting for 33.3%, 19.05%, 23.81% and 23.81% of the total plant height QTLs respectively. This indicated that there were larger genetic differences of ZPH1388 and Zheng 683-1 on ear height and the genetic differences between ZPH5 and Zheng 683-1 were increased. On the traits of plant height, there were significant genetic differences between the 4 donor parents and Zheng 683-1.

Although ZPH1388-IL and Dong 237-IL were both detected with more QTLs of plant type traits, the two introgression lines had no same QTLs. The reason for the huge difference in QTL detected may be that ZPH1388 was exogenous, and Dong 237 was cultivated and bred from local varieties in northeast China. The two were far apart in blood and differed greatly in heredity. Even for the same chromosome regions, the differences in genetic effects were also relatively large. This was also the reason for the larger and more differences in the QTLs detected in ZPH1388-IL and Dong 237-IL. At the same time, the introgressed fragments of the two introgression lines were relatively large, so the genetic differences and gene interactions of the introgressed fragments from the donor ZPH1388 and Dong 237 were also important reasons for these results.

The only QTL of plant height trait was detected in Chang 7-2-IL population, but no relevant QTL was detected with the traits ear height and ear angle, which may be due to the small genetic differences between the donor parents Chang 7-2 and the recurrent parents on the height of ear and the leaf angle. Therefore, the phenotypic effect of chromosome exchange may be less. However, for the other 3 donor parents ZPH1388, ZPH5 and Dong 237, there were large genetic differences between them and the recurrent parents on the plant type traits of plant height, ear height and leaf angle, and the phenotype effect of chromosome replacement was large. Hence, there were more QTLs related to plant traits detected.

Genetic basis of maize plant type traits QTL

Phenotypic and physiological correlations between different traits are widespread in biology. The method of QTL analysis provides available genetic information for the correlation between biological traits. There are four possible reasons for the correlation between different biological traits (23): (1) the two genes involved in controlling different biological traits are closely linked; (2) the same gene with a single function is involved in regulating a series of genes; (3) the same gene may independently control two or more different traits of organisms; (4) two closely linked genes on the chromosome may simultaneously control the same biological trait. The results of this study indicated that the partial QTLs which controlled the different traits of maize plant type were located in the same section of a chromosome. For instance, the QTL (qEHa10) related to ear height and QTL (qPHa7) related to plant height were located in section 1.02, The QTLs related to plant height, ear height and leaf angle qPHa19, qEHa3 and qLAd2 were located together in the chromosome section 2.02. Five pairs of QTLs (qPHa1 and qPHa7), (qPHa2 and qPHa3), (qPHa10 and qPHa9), (qPHa11 and qPHa17), (qPHa12 and qPHa13), which controlled plant height. They were located in chromosome sections 1.02, 1.12, 5.03, 5.05 and 5.09, respectively, showing close linkage relation. QTLs (qEHa15 and qEHa7) in chromosome section 7.03 controlled the ear height of maize, while the three QTLs (qLAa1, qLAa3 and qLAZPH5) related to the maize leaf angle were located in the 3.06 region of the chromosome, suggesting that closely linked genes may simultaneously control the same trait.

QTL mapping of maize plant type traits and genetic improvement

Most of the traits of crops are quantitative traits, which are controlled by a number of micro genes, and the genetic mechanism is very complicated(24). Chinese and foreign scholars have conducted plenty of QTL mapping analyses on these quantitative traits. So far, the MaizeGDB (http://www.maizegdb.org/) website has recorded a total of 2,284 QTLs related to the traits of maize plant height, ear height, ear row number and ear length. How to use this information in breeding has long been a common concern of all breeders and geneticists? At present, the primary effect QTLs related to quality traits and few quantity traits have been cloned, and molecular marker-assisted selection (MAS) was applied to breeding, but most QTLs are hard to be utilized. There are the following difficulties: (1) for a single QTL of a trait, the general genetic effect is very low and the contribution to phenotypic genetic variation is relatively small (25-26). In this study, 51 QTLs of plant type traits were identified, but there were 45 QTLs with a contribution rate of less than 20%, accounting for 88.23% of the total QTLs detected. Molecular marker-assisted selection (MAS) was conducted the individual QTL of plant height, ear height and leaf angle. In general, it is difficult to achieve the ideal effect of improving these three traits. However, if two or more QTLs are selected with the molecular marker-assisted method at the same time, more manpower and material resources will be needed and the cost will go up a lot; (2) due to different materials and environments, the QTL of the same trait identified by different scholars is often very different. In practice, there is not much genetic information of the QTL of the same trait that can be commonly used. These factors also greatly limit the use value of QTL (27-28). In this study, a total of 51 QTLs of plant type traits were identified in 4 introgression lines. Although the QTL with the same trait was located in the same section of chromosome, no QTL with the same trait was detected in different lines; (3) due to small population or insufficient marking density, as most QTLs did not have closely linked molecular markers and the mapping accuracy was not high, so finally they could not be used for molecular marker-assisted selection. Therefore, most of the QTLs are directly applied to crop breeding or germplasm genetic improvement (29-31). At present, there still is a long way to go and further fine mapping and cloning are required so that they can be widely used in genetic improvement.

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Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Author contributions

Dingming Kang designed the research. Yikun Zhao, Jingbao Liu, Lu Huang, Xiaomin Lu performed the cytotoxicity and reversal experiments. Weihong Zhu performed PCR and Western blot experiments. All authors analyzed the results and took part in preparing the manuscript.

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