Heterotic loci for various morphological traits of maize detected using a single segment substitution lines test-cross population

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Abstract Heterosis has been exploited to increase grain yield, quality, and resistance in many crops, and it plays an important role in plant breeding. However, the genetic mechanism of heterosis remains unclear. To dissect the genetic basis of heterosis, a set of 203 single segment substitution lines (SSSLs) was developed, and its test-cross population was used to identify heterotic loci (HL) for plant morphological traits in maize, including plant height (PH), ear height (EH), leaf number (LN), tassel main axis length (TMAL), and tassel branch number (TBN). A total of 41 QTLs and 37 HL were identified for five morphological traits in the test-cross population derived from the 203 SSSLs and the parent, Xu178. Nine HL for PH, nine HL for EH, seven HL for LN, seven HL for TMAL, and five HL for TBN were detected in three different environments, respectively. Eight HL, *ph1a*, *ph1b*, *ph2*, *ph5*, *eh3a*, *eh3b*, *eh10*, and *tmal1b*, were simultaneously detected in the three environments. Among the 37 HL, only 10 (27.03 %; for PH, EH, LN, and TBN) had a corresponding QTL (24.39 %) sharing the same chromosomal region. Of all the HL, 21.4 % showed dominance effects, 76.8 % showed over-dominance effects, and only one (1.8 %) showed a partial-dominance effect. This result illustrated that heterosis and performance was controlled by different genetic mechanisms, and over-dominance effects were the main contributors to heterosis for plant-related traits at the single-locus level in maize.

Keywords Maize · Single segment substitution lines · Test-cross population · Heterosis · Over-dominance

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Introduction

Heterosis, also known as hybrid vigor, refers to the phenomenon in which the progeny of a cross between different pure lines exhibit greater biomass, faster development, and higher fertility than either of the parents (Birchler et al. 2010). As previous study, heterosis plays an important role in increasing crop productivity, nutrient quality, and resistance to biotic and abiotic environmental stresses (Zhou et al. 2012; Guo et al. 2013). In plants, heterosis is a complex trait, and it can be extrapolated as the sum of changes in many physiological and phenotypic traits, including the magnitude and rate of vegetative growth, flowering time, yield, etc. (Lippman and Zamir 2007). Through one century study, three important hypotheses, including dominance (Bruce 1910; Jones 1917), the over-dominance hypothesis (Shull 1908; East 1936), and epistasis (Powers 1944; Williams 1959), have been proposed to explain the genetic basis of heterosis.

Studies based on molecular markers and genomic as well as bioinformatics analyses have shown that heterosis of grain yield and its traits are generally controlled by the dominance of favorable genes, although epistasis may also play an important role (Cockerham and Zeng 1996). Yu et al. (1997) reported that epistasis plays a major role in the genetic basis of heterosis for yield and three traits that were components of yield in rice. Lu et al. (2003) analyzed an F_2 population and observed that 24 quantitative trait loci (QTL) (86 %) had over-dominant effects on grain yield in maize. Melchinger et al. (2007) have analyzed the QTL effects and heterosis using "design III" experiments on test-crosses derived from recombinant inbred lines in maize, and identified genomic regions relevant for heterosis. Li et al. (2008) reported that heterosis for grain yield and eight other yield-related traits was attributable to the combined effects of partial-to-complete dominance, over-dominance, and epistasis in rice. Tang et al. (2010) reported that dominance effects of heterotic loci (HL) at the single-locus level, as well as AD (additive × dominance) interactions, played important roles in the genetic basis of heterosis for grain yield and its components in the maize hybrid Yuyu22. Zhou et al. (2012) reported that the dominance × dominance interaction was important for heterosis of tillers per plant and grain weight, and also yield and grain number in rice. Guo et al. (2014) reported that the yield × environment

interactions differed for each allele of yield in maize. Moreover, when stacked in transgenic plants, the allelic pair resulted in yield and environmental performance advantages over either single allele, resembling heterosis effects.

Among the various types of segregated populations, single segment substitution lines (SSSLs) have been widely used to study heterosis in many crops (Wang et al. 2012; Liu et al. 2012; Shen et al. 2014). Wang et al. (2012) reported on the factors contributing to yield and yield-related traits variations in *japonica–indica* rice hybrids, based on analyses of 66 chromosome segment substitution (CSS) lines. Liu et al. (2012) reported that the 12 dominance and epistasis of QTLs for tiller number in rice using SSSLs. Their data provided information that could be used to improve the tiller number in rice via heterosis and/or QTL pyramiding. Shen et al. (2014) developed a set of 202 CSS lines (CSSLs) for their analyses and concluded that dominance and epistasis were the major factors in the genetic basis of heterosis of plant height (PH), which explained the better-parent heterosis in hybrid rice Shanyou 63. In maize, Lu et al. (2011) reported that chromosomal SSSLs were powerful tools to detect and precisely map QTL and to evaluate the action of genes as single Mendelian factors. In this study, we used a set of 203 SSSLs and its test-cross population to: (1) identify HL for plant morphological traits and (2) dissect the genetic basis of heterosis using various plant morphological traits as a model.

Materials and methods

Materials and field trials

A set of 203 SSSLs of maize and its test-cross population were used as the materials in this study. The SSSLs were produced using two elite inbred lines: The donor parent was the inbred line Zong3, a parent of the elite hybrid Yuyu22, which was a second plant hybrid in China from 1998 to 2002; the receptor parent was the inbred line Xu178, a parent of the elite hybrid Nongda108, which was a first plant hybrid in China from 2002 to 2004. Based on SSR molecular marker linkage map integrated by IBM 2008 Neighbour, a total of 700 SSR markers evenly distributed on ten chromosomes were employed to the two inbred lines Xu178 and Zong3, and 200 polymorphic SSR markers

between the two inbred lines were selected; among this, 172 SSR markers were used for constructing SSSLs population combined with backcross.

The SSSLs included 150 uniquely substituted segments were unevenly distributed on 10 chromosomes (Supplementary Figure 1), the total length of the 150 substitution segments was 11,394.22 cM, covering 4481.74 cM (67.56 %) of the entire maize genome, and the substituted segment length ranged from 0.95 to 186.05 cM with an average of 47.67 cM. Among them, there were 152 substituted segment lengths ranged from 2.25 to 50.00 cM (63.60 %), 66 substituted segment lengths ranged from 50.00 to 100.00 cM (27.61 %), and 21 substituted segment lengths more than 100.00 cM (8.79 %).

The 203 corresponding crosses were produced by manual pollination using the receptor parent Xu178 as the male parent and the SSSLs population as female parents in the winter of 2012, in Hainan Province, China (Sanya, E18°15′, N109°30′). The test-cross population was evaluated in field trials beginning in June, 2013. The field trials were conducted in three locations: at the farms of Hebei Agricultural Institute (Xunxian, E114°33′, N35°41′), Xinxiang Agricultural Institute (Xinxiang, E113°31′, N35°11′), and Xuchang (E113°29′, N34°1′), following a field design of randomized complete blocks with three replicates. Each plot included one row, 4 m long, with 0.6 m distance between rows. The population density was 67,500 plants per hectare. At the three locations, the 203 SSSLs and the two parents were also planted using the same experimental design in the same field. The field was managed using local maize cultivation practices.

Phenotype measurement in the field

The leaves of ten plants were labeled in the field for continuous evaluations of total leaf number (LN). At the filling period, ten typical consecutive plants of each experimental material were selected to measure morphological traits in the field, including PH (cm), ear height (EH, cm), LN, tassel main axis length (TMAL, cm) and tassel branch number (TBN). The PH means the distance from the ground to the top of tassels. The EH means the distance from the ground to the node of attachment of the primary ear. The LN means the total number of leaves on the whole plant. The TMAL means the main axis length of tassel, and the TBN means the first branch number of tassel.

QTL analysis

One-way ANOVA analysis and Duncan's multiple comparisons were conducted using SPSS 17.0 software. A QTL was considered to exist in SSSLs when there was a significant difference in the measured value between the SSSL and Xu178 ($p < 0.05$). The additive effect was calculated using the following equation: $A = (SSSL - Xu178)/2$. The percentage of the additive effect (A %) was calculated using the following equation: $A \% = A/Xu178 \times 100 \%$ (Guo et al. 2013). Here, SSSL refers to the value of the trait in the SSSL, and Xu178 refers to the value of the trait in Xu178.

Heterosis loci analysis

The data for mid-parent heterosis (H_{MP}) for each evaluated trait in the three environments were used to detect HL. Mid-parent heterosis values were calculated using the equation H_{MP} (%) = $(F_1 - MP)/MP \times 100 \%$, where H_{MP} is the percentage of mid-parent heterosis, F_1 is the performance of each testcross hybrid of SSSLs and Xu178 and Mp is the average of SSSLs and Xu178 (Pan 1994).

One-way ANOVA and Duncan's multiple comparisons using SPSS 17.0 software, and heterotic locus (HL) was considered that existed in corresponding chromosomal region between an SSSL and its test cross, when there was a significant difference between the value of the trait in the test-cross hybrid and the mean trait value in Xu178 and SSSLs ($p < 0.05$). According to the experimental design of the test-cross hybrid population, each HL theoretically had only dominance effect (D). The additive effect (A) of the same locus was computed from testcross hybrid performance. The ratio of D/A for a given HL reflects the degree of dominance, a ratio greater than 1 indicates over-dominance (OD) of the HL, a ratio less than 1 indicates partial-dominant (PD) of the HL, and equal to 1 indicates dominant effects, respectively (Tang et al. 2010).

Results

Performance of morphological traits in SSSLs and test-crosses

The performances of the five measured morphological traits in three environments varied over a large range

among the SSSL and the testcross population (Table 1). For PH, the means of the SSSL population in Xunxian location, Xinxiang location, and Xuchang location were 177.78, 169.29, and 156.22, respectively, with ranges of 157.80–199.40, 130.00–201.87, and 127.90–181.00 cm, respectively. The ranges of EH in the SSSL population were 65.40–85.30, 44.25–84.33, and 43.50–84.80 cm in Xunxian, Xinxiang, and in Xuchang location. For TMAL, the mean values for the SSSL population in Xunxian, Xinxiang, and Xuchang location were 27.15, 26.40, and 24.51, respectively.

At the same time, the mean values of PH for the testcross population in the three environments (185.59 cm in Xunxian, 181.50 cm in Xinxiang, 170.72 cm in Xuchang) were higher than the mean PH value of SSSL population and the test parent Xu178. The same pattern was observed for EH and TBN (Table 1). For LN and TMAL, the mean values for the testcross population in the three environments were almost the same as that of the SSSL population and the test parent Xu178. The heritability (h_b^2) of PH, EH, LN, TMAL, and TBN were 62.5, 71.2, 95.9, 99.1, and 48.9 %, respectively (Supplementary Table 1).

Performance of heterosis for plant morphological traits at three environments

The performances of mid-parent heterosis (H_{MP}) for the five measured morphological traits of the test-cross hybrid population in the three environments also showed large variations (Table 2). The mean H_{MP} values for PH in the test-cross population were 1.48, 7.36, and 8.21 % in Xunxian, Xinxiang, and Xuchang location, with ranges of −10.50 to 12.68, −4.08 to 19.76, and −4.25 to 21.07 %, respectively. The means of H_{MP} for EH in the test-cross population were 6.35, 4.87, and 13.73 % in Xunxian, Xinxiang, and Xuchang location, with ranges of −12.32 to 20.84, −6.26 to 21.19, and −6.33 to 30.48 %, respectively. The lowest mean values of H_{MP} were for LN, with 0.83, 0.92 and 4.03 % heterotic performance in Xunxian, Xinxiang, and Xuchang location, respectively. The mean values of H_{MP} for TMAL in the test-cross population in Xunxian, Xinxiang, and Xuchang were 1.57, 1.13, and 1.40 %, with ranges of −12.41 to 16.96, −7.24 to 11.50, and −14.06 to 9.61 % in the three environments, respectively. The mean values of H_{MP} for TBN in the test-cross population in Xunxian,

Xinxiang, and Xuchang were 9.45, 6.62, and 6.45 %, respectively, with ranges of −17.76 to 36.17, −21.45 to 37.52, and −11.11 to 38.35 % in the three environments.

Correlations between phenotype and heterosis for measured traits

PH was significantly correlated with EH in all three environments ($p < 0.01$; Table 3) and also showed significantly correlated with LN and TMAL in Xinxiang location ($p < 0.01$). Additionally, EH showed significant correlations with LN in Xunxian and Xinxiang location ($p < 0.01$) and with TBN in Xinxiang and Xuchang location ($p < 0.01$). For mid-parent heterosis, PH showed significant correlations with EH, LN, and TMAL in the three environments ($p < 0.01$), and EH also showed significant positive correlations with LN in the three environments ($p < 0.01$).

QTL detected for morphological traits of SSSLs in three environments

A total of 41 QTLs were identified for the five morphological traits of SSSLs in the three environments (Table 4). For PH, ten QTLs were identified in three environments, and three QTLs, *qPH4a*, *qPH4b* and *qPH9* were detected in the three environments simultaneously. Among them, the QTL *qPH4a*, located in bin 4.03, accounted for 3.03, 3.29, and 9.96 % of phenotypic variation in Xunxian, Xinxiang and Xuchang location, respectively, and its additive effect came from the nonrecurrent parent Zong3. Another QTL *qPH9*, also derived from the nonrecurrent parent Zong3, accounted for 4.62, 16.09, and 11.82 % of phenotypic variation in the three environments, respectively.

Ten QTLs were identified for EH, located on all chromosomes except for chromosomes 5, 6, 8, and 10. The QTL *qEH2a*, derived from the nonrecurrent parent Zong3, could explain 1.86, 5.00, and 10.20 % phenotypic variation in Xunxian, Xinxiang, and Xuchang location, respectively. The other six QTLs located on chromosomes 1, 2, 3, and 9 were also identified in Xinxiang and Xuchang location simultaneously.

There were five QTLs identified for LN and located on chromosomes 1, 2, 4, and 10, and the percentage of their additive effects ranged from 1.83 to 6.64 %. The

Table 1 Performance of SSSL population and test-cross hybrid population in three environments

Trait	Environment	Xu178 Mean	SSSL population				Test-cross population			
			Mean ± SD	Range	Skewness	Kurtosis	Mean ± SD	Range	Skewness	Kurtosis
PH (cm)	Xunxian	183.50	177.78 ± 7.99	157.80–199.40	−0.56	1.53	185.59 ± 6.93	158.72–202.31	−0.90	0.82
	Xinxiang	168.90	169.29 ± 11.94	130.00–201.87	−0.65	2.01	181.50 ± 7.84	160.19–203.55	0.07	0.06
	Xuchang	159.75	156.22 ± 10.50	127.90–181.00	−0.10	−0.22	170.72 ± 6.86	152.50–185.31	−0.21	−0.22
EH (cm)	Xunxian	64.30	72.18 ± 3.86	65.40–85.30	−0.44	0.09	74.85 ± 4.47	61.54–88.97	−0.37	0.85
	Xinxiang	69.40	68.31 ± 6.38	44.25–84.33	−0.72	1.52	72.12 ± 3.79	63.42–85.68	0.29	0.26
	Xuchang	63.40	62.33 ± 6.66	43.50–84.80	0.08	0.59	71.52 ± 4.46	63.11–84.73	0.17	−0.26
LN	Xunxian	20.20	20.12 ± 0.33	18.80–21.10	−0.15	1.25	20.30 ± 0.45	19.22–21.27	−0.08	−0.08
	Xinxiang	19.40	19.53 ± 0.52	18.11–20.89	0.14	0.06	19.66 ± 0.47	18.32–20.81	0.17	1.23
	Xuchang	19.95	20.47 ± 0.57	18.60–22.30	0.36	0.73	20.91 ± 0.53	19.72–22.59	0.36	0.85
TMAL (cm)	Xunxian	27.00	27.15 ± 0.85	25.10–30.70	0.88	3.05	27.54 ± 1.26	23.91–32.03	0.50	2.78
	Xinxiang	26.52	26.40 ± 1.51	20.13–30.00	−0.37	0.92	26.72 ± 1.04	24.16–30.94	0.27	1.75
	Xuchang	24.13	24.51 ± 1.48	20.30–28.90	0.12	0.87	24.60 ± 0.91	22.26–27.25	−0.04	0.45
TBN	Xunxian	6.70	7.15 ± 0.49	5.40–8.30	−0.41	0.49	7.69 ± 0.84	5.10–9.65	0.16	0.50
	Xinxiang	6.75	6.55 ± 1.06	3.30–9.50	−0.07	0.18	7.12 ± 0.65	5.60–9.00	0.24	−0.19
	Xuchang	6.55	6.52 ± 0.98	4.10–9.80	0.12	0.29	7.05 ± 0.62	5.20–8.41	−0.07	−0.10

PH plant height, *EH* ear height, *LN* leaf number, *TMAL* tassel main axis length, *TBN* tassel branch number

Table 2 Mid-parent heterosis of the test-cross population in three environments

Traits	Environments	Mean ± SD	Range	Skewness	Kurtosis
PH (%)	Xunxian	1.48 ± 4.24	−10.50 to 12.68	−0.19	0.05
	Xinxiang	7.36 ± 4.35	−4.08 to 19.76	0.22	0.40
	Xuchang	8.21 ± 4.63	−4.25 to 21.07	0.23	−0.10
EH (%)	Xunxian	6.35 ± 6.56	−12.32 to 20.84	−0.40	0.11
	Xinxiang	4.87 ± 5.33	−6.26 to 21.19	0.08	−1.40
	Xuchang	13.73 ± 7.30	−6.33 to 30.48	0.02	−0.30
LN (%)	Xunxian	0.83 ± 2.36	−7.19 to 7.42	−0.04	0.62
	Xinxiang	0.92 ± 2.38	−6.63 to 7.53	−0.26	0.50
	Xuchang	4.03 ± 2.93	−3.24 to 12.37	0.23	0.57
TMAL (%)	Xunxian	1.57 ± 4.43	−12.41 to 16.96	0.43	2.06
	Xinxiang	1.13 ± 3.25	−7.24 to 11.50	0.12	0.56
	Xuchang	1.40 ± 3.43	−14.06 to 9.61	−0.47	2.34
TBN (%)	Xunxian	9.45 ± 11.34	−17.76 to 36.17	0.08	−0.24
	Xinxiang	6.62 ± 9.20	−21.45 to 37.52	0.30	0.73
	Xuchang	6.45 ± 6.67	−11.11 to 38.35	0.57	0.74

Table 3 Correlation coefficients between phenotype and mid-parent heterosis for five traits of test-cross hybrid population in three environments

Traits	Environment	PH	EH	LN	TMAL	TBN
PH	Xunxian		0.504**	0.130	0.220*	−0.008
	Xinxiang		0.547**	0.404**	0.557**	0.215**
	Xuchang		0.814**	0.230**	0.048	0.364**
EH	Xunxian	0.597**		0.265**	−0.032	0.115
	Xinxiang	0.681**		0.281**	0.257**	0.249**
	Xuchang	0.786**		0.151	−0.005	0.379**
LN	Xunxian	0.337**	0.414**		0.079	−0.032
	Xinxiang	0.340**	0.451**		0.214**	0.152
	Xuchang	0.291**	0.436**		−0.083	0.173
TMAL	Xunxian	0.457**	0.219**	0.131		0.101
	Xinxiang	0.469**	0.146*	0.179*		0.163*
	Xuchang	0.306**	0.125	0.194**		0.067
TBN	Xunxian	0.119	0.129	0.240**	0.035	
	Xinxiang	0.094	0.205**	0.172*	0.154*	
	Xuchang	0.120	0.321**	0.291**	0.161*	

Correlation coefficients for phenotype and mid-parent heterosis of each trait are listed above and below the diagonal

*, ** Significance at the $p < 0.05$ and $p < 0.01$ levels, respectively

QTL *qLN1* accounted for 4.54 and 6.31 % phenotypic variation in Xinxiang and Xuchang location, respectively. For TMAL, seven QTLs were detected in the three environments, and the QTL *qTMAL3* was detected in the three environments simultaneously, accounted for 5.79, 1.70, and 9.92 % phenotypic variation in Xunxian, Xinxiang, and Xuchang locations, respectively.

Table 4 QTL identified for five traits in three enviroments

Trait	QTL	Bin	Position (cM)	Substitution segment	Percentage of additive effect (A %)		
					Xunxian	Xinxiang	Xuchang
PH	qPH1a	1.01	2.50–190.53	phi097~umc1106~umc1269~phi427913~bnlg1007	4.03		
	qPH1b	1.03	190.53–283.27	bnlg1007~bnlg1203~phi001~phi095			6.53
	qPH1c	1.06	457.00–532.80	umc1703~umc1754~bnlg1598		−8.38	
	qPH2	2.04	294.20–323.30	bnlg1018~umc2079~umc1485	6.52		4.11
	qPH3	3.02	77.00–165.00	bnlg1144~bnlg1647~umc1425	−3.03	−11.52	−17.09
	qPH4a	4.03	81.00–218.50	phi074~umc1550~umc1117		−3.29	−9.96
	qPH4b	4.11	731.60–737.80	phi076~umc1058	4.47	5.28	4.15
	qPH5	5.01	0–79.22	umc1491~bnlg1006~umc1478	−4.28		
	qPH8	8.06	412.90–571.50	umc2356~umc1724~phi080		9.76	
	qPH9	9.04	317.08–321.60	umc1771~umc1990~umc1519	−4.62	−16.09	−11.82
EH	qEH1a	1.01	86.30–190.53	umc1269~phi427913~bnlg1007		4.71	9.52
	qEH1b	1.03	190.53–283.27	bnlg1007~bnlg1203~phi001~phi095			8.36
	qEH1c	1.06	457.00–532.80	umc1703~umc1754~bnlg1598		−14.22	−19.22
	qEH2a	2.04	250.10–284.70	umc1024~umc1541~umc1579~phi083	−1.86	−5.00	−10.20
	qEH2b	2.04	294.20–323.30	bnlg1018~umc2079~umc1485		−9.17	−10.28
	qEH3	3.02	77.00–165.00	bnlg1144~bnlg1647~umc1425		−13.83	−21.24
	qEH4	4.03	81.00–252.52	phi074~umc1550~umc1117~umc1662	−5.78		
	qEH7	7.05	472.90–598.35	phi328175~phi069~umc2222~umc2333	−6.76		
	qEH9a	9.03	147.50–274.25	bnlg1401~umc1271~umc2119		−4.42	−15.7
	qEH9b	9.04	317.08–321.60	umc1771~umc1990~umc1519		−18.12	−14.59
LN	qLN1	1.01	86.30–190.53	umc1269~phi427913~bnlg1007		4.54	6.31
	qLN2	2.04	274.90–284.70	umc1541~umc1579~phi083	−2.56		
	qLN4	4.11	599.63–731.60	umc1989~umc1109~phi076		−3.25	
	qLN10a	10.04	245.90–344.80	umc1995~umc1077~umc1053~umc2350~umc1506	1.83		
	qLN10b	10.05	283.50–380.50	umc2350~umc1506~bnlg1028			6.64
TMAL	qTMAL1a	1.03	259.30–287.20	bnlg1203~phi001~phi095~bnlg182		6.60	
	qTMAL1b	1.04	283.27–417.00	phi095~bnlg182~umc2217~bnlg2295~umc2025	6.19		
	qTMAL3	3.02	77.00–165.00	bnlg1144~bnlg1647~umc1425	5.79	1.70	9.92
	qTMAL4a	4.04	185.17–252.52	umc1550~umc1117~umc1662		4.59	8.64
	qTMAL4b	4.11	731.60–737.80	phi076~umc1058~		−6.35	
	qTMAL9	9.04	317.08–321.60	umc1771~umc1990~umc1519		−12.00	

Table 4 continued

Trait	QTL	Bin	Position (cM)	Substitution segment	Percentage of additive effect (A %)		
					Xunxian	Xinxiang	Xuchang
	qTMAL10	10.04	253.30–283.50	umc1077~umc1053~umc2350			8.81
TBN	qTBN1a	1.01	86.30–190.53	umc1269~phi427913~bnlg1007	11.69	15.68	15.65
	qTBN1b	1.05	417.00–439.11	umc2025~umc1461~umc1689	10.20	11.24	24.81
	qTBN2a	2.04	274.90–320.70	umc1541~umc1579~phi083~bnlg1018~umc2079		−25.31	−18.36
	qTBN2b	2.08	414.10–548.30	umc2129~phi328189~phi090		−19.88	−14.38
	qTBN2c	2.08	414.10–548.30	umc2129~phi328189~phi090		−20.37	
	qTBN3	3.02	77.00–165.00	bnlg1144~bnlg1647~umc1425			14.97
	qTBN4	4.03	81.00–252.52	phi074~umc1550~umc1117~umc1662		20.62	19.38
	qTBN5	5.01	47.50–156.90	bnlg1006~umc1478~umc1587	11.94		
	qTBN9	9.04	317.08–321.60	umc1771~umc1990~umc1519		−14.94	−15.39

A means additive effect percentages, A % = (SSSLs − Xu178)/(2 × Xu178) × 100; A % positive means that it comes from the parent Xu178, A % negative means that it comes from the parent Zong3

Nine QTLs were identified for TBN, and two of the QTLs were detected in the three environments simultaneously. Among them, the QTL *qTBN1a* could explain 11.69, 15.68, and 15.65 % phenotypic variation in Xunxian, Xinxiang, and Xuchang locations, respectively. Another QTL, *qTNB1b*, accounted for 10.20, 11.24, and 24.81 % of phenotypic variation in the three environments.

Heterotic loci identification

In this study, a total of 37 HL for the five measured traits were identified in three environments, and eight HL for PH, EH and TMAL were detected in the three environments simultaneously, including *ph1a*, *ph1b*, *ph2*, *ph5*, *eh3a*, *eh3b*, *eh10*, and *tmal1b* (Table 5). Nine HL were detected for PH, distributed on all chromosomes except for chromosomes 6, 8, and 9, and four HL were detected in the three environments simultaneously. Out of them, the HL, *ph1a*, showed over-dominant effect and had 10.5, 7.8 and 11.6 % phenotypic contribution of mid-parent heterosis to PH in Xunxian, Xinxiang and Xuchang, respectively. Another HL, *ph1b*, showed a dominant effect, made a 3.4, 18.7, and 5.6 % mid-parent heterosis contribution to PH in the three environments, respectively. The other HL *ph2* had 1.8, 10.1, and 14.3 % phenotypic contribution of mid-parent heterosis to PH in the same environments, respectively, and also showed an over-dominant effect. Additionally, the HL *ph5*, showed an over-dominant effect, with 8.1, 12.0, and 9.5 % phenotypic contribution of mid-parent heterosis to PH in the three environments, respectively.

Nine HL were identified for EH, and three HL were detected in three environments simultaneously. The HL, *eh3a*, which showed over-dominant effect, could explain 13.5, 13.5, and 18.3 % phenotypic contribution of mid-parent heterosis for EH in Xunxian, Xinxiang, and Xuchang, respectively. Another HL *eh3b* that showed over-dominant effect had 3.8, 3.5 and 18.0 % mid-parent heterosis contribution to EH in the three environments, respectively. The third HL, *eh10*, made a 4.7, 5.8, and 35.6 % mid-parent heterosis contribution to EH in Xunxian, Xinxiang, and Xuchang, respectively, also showed over-dominant effect.

There were seven HL detected for LN, located on chromosomes 1, 2, 4, 7, 9, and 10. None of these seven HL were detected in the three environments simultaneously.

Table 5 Heterotic loci identified for five traits in testcross hybrid population derived from SSSLs and Xu178

Trait	Heterotic loci	Bin	Position (cM)	Substitution segment	H_{MP} (Mode of effect)		
					Xunxian	Xinxiang	Xuchang
PH	ph1a	1.01	2.50–190.53	phi097~umc1106~umc1269~phi427913~bnlg1007	10.5 (OD)	7.8 (OD)	11.6 (OD)
	ph1b	1.03	190.53–283.27	bnlg1007~bnlg1203~phi001~phi095	3.4 (D)	18.7 (D)	5.6 (D)
	ph1c	1.05	439.11–457.00	umc1689~umc1124~umc1703		19.8 (OD)	
	ph2	2.06	323.30–376.39	umc1485~bnlg1831~bnlg1396	1.8 (OD)	10.1 (OD)	14.3 (OD)
	ph3	3.02	77.00–165.00	bnlg1144~bnlg1647~umc1425			10.9 (D)
	ph4	4.11	731.60–737.80	phi076~umc1058		11.0 (OD)	
	ph5	5.06	156.90–609.40	umc1587~mmc0081~umc2072~umc2305~umc1680~bnlg118	8.1 (OD)	12.0 (OD)	9.5 (OD)
	ph7	7.00	13.80–118.50	umc1241~umc1642~umc2160		4.5 (OD)	15.6 (OD)
	ph10	10.03	165.52–226.10	umc1863~umc2067~phi050			18.1 (OD)
EH	eh1	1.05	438.80–457.00	umc1461~umc1689~umc1124	18.9 (OD)		19.9 (OD)
	eh2	2.04	294.20–323.30	bnlg1018~umc2079~umc1485		9.1 (D)	
	eh3a	3.02	77.00–165.00	bnlg1144~bnlg1647~umc1425	13.5 (OD)	13.5 (OD)	18.3 (OD)
	eh3b	3.08	608.17–728.65	umc1844~umc1320~bnlg1182	3.8 (OD)	3.5 (OD)	18.0 (OD)
	eh4	4.11	599.63–737.80	umc1989~umc1109~phi076~umc1058	9.6 (OD)		
	eh6	6.00	0–23.20	~bnlg1043~bnlg161~bnlg238	1.8 (OD)		
	eh8	8.03	244.90–274.90	umc2075~bnlg1863~phi100175	31.2 (OD)		
	eh9	9.04	317.08–321.60	umc1771~umc1990~umc1519	9.0 (OD)		
	eh10	10.03	165.52–226.10	umc1863~umc2067~phi050	4.7 (OD)	5.8 (OD)	35.6 (OD)
LN	ln1a	1.01	86.30–190.53	umc1269~phi427913~bnlg1007			6.1 (D)
	ln1b	1.03	190.53–287.20	bnlg1007~bnlg1203~phi001~phi095~bnlg182		5.8 (OD)	
	ln2	2.04	274.90–284.70	umc1541~umc1579~phi083	7.4 (OD)		
	ln4	4.04	185.17–252.52	umc1550~umc1117~umc1662			11.5 (OD)
	ln7	7.05	320.91–598.35	umc1456~bnlg1805~phi328175~phi069~umc2222			12.2 (OD)
	ln9	9.04	317.08–321.60	umc1771~umc1990~umc1519	7.0 (OD)		
	ln10	10.04	253.30–283.50	umc1077~umc1053~umc2350		3.7 (D)	
TMAL	tmal1a	1.01	86.30–190.53	umc1269~phi427913~bnlg1007			9.1 (OD)
	tmal1b	1.05	439.11–457.00	umc1689~umc1124~umc1703	3.6 (D)	9.7 (D)	2.5 (D)
	tmal2	2.06	368.80–408.89	bnlg1831~bnlg1396~umc1637			4.9 (D)
	tmal4	4.08	462.50–481.19	bnlg1444~phi066~umc2041	14.5 (OD)		
	tmal6	6.00	0–23.20	~bnlg1043~bnlg161~bnlg238	16.4 (OD)		
	tmal8	8.03	244.90–274.90	umc2075~bnlg1863~phi100175	17.0 (OD)		

Table 5 continued

Trait	Heterotic loci	Bin	Position (cM)	Substitution segment	H_{MP} (Mode of effect)		
					Xunxian	Xinxiang	Xuchang
	tmal10	10.03	165.52–226.10	umc1863 ~ umc2067 ~ phi050			
TBN	tbn1	1.08	722.40–842.30	umc1013 ~ dupssr12 ~ umc2047	36.2 (OD)	1.6 (PD)	
	tbn2	2.06	368.80–408.89	bnlg1831 ~ bnlg1396 ~ umc1637	32.4 (OD)		
	tbn3	3.08	608.17–728.65	umc1844 ~ umc1320 ~ bnlg1182		26.6 (OD)	15.5 (OD)
	tbn9	9.04	317.08–321.60	umc1771 ~ umc1990 ~ umc1519			38.3 (OD)
	tbn10	10.04	253.30–283.50	umc1077 ~ umc1053 ~ umc2350		13.6 (D)	

For TMAL, seven HL were detected and distributed on chromosomes 1, 2, 4, 6, 8, and 10. Out of them, the HL *tmal1b* (in bin 1.05) showed dominant effect, made a 3.6, 9.7, and 2.5 % mid-parent heterosis contribution to TMAL in Xunxian, Xinxiang, and Xuchang, respectively.

Five HL for TBN were detected in the three environments, located on chromosomes 1, 2, 3, 9, and 10. Only one HL, *tbn3*, was detected in both Xinxiang and Xuchang locations, with a 26.6 and 15.5 % mid-parent heterosis contribution, respectively, and showed over-dominant effect.

Discussion

It is important to use appropriate experimental materials for the genetic analysis of heterosis. Several types of segregated populations have been used in previous studies, for example, F_2 populations, DH and RIL test populations, and IF_2 populations (Koester et al. 1993; Yu et al. 1997; Melchinger et al. 1998; Zhuang et al. 2001; Syed and Chen 2005; Guo et al. 2008; Melchinger et al. 2008; Luo et al. 2009; Ma et al. 2009; Li et al. 2010; Song et al. 2011). The F_2, BC_1, $F_{2:3}$, and $F_{2:4}$ populations can provide relatively complete information and have proven to be good populations for the mapping and analysis of additive effects, dominance effects, and their interactions. However, the genotype of each family in these populations is only temporary. To overcome the disadvantages of these populations, Hua et al. (2003) reported a new type of population for genetic studies, the IF_2 population, which has the same genetic structure as the F_2 population, and can be crossed repeatedly. Additionally, Song et al. (2011) reported the genetic basis of PH and ear position for a highly heterotic maize hybrid Yuyu 22 by using triple testcross (TTC) genetic mating design. In comparison with previous populations, introgression lines (ILs) have relative simple genetic background and would have more advantages in dissection of the genetic basis of complex traits (Qi et al. 2013). Unlike the genomic construct of DH and RIL populations, the genomes of SSSL population are basically the same as those of the recipient parent except for one homozygous chromosome segment from the donor parent. When SSSL population is tested using the recipient parent, the SSSL test population has only one heterozygous chromosomal region comparing to the

recipient parent. If individuals in the SSSL test population show heterosis for some traits comparing to the recipient parent, then the HL can be analyzed directly as the Mendelian genetic model. Recently, Guo et al. (2013) detected HL for yield and agronomic traits using chromosome segment ILs in cotton. So the SSSL test population was used to identify HL for plant morphological traits in maize in this study.

Schnable and Springer (2013) have summarized that heterosis is generally the result of the action of multiple loci and that different loci affect heterosis for different traits and in different hybrids, so it is challenging to unify a common genetic basis of heterosis even within a species for different traits or experimental design (Shen et al. 2014). Although each trait in different hybrids perhaps has different HL, the morphological traits and grain yield as well as its components should same genetic mechanism of heterosis in maize. In this study, the performance of mid-parent heterosis of the test-cross population in three environments was PH > EH > TBN > T-MAL > LN (Table 2). Out of the five morphological traits, PH and EH are two important agricultural traits in maize and highly associated with biomass, grain yield, and lodging resistance. According to the results of previous studies, the PH and EH have high heterosis and were extremely easy to measure and obtain accurate phenotypic value comparing to the other morphological traits, so it was used as a model for genetic analysis of heterosis in previous researches and this study (Beavis et al. 1991; Tang et al. 2006; Lu et al. 2011; Shen et al. 2014). For the other three morphological traits used in this study, LN had significant correlation with PH and EH in several environments (Table 3), and TMAL and TBN have the similar differentiation procedure of ear length and row number; thus, the three morphological traits also were used to detect the HL in this study. Additionally, this study also showed that the experimental design of the SSSLs test population constructed with SSSLs population and its receptor parent was suit to dissect the genetic basis of heterosis for grain yield and its components. Owing to plant height and ear height is high correlation with lodging in the field, and the two traits has strong heterosis, so in the procedure of maize breeding, maize breeder should select the hybrids with low heterosis of the two morphological traits. Many architecture-correlated traits in plants are complex quantitative traits controlled by multiple genes and

show obvious heterosis (Yu et al. 2002). However, the genetic effects of additive genes and HL for quantitative traits are superimposed in a single hybrid, and it is very difficult to distinguish accurately the performance values of two kinds of genes. In previous studies, two different strategies have been used to dissect the genetic mechanism of heterosis, the QTL mapping method and the HL mapping method, and are based on different genetically segregated populations (Yu et al. 1997; Luo et al. 2009; Li et al. 2010; Hua et al. 2003; Guo et al. 2013). Shen et al. (2014) reported that 15 QTLs were also HL contributing to heterosis with dominance effects in rice. Guo et al. (2013) reported that among 58 HL, only seven HL were also detected by QTL analysis using the data of the chromosome segment ILs (CSILs) population in cotton. Tang et al. (2010) reported that 13 heterotic (HL) were detected for grain yield and its three components using a IF2 population in maize, and a total of 143 digenic interactions contributing to mid-parent heterosis were detected at the two-locus level involving all three types of interactions. Additionally, Tang et al. (2006) reported that only three QTLs (qPH1b, qPH5 and qPH9) and HL (qPHH1e, qPHH5 and qPHH9) had the same chromosome locus in the "immortalized F_2" (IF_2) population in maize, accounted for the detected 25 % of QTLs and 30 % of HL in IF_2 population, respectively. In this study, 41 QTLs and 37 HL were identified for the five measured traits in three environments, and only 10 of the HL (27.03 %) were located in the same position as a corresponding QTL (24.39 %). Comparing this results with previous study, one HL for PH (ph1b) detected in this study locate in similar regions of corresponding chromosomal region with qPHH1b and qPHH1c (Tang et al. 2006). In a word, the phenotype trait and heterosis should be controlled by two different genetic and molecular mechanisms.

Dominance and over-dominance are the two most important hypotheses to explain the genetic basis of heterosis. Li et al. (2001) reported that most QTL associated with heterosis in rice appeared to be involved in epistasis, and most (approximately 90 %) of the QTL contributing to heterosis appeared to be over-dominant. Hua et al. (2003) reported that over-dominance/pseudo-overdominance were the most important contributors to heterosis of yield, number of grains per panicle, and grain weight. The dominance × dominance interaction was important

for heterosis of tillers per plant and grain weight and also affected yield and grain number. In F_2 populations derived from the commercial hybrid rice line Shanyou 10, five QTLs were detected for grain yield and 13 QTLs were detected for number of panicles, and most of these QTLs showed over-dominance effects (Zhuang et al. 2001). Kusterer et al. (2007) showed that dominance, as well as digenic and possibly higher-order epistatic effects, played important roles in heterosis for biomass-related traits in *Arabidopsis thaliana*. There are many reports that over-dominance is the most important factor in the genetic basis of yield and its components in maize (Stuber et al. 1992; Larièpe et al. 2012). However, other studies on maize (Cockerham and Zeng 1996; Garcia et al. 2008) have provided evidence for the dominance hypothesis. In this study, 21.4 % of the HL for five measured traits showed dominance effects, 76.8 % of the HL showed over-dominance effects, and only one HL (1.8 %) showed a partial-dominance effect. These results implied that the over-dominance effect is the main contributor to heterosis for these five traits at the single-locus level in maize, and the result is similar to the conclusion reported by Zhuang et al. (2001) in rice.

References

Beavis WD, Grant D, Albertsen M, Fincher R (1991) Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor Appl Genet 83(2):141–145

Birchler JA, Yao H, Chudalayandi S, Vaiman D, Veitia RA (2010) Heterosis. Plant Cell 22:2105–2112

Bruce AB (1910) The Mendelian theory of heredity and the augmentation of vigor. Science 32:627–628

Cockerham CC, Zeng ZB (1996) Design III with marker loci. Genetics 143:1437–1456

East EM (1936) Heterosis. Genetics 21:375–397

Garcia AA, Wang SC, Melchinger AE, Zeng ZB (2008) Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. Genetics 180:1707–1724

Guo JF, Su GQ, Zhang JP, Wang GY (2008) Genetic analysis and QTL mapping of maize yield and associate agronomic traits under semi-arid land condition. Afr J Biotechnol 7(12):1829–1838

Guo X, Guo YP, Ma J, Wang F, Sun MZ, Gui LJ, Zhou JJ, Song XL, Sun XZ, Zhang TZ (2013) Mapping heterotic loci for yield and agronomic traits using chromosome segment

introgression lines in cotton. J Integr Plant Biol 55(8):759–774

Guo M, Rupe MA, Wei J, Winkler C, Goncalves-Butruille M, Weers BP, Cerwick SF, Dieter JA, Duncan KE, Howard RJ, Hou ZL, Löffler CM, Cooper M, Simmons CR (2014) Maize ARGOS1 (ZAR1) transgenic alleles increase hybrid maize yield. J Exp Bot 65(1):249–260

Hua JP, Xing YZ, Wei WR, Xu CG, Sun XL, Yu SB, Zhang QF (2003) Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. PNAS 100:2574–2579

Jones DF (1917) Dominance of linked factors as a means of accounting for heterosis. Genetics 2:466–479

Koester R, Sisco PH, Stuber CW (1993) Identification of quantitative trait loci controlling days to flowering and plant height in two near isogenic lines of maize. Crop Sci 33(6):1209–1216

Kusterer B, Muminovic J, Utz HF, Piepho HP, Barth S, Heckenberger M, Meyer RC, Altmann T, Melchinger AE (2007) Analysis of a triple testcross design with recombinant inbred lines reveals a significant role of epistasis in heterosis for biomass-related traits in Arabidopsis. Genetics 175:2009–2017

Larièpe A, Mangin B, Jasson S, Combes V, Dumas F, Jamin P, Lariagon C, Jolivot D, Madur D, Fiévet J, Gallais A, Dubreuil P, Charcosset A, Moreau L (2012) The genetic basis of heterosis: multiparental quantitative trait loci mapping reveals contrasted levels of apparent over-dominance among traits of agronomical interest in maize (*Zea mays* L.). Genetics 190:795–811

Li ZK, Luo LJ, Mei HW, Wang DL, Shu QY, Tabien R, Zhong DB, Ying CS, Stansel JW, Khush GS, Paterson AH (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics 158(4):1737–1753

Li LZ, Lu KY, Chen ZM, Mu TM, Hu ZL, Li XQ (2008) Dominance, overdominance and epistasis condition the heterosis in two heterotic rice hybrids. Genetics 180:1725–1742

Li ZK, Xie QG, Zhu ZL, Liu JL, Han SX, Tian B, Yuan QQ, Tian JC (2010) Analysis of plant height heterosis based on QTL mapping in wheat. Acta Agron Sin 36:771–778

Lippman Z, Zamir D (2007) Heterosis: revisiting the magic. Trends Genet 23:60–66

Liu GF, Zhu HT, Zhang GQ, Li LH, Ye GY (2012) Dynamic analysis of QTLs on tiller number in rice (*Oryza sativa* L.) with single segment substitution lines. Theor Appl Genet 125:143–153

Lu H, Romero-Severson J, Bernardo R (2003) Genetic basis of heterosis explored by simple sequence repeat markers in a random-mated maize population. Theor Appl Genet 107:494–502

Lu MY, Li XH, Shang AL, Wang YM, Xi ZY (2011) Characterization of a set of chromosome single-segment substitution lines derived from two sequenced elite maize inbred lines. Maydica 56(4):399–407

Luo XJ, Fu YC, Zhang PJ, Wu S, Tian F, Liu JY, Zhu ZF, Yang JS, Sun CQ (2009) Additive and over-dominant effects resulting from epistatic loci are the primary genetic basis of heterosis in rice. J Integr Plant Biol 51:393–408

Ma LY, Bao JS, Guo LB, Zeng DL, Li XM, Ji ZJ, Xia YW, Yang CD, Qian Q (2009) Quantitative trait loci for panicle layer uniformity identified in doubled haploid lines of rice in two environments. J Integr Plant Biol 51:818–824

Melchinger AE, Utz HF, Schön CC (1998) Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 149(1):383–403

Melchinger AE, Utz HF, Piepho H-P, Zeng ZB, Schon CC (2007) The role of epistasis in the manifestation of heterosis: a systems oriented approach. Genetics 177:1815–1825

Melchinger AE, Utz HF, Schon CC (2008) Genetic expectations of quantitative trait loci main and interaction effects obtained with the triple testcross design and their relevance for the analysis of heterosis. Genetics 178:2265–2274

Pan JJ (1994) Crop breeding. China Agriculture Press, Beijing, pp 6–95

Powers L (1944) An expansion of Jones's theory for the explanation of heterosis. Am Nat 78:275

Qi HH, Huang J, Zheng Q, Huang YQ, Shao RX, Zhu LY, Zhang ZX, Qiu FZ, Zhou GC, Zheng YL, Yue B (2013) Identification of combining ability loci for five yield-related traits in maize using a set of testcrosses with introgression lines. Theor Appl Genet 126:369–377

Schnable PS, Springer NM (2013) Progress toward understanding heterosis in crop plants. Annu Rev Plant Biol 64:71–78

Shen GJ, Zhan W, Chen HX, Xing YZ (2014) Dominance and epistasis are the main contributors to heterosis for plant height in rice. Plant Sci 215–216:11–18

Shull GH (1908) The composition of a field of maize. J Hered 1:296–301

Song FW, Peng HR, Liu T, Zhang YR, Sun QX, Ni ZF (2011) Heterosis for plant height and ear position in maize revealed by quantitative trait loci analysis with triple testcross design. Acta Agron Sin 37(7):1186–1195

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Syed NH, Chen ZJ (2005) Molecular marker genotypes, heterozygosity and genetic interactions explain heterosis in *Arabidopsis thaliana*. Heredity 94:295–304

Tang JH, Ma XQ, Teng WT, Yan JB, Wu WR, Dai JR, Li JS (2006) Detection of quantitative trait loci and heterosis for plant height in maize in "immortalized F_2" (IF_2) population. Chin Sci Bull 51(24):2864–2869

Tang JH, Yan JB, Ma XQ, Teng WT, Wu WR, Dai JR, Dhillon BS, Melchinger AE, Li JS (2010) Dissection of the genetic basis of heterosis in an elite maize hybrid by QTL mapping in an immortalized F_2 population. Theor Appl Genet 120:333–340

Wang ZQ, Yu CY, Liu X, Liu SJ, Yin CB, Liu LL, Lei JG, Jiang L, Yang C, Chen LM, Zhai HQ, Wan JM (2012) Identification of indica rice chromosome segments for the improvement of japonica inbreds and hybrids. Theor Appl Genet 124(7):1351–1364

Williams W (1959) Heterosis and the genetics of complex characters. Nature 184:527–530

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang QF, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231

Yu SB, Li JX, Xu CG, Tan YF, Li XH, Zhang QF (2002) Identification of quantitative trait loci and epistatic interactions for plant height and heading date in rice. Theor Appl Genet 104:619–625

Zhou G, Chen Y, Yao W, Zhang CJ, Xie WB, Hua JP, Xing YZ, Xiao JH, Zhang QF (2012) Genetic composition of yield heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 109(39):15847–15852

Zhuang JY, Fan YY, Wu JL, Xia YW, Zheng KL (2001) Importance of over-dominance as the genetic basis of heterosis in rice. Sci China Ser C 44(3):327–336