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REVIEW

QTL mapping and marker analysis of main stem height and the first lateral branch length in peanut (*Arachis hypogaea* L.)

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Abstract Main stem height (MSH) and the first lateral branch length (LBL) of peanut (*Arachis hypogaea* L.) are important traits affecting plant shape and yield. Identification of quantitative trait loci (QTLs) related to these two traits, the prediction and cloning of candidate genes, and identification of plant height-related molecular markers are the basis for analysis of the molecular genetic mechanism of plant shape in peanut. In this study, a population of 151 recombinant inbred lines from a single seed, derived from a cross between variety 79266 (P1) and its variant progeny D893 (P2), was used to construct a peanut genetic map. The map consisted of 231 simple sequence repeat markers in 23 linkage groups, had a total length of 905.18 cM with average and minimum marker intervals of 3.92 and 0.1 cM, respectively.

There were 11 and 16 QTLs detected in six environments for MSH and LBH with 6.26–22.53 and 5.89–21.63% phenotypic variation explained (PVE), respectively. Seven QTLs were detected in two or more environments: 3 QTLs for MSH (including Qmsh-14-3) with 7.66–22.53% PVE, and 4 QTLs for LBL (including Qllb-11-1) with 6.12–21.63% PVE. Qmsh-14-3 was steadily detected in five environments, localized between two markers, ARS376 and SEQ4G02, exhibited a genetic distance of 0.59–2.59 and 4.11–6.11 cM from the two markers, respectively. Qllb-11-1 was steadily detected in five environments, localized between two markers, ARS203 and AHS1413, exhibited a genetic distance of 1.06–3.06 and 0.23–2.23 cM from the two markers, respectively. There were 220 germplasm accessions used to detect the relationship between genotype and phenotypic values of traits at the marker loci ARS376 and SEQ4G02, average values of MSH and LBL were significantly higher for germplasm with the P1 compared to the P2 genotype. Determination of the marker loci ARS203 and AHS1413 indicated that average values of LBL were greater in germplasm with the P1 than the P2 genotype. The results provide references for fine mapping of QTLs for MSH and LBL, as well as breeding optimum plant-types in peanut.

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Abbreviations

QTL Quantitative trait locus/loci
RIL Recombinant inbred line
SSR Simple sequence repeat
MSH Main stem height
LBL The first lateral branch length

Introduction

Peanut (*Arachis hypogaea* L.), also known as ground-nut, is a grain legume and oilseed crop widely cultivated in tropical and subtropical regions (annual production of ~ 46 million tonnes) (David et al. 2016). Main stem height (MSH) and the first lateral branch length (LBL) of peanut are important agricultural traits affecting plant shape and yield. Moderate plant height, well-developed branches and appropriate bunch density are ideal goals for high-yield peanut breeding. Lan et al. (2011) used the gray relational analysis grade method to study the correlation between peanut yield and main agricultural traits, and found that MSH and LBL were some of the leading factors affecting peanut yield. Correlation and cluster analyses of main agricultural traits in 18 varieties showed that MSH and LBL were highly correlated with peanut yield (Yin et al. 2006). The identification of quantitative trait loci (QTLs) related to MSH and LBL, the prediction and cloning of candidate genes, as well as the identification of molecular markers related to plant height are the research basis for analyzing the molecular genetic mechanism of plant shape, and are important for optimum plant-type breeding in peanut.

The construction of a genetic map is an important basis for QTL identification. Cultivated peanut is an allotetraploid ($2n = 4X = 40$, AABB) with closely related subgenomes of a total size of ~ 2.7 Gb (David et al. 2016). The peanut subgenomes are closely related (Moretzsohn et al. 2013; Nielen et al. 2012), which to some extent increase the difficulties of molecular marker genetic map construction and molecular marker development. Since Halward et al. (1993) crossed two diploid wild types of *Arachis stenosperma* and *Arachis cardenasii*, and constructed the first genetic linkage map of peanut using an F_2 population and amplified fragment length polymorphism markers, studies on peanut genetic linkage

maps have made great progress. Especially in recent years, due to the maturity of simple sequence repeat (SSR) marker technology, the construction of genetic linkage maps in cultivated peanut has rapidly developed (Hong et al. 2009, 2010; Li et al. 2015; Qin et al. 2012; Varshney et al. 2009; Wang et al. 2013, 2015). Shirasawa et al. (2013) used 15 genetic linkage maps to integrate and construct a genetic map consisting of 3693 SSR markers in 20 lineage groups, which is currently the peanut integrated genetic map with the highest SSR marker density. David et al. (2016) completed the genome sequencing of diploid ancestors *Arachis duranensis* and *Arachis ipaensis* of cultivated varieties, and obtained sequence coverage of 96% of the genomes of the diploid wild species (the website for inquiry of genome sequence information is http://peanutbase.org). The genome sequence information of the diploid wild species provided important references for distinguishing the two subgenomes (A and B) in cultivated varieties, the development of molecular markers, increasing the density of the molecular genetic linkage map and searching for the physical identification of functional genes in chromosomes.

MSH and LBL in peanut are quantitative traits. Recently, several QTLs related to the above traits were identified. Shirasawa et al. (2012) identified 3 QTLs for MSH with 4.8, 19.2 and 15.7% phenotypic variation explained (PVE) respectively, and detected 2 QTLs for LBL with 21.1 and 14.2% PVE, respectively, on the genetic map of cultivated peanut. Cheng et al. (2015) constructed a genetic map consisting of 234 markers using an F_2 population from crossing between cultivated varieties, and identified six stable QTLs for MSH in two environments. Therein, qMHA091.1 and qMHA062.1 with highest PVE of 9.43 and 8.95%, respectively, were separately mapped in the A09 and A06 linkage groups on the integrated map constructed by Shirasawa et al. (2013). Li et al. (2015) identified 3 QTLs for MSH with 8.31, 6.12 and 8.90% PVE, respectively.

In this study, a recombinant inbred lines (RILs) population, constructed from the generations of a cross between the cultivated variety 79266, widely cultivated by farmers in China, and its dwarf mutant line D893, and 220 germplasm accessions of peanut were used to identify QTLs and markers related to MSH and LBL in peanut. The results will be used to identify more QTLs related to plant height and shape, develop

more molecular markers and provide references for genetic improvement in future breeding in peanut.

Materials and methods

Materials and field planting

The female parent, 79266 (P1), was a peanut variety bred by the Shandong Peanut Research Institute with characteristics of early maturity and high yield. The male parent, D893 (P2), had characteristics of dwarf and super-large pods, and was developed by our research laboratory. It was derived from the variant progeny by introduction of *Sophora japonica* Linn DNA into 79266 after pollination via the pollen tube pathway (Zhou 1992). There were significant differences in MSH and LBL between parents (Table 1). The cross 79266 × D893 was performed at Tai'an, Shandong in 2005, false hybrids were removed at F_1 generation, single-seed descent method was applied from F_2 generation to F_6. The $F_{6:10}$ RIL population

consisting of 151 stable families was obtained after successive inbreeding.

A randomized block design with two replications were applied in the field experiment, with planting conducted under six environmental conditions at Tai'an/Shandong of China from 2011 to 2015 and at Ji'ning/Shandong in 2013 (the environments were named E1–E6, respectively). Each material was planted in a row with length of 280 cM and row spacing of 40 cM, and each row had 14 holes. Each hole was reduced to a single plant after germination, and management was the same as for field production.

There were 220 germplasm accessions, collected from China and abroad, planted in in China at Tai'an,Shandong in 2013 and 2014 as a validation population. These were applied to the validation of linkage markers for identified QTLs.

Trait measurement and data analysis

The plants on edges of each row were removed during harvest. Five single plants in succession were selected from 12 plants in the middle and used to measure MSH

Table 1 Phenotypic value for the two traits of RILs and their parents

Trait	Environment	Parents		RIL population						
		79266 (P1)	D893 (P2)	Min	Max	Mean	SD	CV%	Skewness	Kurtosis
MSH (cM)	E1	58.20	40.60[**]	28.00	72.33	45.82	8.28	18.08	0.46	0.68
	E2	55.80	30.20[**]	23.20	64.25	40.59	8.31	20.46	0.30	0.24
	E3	61.83	45.08[**]	26.63	86.00	53.43	11.00	20.58	0.37	0.58
	E4	52.50	34.50[**]	23.00	66.25	42.13	7.66	18.19	0.08	0.17
	E5	35.00	27.50[*]	17.50	52.50	32.22	6.18	19.18	0.56	1.09
	E6	83.25	44.88[**]	32.25	92.69	54.31	11.63	21.42	0.68	0.59
	Average	57.76	37.13	25.10	72.34	44.75	8.84	19.65		
LBL (cM)	E1	139.00	95.00[**]	68.00	169.67	111.97	24.30	21.70	0.39	-0.55
	E2	121.20	70.80[**]	52.60	184.25	102.91	22.27	21.64	0.60	0.97
	E3	149.30	111.70[**]	74.25	216.25	134.92	29.12	21.59	0.26	-0.22
	E4	114.00	85.00[**]	55.50	165.00	100.23	19.40	19.36	0.67	0.88
	E5	90.00	76.00[**]	44.50	121.25	82.01	14.61	17.82	-0.08	-0.18
	E6	180.90	108.40[**]	75.94	209.25	136.42	29.84	21.88	0.28	-0.58
	Average	132.40	91.15	61.80	177.61	111.41	23.26	20.66		

Environments for RILs are indicated with an 'E': E1, E2, E3, E4 and E5 are Tai'an 2011, 2012, 2013, 2014 and 2015, respectively; and E6 is Ji'ning 2013. *MSH* Main stem height; *LBL* the first lateral branch length

[*] Significant difference of phenotypic values between 79266 and D893 at P < 0.05

[**] Significant difference of phenotypic values between 79266 and D893 at P < 0.01

and LBL according to the standard procedures previously described by Jiang et al. (2006).

SPSS 20.0 (IBM Company, Armonk, NY, USA) was used for analysis of variance, genetic variation coefficient analysis and descriptive statistics for the data.

DNA extraction and SSR genotyping

Genomic DNA was extracted from young leaves collected from each line using a modified cetyltrimethyl ammonium bromide method (Paterson et al. 1993). The quality and quantity of DNA were evaluated using a Nano Drop 2000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA).

PCR reactions followed the protocol of Chen et al. (2008). The PCR products were separated through 8% non-denaturing polyacrylamide gel electrophoresis under constant voltage of 150 V for 2 h, silver stained and photographed for preservation after film development.

A total of 4438 SSR primer pairs from different resources were used to screen for polymorphism between the two parental genotypes. The SSR primers were derived from the following: Bertioli et al. (2009); Budiman et al. 2006; Cuc et al. (2008); Ferguson et al. (2004); Gimenes et al. (2007); Guo et al. 2008; He et al. (2003); Hopkins et al. (1999); Koilkonda et al. 2012; Moretzsohn et al. (2005); Nagy et al. (2010); Naito et al. 2008; Proite et al. (2007); Shirasawa et al. (2012); and Wang et al. 2007. All primers were synthesized by Shanghai Sangon Biotech Co. Ltd. The Taq polymerase, dNTP and other reagents for PCR reaction were purchased from Beijing TransGen Biotech Co. Ltd. Acrylamide, ammonium persulfate, TEMED and other reagents for gel electrophoresis were purchased from Taian Keshang Biotech Co. Ltd.

Construction of genetic linkage map and QTL analysis

The results of the experiment banding pattern were digitized. In the mapping population, the families with the same banding pattern as the female parent were recorded as 'a', those with the same banding pattern as the male parent were recorded as 'b', those with a hybridization banding pattern were recorded as 'h' and those without or with unclear banding pattern were recorded as '−'. Join Map 4.0 (Van 2006) was used to construct the genetic linkage map. QTL IciMapping V4.0 was used to identify QTLs by Inclusive Composite Interval Mapping (ICIM) (Li et al. 2008).

Validation of QTL linkage markers in the germplasm population

The MSH and LBL of 220 germplasm accessions were measured during the harvest period, using the same methods of measurement and statistics as for the RIL population. The detected QTL linkage markers were validated in the germplasm population and statistical analysis of banding patterns conducted. The individuals with different banding patterns were compared and analyzed to validate the detected markers.

Results

Characterization of phenotypic distribution of parents and RIL population

The results for the six planting environments showed significant differences in MSH and LBL between the two parents. There was a greater variation range of characteristics in the RIL population, showing transgressive segregation. The standard deviations (SDs) and coefficient of variations (CVs) indicated abundant population variation, with all CVs >20% (Table 1). The population distribution histogram (Fig. 1) showed continuous variation with a normal distribution for MSH and LBL, which are typical characteristics of quantitative traits. Therefore, this population was suitable for the construction of the genetic linkage map and detection of QTLs.

Construction of the genetic linkage map

Of the 4438 SSR primer pairs, there were 252 detected polymorphic DNA fragments between the parents 79266 and D893. These 252 primer pairs were used to genotype the 151 RILs. Using Join Map 4.0 with the minimum logarithm of odds (LOD) of 3.0, a genetic linkage map for cultivated peanut was constructed consisting of 231 markers in 23 linkage groups. The map had a total length of 905.18 cM with an average marker interval of 3.92 cM and minimum marker interval of 0.1 cM. The average length of each linkage group was 39.56 cM with 2–63 markers (Table 2).
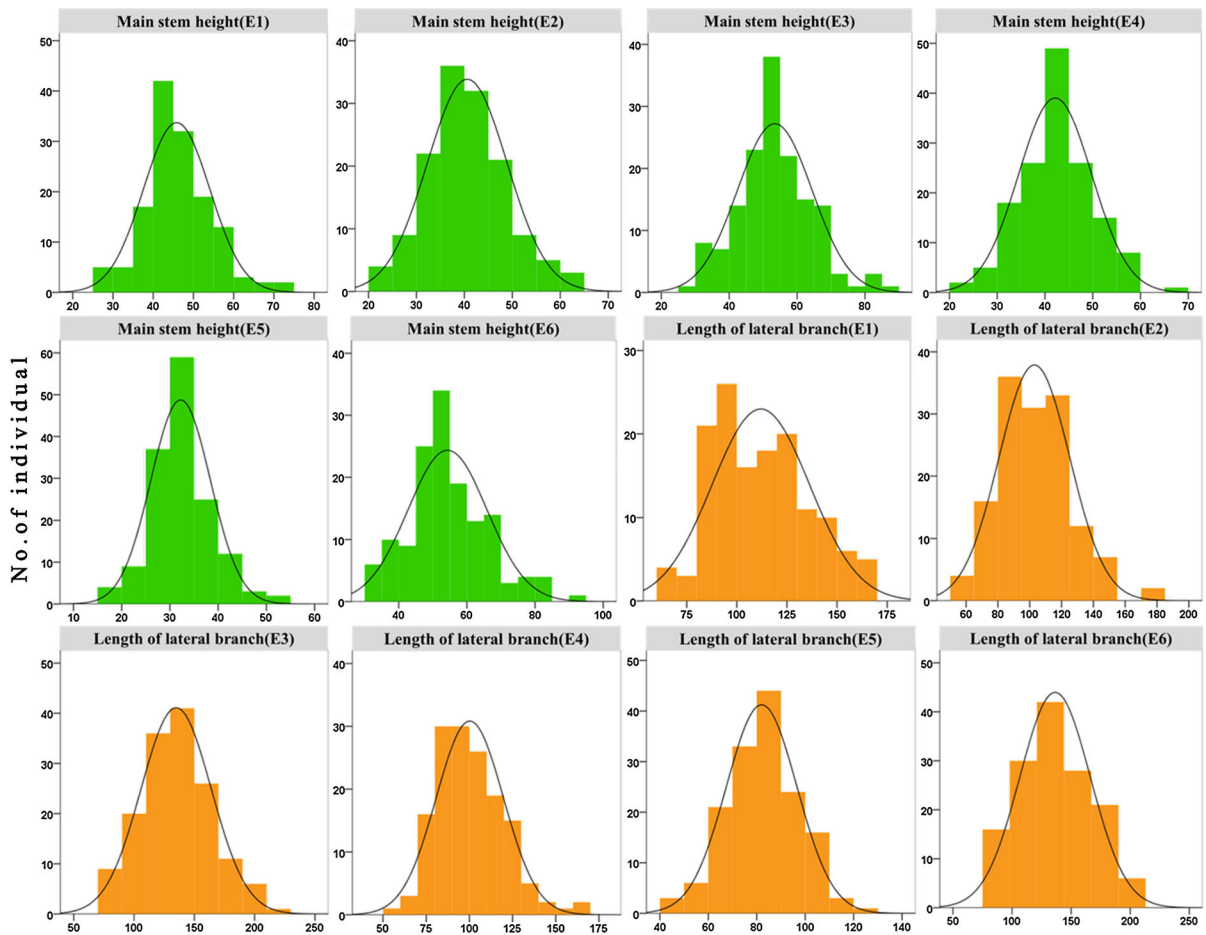
Fig. 1 Distribution of the main stem height and length of lateral branch in the RIL population. The *horizontal axis* shows the values of main stem height (cM) or lateral branch length (cM):

E1, E2, E3, E4 and E5 are Tai'an 2011, 2012, 2013, 2014 and 2015, respectively; and E6 is Ji'ning 2013

Identification of QTLs for main stem height and lateral branch length in peanut

Based on the constructed linkage map, combined with the phenotypic data of six environments, QTL identification of MSH and LBL in peanut was conducted using QTL IciMapping V4.0 software and the ICIM method. A total of 27 QTL sites were detected using LOD ≥ 2.5.

A total of 11 QTLs for MSH were detected in six environments (Table 3; Fig. 2) and distributed on six linkage groups, with PVE of 6.26–22.53% and the additive effects of 3.48–5.29. Of these, 8 QTLs were detected in only one environment. Two QTLs, Qmsh-14-2 and Qmsh-21-1, were detected in two environments. The QTL Qmsh-14-3 was detected in all environments except for E5 and showed positive

additive effects, with PVE >10%. The largest PVE and additive effect values of Qmsh-14-3 were 22.53 and 6.27, respectively. Thus, Qmsh-14-3 was considered a stable and main-effect QTL site, being insensitive to environment, for MSH in peanut.

A total of 16 QTLs for LBL were detected in six environments (Table 4; Fig. 2) and distributed on eight linkage groups, with PVE of 5.89–21.63% and additive effects of −8.71 to 12.23. Of these, 12 QTLs were detected in only one environment, Qllb-7-3 and Qllb-14-1 were detected in two environments, and Qllb-14-4 was detected in three environments. Qllb-11-1 was detected in all environments except for E1, with PVEs of 12.18–15.05% and additive effect of 5.24–12.17. Qllb-11-1 was considered a stable and main-effect QTL for lateral branch length in peanut.

Table 2 Main characteristics of genetic linkage map in peanut (*Arachis hypogaea* L.)

Linkage group	Length (cM)	No. of markers	Average distance (cM)
LG1	52.648	4	17.55
LG2	12.249	3	6.12
LG3	68.205	13	5.68
LG4	62.666	8	8.95
LG5	18.137	5	4.53
LG6	24.983	3	12.49
LG7	92.829	63	1.50
LG8	25.315	8	3.62
LG9	4.967	8	0.71
LG10	52.444	13	4.37
LG11	8.887	4	2.96
LG12	77.504	25	3.23
LG13	22.632	4	7.54
LG14	53.395	9	6.67
LG15	39.266	5	9.82
LG16	47.267	8	6.75
LG17	98.55	16	6.57
LG18	5.338	2	5.34
LG19	10.331	6	2.07
LG20	89.516	15	6.39
LG21	24.578	5	6.14
LG22	6.343	2	6.34
LG23	7.131	2	7.13
Total or mean	905.181	231	3.94

Qmsh-14-3 and Qllb-14-4 were simultaneously detected in three environments (E1, E3 and E6), with map distances of 2, 1 and 1 cM, respectively, indicating that these two sites might be pleiotropic.

Prediction of corresponding physical map positions for marker intervals of QTLs Qmsh-14-3 and Qllb-11-1.

In this study, the located site Qmsh-14-3 related to MSH was steadily detected in five environments, comparing with the statistics on QTLs for MSH of Shirasawa et al. (2012) and the Legume Information System (LIS, http://legumeinfo.org/cmap/feature_search). The left marker ARS376 was the same as qML-12-1, which was located by Zhang (2011). The flanking markers of Qmsh-14-3 were ARS376 (27.4) and SEQ4G02 (34.1). ARS376 did not occur in the integration map (Shirasawa et al. 2013), but GM849 outside ARS376 of the map constructed in this study occurred in the integration map. Therefore, the marker regions at both ends of Qmsh-14-3, ARS376 (27.4) and SEQ4G02 (34.1), were detected in the regions of GM849 (74.92)–SEQ4G02 (71.98) in A09 linkage group on the integration map (Shirasawa et al. 2013). The DNA sequence corresponding to marker ARS376 (He et al. 2003) was *Aradu.*A09: 104,573,697–104,577, 899 bp in the genome physical map of *A. duranensis* (http://peanutbase.org). The DNA sequence corresponding to marker SEQ4G02 (Ferguson et al. 2004) was *Aradu.*A09: 111,380,567–111, 444,791 bp in the genome physical map of *A. duranensis*. It was concluded that the physical map position corresponding to the marker region of Qmsh-14-3 was *Aradu.*A09: 104,573,697–111,444,791 bp with DNA length of 6,871,094 bp. The Qllb-14-4 related to LBL, which was steadily detected in three environments, was also detected in this region. The study provides a reference sequence for the fine mapping of Qmsh-14-3 and Qllb-14-4.

Table 3 Parameters of QTLs for main stem height in RIL population

QTL name	Linkage group	Flanking markers	Interval distance (cM)	Peak position (cM)	LOD score	PVE (%)	Additive	Environment
Qmsh-14-3	LG 14	ARS376–SEQ4G02	6.71	28	8.51	22.53	4.24	E1
				29	5.79	13.78	3.24	E2
				28	7.17	13.84	4.32	E3
				30	4.67	11.88	2.89	E4
				28	6.77	17.94	5.29	E6
Qmsh-14-2	LG 14	AHGS2635–AhTE0915	5.97	11	4.53	9.68	2.48	E4
				11	5.71	16.61	2.57	E5
Qmsh-21-1	LG 21	AHGS2573–GM1798	6.11	12	3.64	8.70	2.49	E1
				12	3.45	8.58	3.47	E6
Qmsh-3-1	LG 3	AHGS1163–AhTE0951	12.58	13	3.00	6.26	−2.08	E2
Qmsh-7-1	LG 7	AhTE0882–SEQ17F06	0.76	38	5.22	10.11	−3.48	E3
Qmsh-7-2	LG 7	AhTE1003–GM2637	2.00	66	3.51	8.93	2.60	E1
Qmsh-7-3	LG 7	AHS1000–AHGS1353	7.60	78	4.58	9.74	3.52	E3
Qmsh-10-1	LG 10	GM623–TC7C06	1.60	24	3.34	7.09	2.21	E2
Qmsh-13-1	LG 13	AhTE0843–AhTE0122	7.41	17	2.71	6.00	1.92	E4
Qmsh-14-1	LG 14	TC5D06–AHGS2635	5.63	9	5.61	14.31	3.18	E2
Qmsh-21-2	LG 21	GM1798–AhTE0846	7.15	13	6.58	13.19	3.98	E3

Environments for RILs are indicated with an 'E': E1, E2, E3, E4 and E5 are Tai'an 2011, 2012, 2013, 2014 and 2015, respectively; and E6 is Ji'ning 2013

In this study, the located Qllb-11-1 for LBL was steadily detected in five environments, and was considered a novel site by comparison with the statistics on QTLs for LBL from previous research (Huang et al. 2015; Liu et al. 2013) and LIS (http://legumeinfo.org/cmap/feature_search). The flanking markers of Qllb-11-1 were ARS203 (1.9) and AHS1413 (5.2). The DNA sequence, corresponding to marker ARS203 (He et al. 2003), was *Aradu*.A06: 110,497,201–110,498,111 bp in the genome physical map of *A. duranensis*; and that for marker AHS1413 (Koilkonda et al. 2012) was *Aradu*.A06:110,540,970–110,541,873 bp. It was concluded that the physical map position corresponding to the marker region of Qllb-11-1 was *Aradu*.A06: 110,497,201–110,541, 873 bp with DNA length of 44,672 bp, which provided a reference sequence for the fine mapping of Qllb-11-1 and prediction of functional genes.

Analysis of Qmsh-14-3 and Qllb-11-1 in the germplasm population

There were 220 germplasm accessions of peanut used for the phenotypic value investigation of MSH and

LBL in 2013 and 2014. Phenotypic frequency showed continuous variation and an approximately normal distribution (Fig. 3).

The linkage markers of Qmsh-14-3 for MSH, ARS376 and SEQ4G02, were applied for genotypic testing in the germplasm population (Fig. 4). Correlation analysis was conducted with the genotypic data and the phenotypic variation in 2013 and 2014 (Table 5).

For marker ARS376, there were 28 accessions with the P1 genotype, representing 12.7% of the population, with MSH averages of 70.16 cM (2013) and 62.85 cM (2014). There were 190 accessions with the P2 genotype, representing 86.4% of the population, with MSH averages of 67.39 cM (2013) and 56.94 cM (2014). The averages of MSH in accessions with the P1 genotype were 4.11% (2013) and 10.04% (2014) higher than those with the P2 genotype. For marker SEQ4G02, there were 86 accessions with the P1 genotype, accounting for 39.1% of the population, with MSH averages of 68.26 cM (2013) and 58.91 cM (2014). Meanwhile, 130 accessions with the P2 genotype accounted for 59.1% of the population, with MSH averages of 67.41 cM (2013) and 56.94 cM

LG1
- 0.0 AHGS1846
- 32.2 AhTE0994
- 38.1 AHGS1813
- 52.6 AHGS1650

LG2
- 0.0 RN17F12
- 9.2 Ah3
- 12.2 Leg37

LG3
- 0.0 AhTE0465
- 12.0 AHGS1163
- 24.6 AhTE0951
- 28.6 AHGS1410
- 30.3 AHGS0655
- 31.3 AHGS1360
- 31.9 AHGS1463
- 32.9 AHGS1519
- 36.4 ARS654
- 39.0 Seq1B09
- 43.5 Seq12E03
- 54.3 pPGPseq4D10
- 68.2 Seq14F04

LG4
- 0.0 TC11E04
- 16.5 Ah1TC1E06-1
- 23.6 AHGS1843
- 33.9 AhTE0534
- 47.6 GM2402
- 53.3 AHGS1239
- 58.2 PM15
- 62.7 Seq18A05

LG5
- 0.0 AHGS1986
- 5.3 Seq19D09
- 10.5 TC7E04-2
- 15.0 AHGS1951
- 18.1 TC7E04

LG6
- 0.0 TC2A02
- 10.7 TC3E02
- 25.0 AhTE0178_c1

LG8
- 0.0 ARS141
- 2.7 AHS2705
- 5.6 AHGS2244
- 12.0 AhTE0839
- 14.9 GM1788
- 17.7 GM1878
- 19.3 GA28
- 25.3 AhTE0711

LG9
- 0.0 TC3A12
- 0.4 GM630
- 1.0 GM1049
- 1.6 TC2B09
- 2.8 TC6E01 GM1577
- 4.1 AhTE0540
- 5.0 AHS1250

LG10
- 0.0 IPAHM659
- 7.5 Seq15D03
- 12.9 IPAHM509
- 23.0 GM623
- 24.6 TC7C06
- 27.5 AhTE0372
- 29.4 AHGS1675
- 31.8 ARS816
- 33.0 RN34H10
- 34.1 AHGS1173
- 36.4 AHGS1844
- 40.9 AHS0305
- 52.4 TC1A01

LG11
- 0.0 Seq4B11
- 1.9 ARS203
- 5.2 AHS1413
- 8.9 AHS2580

LG15
- 0.0 AHGS1211
- 12.0 TC11B04
- 28.9 AHGS2006
- 31.9 AHGS1390
- 39.3 Seq3E10

LG16
- 0.0 AhTE0737
- 5.6 TC30D04
- 8.2 AHS0487
- 10.5 AHGS1246
- 14.5 AhTE0251
- 22.5 AhTE0489
- 28.2 AhTE0426
- 47.3 AhTE0771

LG7
- 0.0 AhTE0872
- 12.2 AhTE0682
- 14.3 AhTE0107
- 15.6 Seq15C12-1
- 19.0 AHGS1552
- 21.9 AhTE0116
- 22.6 AhM091
- 25.5 AS1RN17A01
- 30.1 IPAHM108
- 31.3 AhTE0237
- 34.3 AhTE0189
- 34.6 AhTE0475
- 35.1 IPAHM105
- 36.0 GM2033
- 37.5 AhTE0882
- 38.2 Seq17F06
- 38.7 AhTE0910
- 39.6 AhTE1017
- 40.0 AhTE0691
- 41.2 AhTE0908
- 41.3 AhTE0335
- 41.6 AHS2274
- 41.7 TC39B04 AhTE0903
- 41.8 AHGS0936
- 41.8 AhTE1033
- 41.9 AhTE0416
- 42.1 PMc348
- 42.2 AhTE0261
- 43.3 AHGS2100
- 43.6 AHGS1311
- 44.2 AHGS2012
- 44.6 ARS306
- 45.5 AHGS0390
- 45.9 EM88
- 46.8 gi832
- 47.1 TC4H07
- 47.8 AhTE0777
- 48.8 Seq15C12-2
- 49.3 PMc297
- 52.5 AhTE0191
- 55.6 GM1641 GM2638
- 55.9 AhTE0001
- 57.6 AhTE0945
- 58.4 AHGS1382
- 59.1 AhTE0959
- 59.5 AhTE0999
- 61.5 AhTE0347
- 63.9 AHS2310
- 64.9 AhTE1003
- 66.9 GM2637
- 69.8 AhTE0796
- 71.9 AHS1000
- 79.5 AHGS1353
- 80.0 AHGS1160
- 81.6 AHS1741
- 82.9 AHGS2481
- 84.8 AHS2312
- 85.4 AHGS1471
- 86.9 GM1720
- 89.0 GM2313
- 92.8 AHGS1279

LG12
- 0.0 AHGS1525
- 5.0 TC3B05
- 9.0 GM2788
- 17.1 AHS2118
- 21.7 AhTE0929
- 25.4 AhTE0851
- 29.4 AHS0332
- 31.0 AhTE0819
- 32.1 GM2289
- 36.7 pPGPseq4E10
- 39.4 TC7A02
- 40.4 TC3B04
- 44.7 ARS120
- 47.6 GM1901
- 49.2 Seq2G03
- 52.0 AhTE0824
- 53.7 ML1G04
- 55.1 Seq5D01
- 56.9 RN18H05
- 60.9 AHGS1888
- 66.4 TC9F10
- 67.7 TC6H03
- 71.1 AHGS1470
- 75.1 ARS812
- 77.5 AhTE0658

LG13
- 0.0 AhTE0254
- 10.5 AhTE0843
- 17.9 AhTE0122
- 22.6 AhTE0010

LG14
- 0.0 GM2450
- 4.9 TC5D06
- 10.6 AHGS2635
- 16.5 AhTE0915
- 20.9 GM849
- 27.4 ARS376
- 34.1 Seq4G02
- 39.1 GM1047
- 53.4 AHGS1283

LG18
- 0.0 AHGS2781
- 5.3 AhTE0211

LG17
- 0.0 Seq3D09
- 1.6 ARS535-1
- 3.9 ARS534
- 6.4 GM1733
- 8.8 ARS535-2
- 32.0 Ah1TC1E06-2
- 47.8 AHGS1201
- 54.0 PM238
- 58.3 ARS338
- 63.4 RM14E11
- 72.8 AHGS2299
- 77.1 Ah-590
- 79.2 Seq2H08
- 81.4 Seq14C11
- 87.8 Seq16F01
- 98.6 GM1954

LG19
- 0.0 ARS122
- 0.7 GM2769
- 0.9 GM2137
- 1.6 EM-31
- 4.9 AhTE0319
- 10.3 AhTE0446

LG20
- 0.0 AhTE0143
- 12.1 AhTE0633
- 25.6 GM1254
- 32.9 ARS333
- 34.5 ARS227
- 38.4 Seq5D05
- 53.9 GM2067
- 58.0 AhTE0282
- 63.5 Seq2E06
- 68.5 AhTE0047
- 70.2 GM2156
- 73.4 AhTE0896
- 81.4 AHGS3217
- 84.8 AhTE0996
- 89.5 RN13D04

LG21
- 0.0 AHGS1616
- 6.0 AHGS2573
- 12.1 GM1798
- 19.3 AhTE0846
- 24.6 AhTE0724

LG22
- 0.0 AhTE0562
- 6.3 AhTE0999

LG23
- 0.0 S49
- 7.1 S76

Legend:
- ■ main stem height （E1）
- ■ main stem height （E2）
- ■ main stem height （E3）
- ■ main stem height （E4）
- ■ main stem height （E5）
- ■ main stem height （E6）
- ◆ length of lateral branch （E1）
- ◆ length of lateral branch （E2）
- ◆ length of lateral branch （E3）
- ◆ length of lateral branch （E4）
- ◆ length of lateral branch （E5）
- ◆ length of lateral branch （E6）

◄ **Fig. 2** Peanut genetic linkage map and QTLs for the main stem height and length of lateral branch detected in the RILs E1, E2, E3, E4 and E5 are Tai'an 2011, 2012, 2013, 2014 and 2015, respectively; and E6 is Ji'ning 2013

(2014). The averages of MSH in accessions with P1 genotype were 1.26% (2013) and 3.46% (2014) higher than those with the P2 genotype.

The main-effect QTL, Qllb-14-4 for LBL was simultaneously detected in the region of ARS376 and SEQ4G02. The averages of LBL in accessions with the P1 genotype for marker ARS376 were 8.67% (2013) and 9.03% (2014) higher than those with the P2 genotype. The averages of LBL in accessions with the P1 genotype for marker SEQ4G02 were 4.02% (2013) and 6.57% (2014) higher than those with the P2 genotype (Table 5). This indicated that ARS376 had stronger linkage with Qmsh-14-3 and Qllb-14-4 than SEQ4G02.

The linkage markers ARS203 and AHS1413 of the main-effect QTL site Qllb-11-1 for LBL were applied for genotypic testing in the germplasm population (Fig. 5). Correlation analysis was conducted with the genotypic data and the phenotypic variation in 2013 and 2014 (Table 5). For marker ARS203, 31 accessions with the P1 genotype accounted for 14.1% of the population, with LBL averages of 77.10 cM (2013) and 68.75 cM (2014). There were 179 accessions with the P2 genotype, accounting for 81.4% of the population, with LBL averages of 72.92 cM (2013) and 64.60 cM (2014). The averages of LBL in accessions with the P1 genotype were 5.73% (2013) and 6.42% (2014) higher than those with the P2 genotype. For marker AHS1413, 33 accessions with the P1 genotype accounted for 15% of the population, with LBL averages of 77.3 cM (2013) and 68.77 cM (2014). Meanwhile, there were 186 accessions with the P2 genotype, accounting for 84.5% of the population, with LBL averages of 72.82 cM (2013) and 64.47 cM (2014). The averages of LBL in accessions with the P1 genotype were 6.15% (2013) and 6.67% (2014) higher than those with the P2 genotype (Table 5). In the germplasm population, ARS203 and AHS1413 showed close linkage, and only four accessions showed recombination between the two markers, accounting for 1.81% of the population.

Discussion

Polymorphism analysis of markers between parents

Most previous peanut mapping populations were generated from crosses between two parents, such as between two wild species, a wild species and a cultivar, or two cultivated varieties with quite different genetic backgrounds. Abundant allelic differences were easily generated as a result of the distant phylogenetic relationships. In the present study, the RIL population derived from the cross between 79266 and D893 was applied for genetic linkage map construction. Because of the close phylogenetic relationship between the two parents, the polymorphic rate of markers between parents was relatively low and accounted for 5.67% of the 4438 SSRs in the study, which was lower than that in general mapping populations. However, the significant differences in phenotypic values of plant shape and pod traits between the parents were convenient for targeted research on the QTLs for traits.

Comparison with integration map

Of the 231 SSR markers on the genetic map constructed in this study, 205 markers were the same as for the integrated consensus map developed by Shirasawa et al. (2013). There were 149 markers of the linkage map identified as having corresponding markers in the 20 chromosomes of the integrated consensus map (Fig. 6), with the corresponding rate of 71.2%. In addition, the 3693 SSR markers on the integration map were used to detect the DNA of two parents in this experiment, and only 205 markers showed polymorphism. The constructed genetic map covered only 16.81% of the integration map, indicating that changes in genome DNA occurred in local regions of the chromosome after the exogenous DNA was introduced into the recipient cells. The mechanism of the genetic change in multiple chromosomes of the mutant line D893, resulting from the exogenous DNA introduction, is not yet clear and requires more research.

Table 4 Parameters of QTLs for lateral branch length in RIL population

QTL name	Linkage group	Flanking markers	Interval distance (cM)	Peak position (cM)	LOD score	PVE (%)	Additive	Environment
Qllb-11-1	LG 11	ARS203–AHS1413	3.29	5	5.34	12.69	8.06	E2
				4	10.18	14.39	11.44	E3
				4	6.14	14.83	7.74	E4
				4	5.19	12.18	5.24	E5
				3	8.91	15.05	12.17	E6
Qllb-14-4	LG 14	ARS376–SEQ4G02	6.71	30	6.16	21.63	12.23	E1
				29	4.27	6.12	7.82	E3
				29	5.71	10.03	10.17	E6
Qllb-7-3	LG 7	AHGS1160–AHS1741	1.60	80	2.83	6.45	5.84	E2
				80	5.27	6.68	7.90	E3
Qllb-14-1	LG 14	TC5D06–AHGS2635	5.63	10	5.05	12.01	6.97	E4
				10	5.21	14.20	5.57	E5
Qllb-7-1	LG 7	AhM091–AS1RN17A01	2.90	25	4.06	6.77	-7.93	E6
Qllb-7-2	LG 7	ARS306–AHGS0390	0.88	45	6.03	8.43	-8.71	E3
Qllb-7-4	LG 7	AHGS1471–GM1720	1.58	86	2.96	8.25	7.31	E1
Qllb-10-1	LG 10	GM623–TC7C06	1.60	24	4.62	6.81	7.79	E3
Qllb-13-1	LG 13	AhTE0254–AhTE0843	10.51	5	3.06	5.89	7.38	E6
Qllb-13-2	LG 13	AhTE0843–AhTE0122	7.41	16	2.86	6.84	5.18	E4
Qllb-14-2	LG 14	AHGS2635–AhTE0915	5.97	15	4.39	6.40	7.83	E3
Qllb-14-3	LG 14	GM849–ARS376	6.46	24	6.85	19.02	10.20	E2
Qllb-18-1	LG 18	AHGS2781–AhTE0211	5.34	0	4.08	6.12	7.65	E6
Qllb-20-1	LG 20	AhTE0633–AhTE0143	12.07	80	3.59	9.56	4.52	E5
Qllb-21-1	LG 21	AHGS2573–GM1798	6.11	12	4.70	7.16	8.14	E6
Qllb-21-2	LG 21	GM1798–AhTE0846	7.15	13	3.33	4.47	6.27	E3

Environments for RILs are indicated with an 'E': E1, E2, E3, E4 and E5 are Tai'an 2011, 2012, 2013, 2014 and 2015, respectively; and E6 is Ji'ning 2013
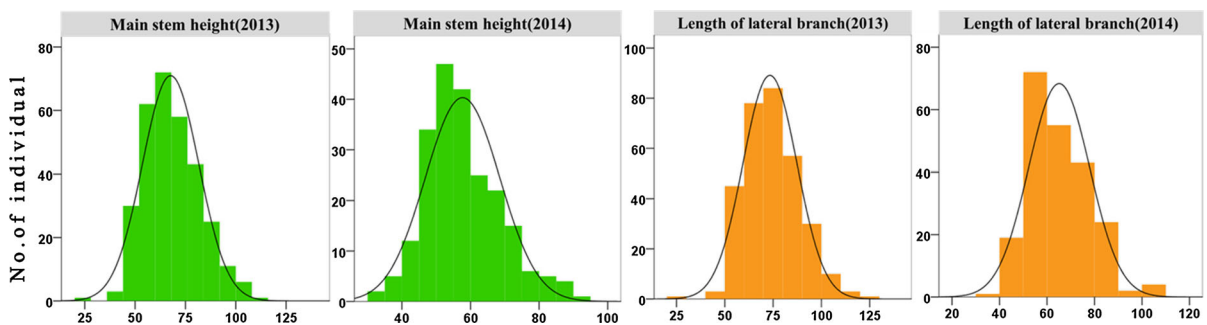
Fig. 3 Phenotypic variation of main stem height and lateral branch length in germplasm population. The *horizontal axis* shows the values of main stem height (cM) or lateral branch length (cM)

Analysis of multiple-effect QTLs

Clustering of QTLs for two traits (MSH and LBL) were mapped in the same region of linkage groups

(Fig. 2). Qmsh-14-3 related to MSH and Qllb-14-4 controlling LBL were steadily detected in the region of ARS376-SEQ4G02 of LG14. It was considered that there were multiple-effect QTLs between markers

Fig. 4 The PCR amplification profiles of marker ARS376 (*left*) and SEQ4G02 (*right*) primers in the germplasm population M, DL1000 DNA marker; P1, 79266 banding pattern; P2, 04D893 banding pattern; 157–163, the number of accessions in the germplasm population
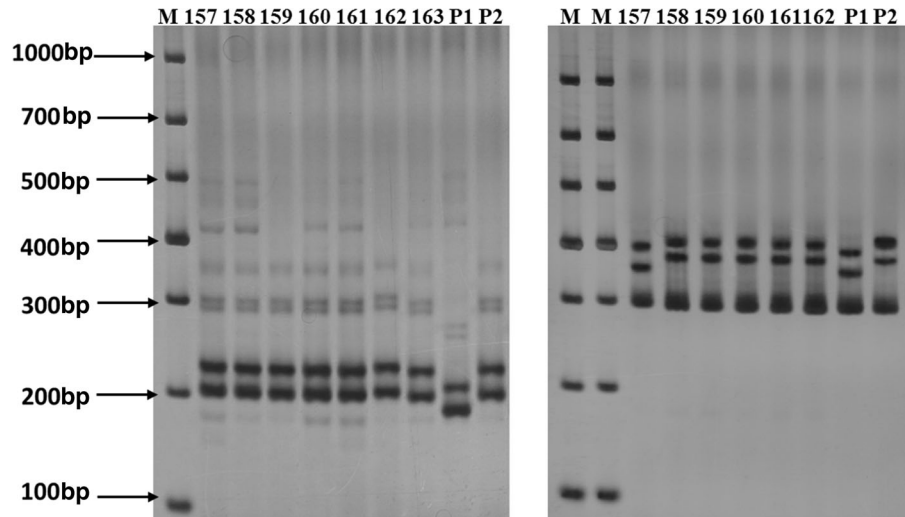

Table 5 Distribution of QTL linkage markers in the germplasm population

QTL name	Marker name	Banding pattern	Material number	2013		2014	
				Main stem height (cM)	Lateral branch length (cM)	Main stem height (cM)	Lateral branch length (cM)
Qmsh-14-3	ARS376						
Qllb-14-4		79266	28	70.16	78.86	62.85	70.12
		D893	190	67.39	72.57	56.94	64.31
	SEQ4G02						
		79266	86	68.26	75.12	58.91	67.63
		D893	130	67.41	72.22	56.93	63.46
Qllb-11-1	ARS203						
		79266	31	68.64	77.10	57.29	68.75
		D893	179	67.54	72.92	57.85	64.60
	AHS1413						
		79266	33	68.65	77.3	57.50	68.77
		D893	186	67.55	72.82	57.71	64.47

ARS376 (27.4) and SEQ4G02 (34.1). Simultaneously, multiple-effect QTLs for the above two traits were also detected in LG10, LG13, LG14 and LG21, respectively. These results support the attribution of the correlation of MSH and LBL to the effect of pleiotropy or to the very close linkage of genes.

Validation of molecular markers in peanut germplasm population

The detection result of identified markers ARS376 and SEQ4G02 in 220 accessions indicated that, the averages of MSH and LBL were significantly higher in varieties with genotype 79266 than D893. In the markers ARS203 and AHS1413 on both sides of the QTL Qllb-11-1 related to LBL, the average LBL was significantly higher in varieties with genotype 79266 than D893. The above four markers had correlations with the corresponding traits; however, analysis showed that the differences were not significant ($P > 0.05$). The possible reason was that both MSH and LBL were quantitative traits and controlled by multiple genes. In the RIL population of this experiment, the phylogenetic relationship between two

Fig. 5 The PCR amplification profiles of marker ARS203 (*left*) and AHS1413 (*right*) primers in the germplasm population M, DL1000 DNA marker; P1, 79266 banding pattern; P2, 04D893 banding pattern; 81–88 and 173–179, the number of accessions in the germplasm population
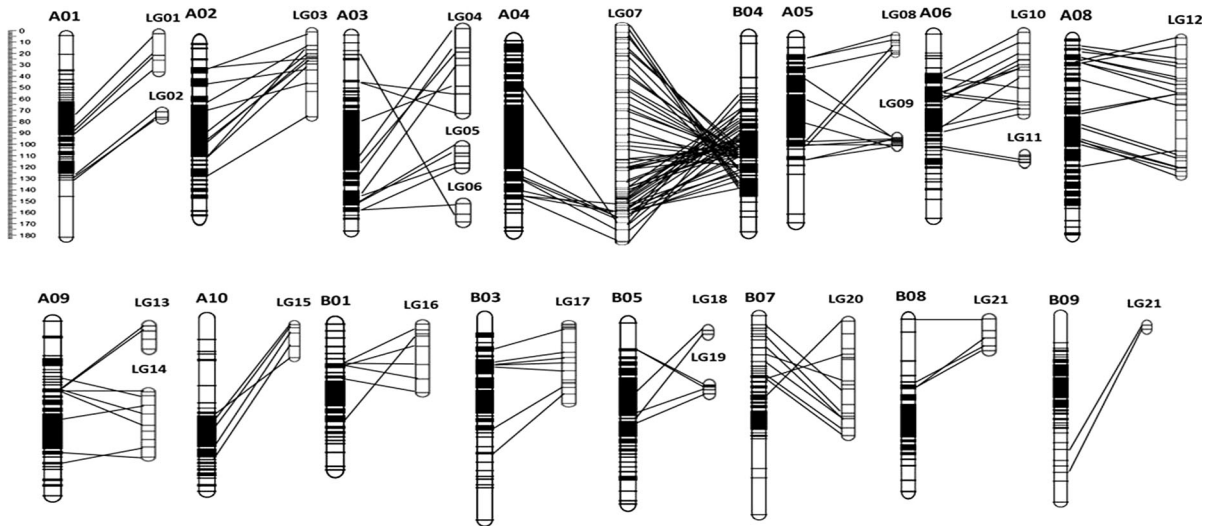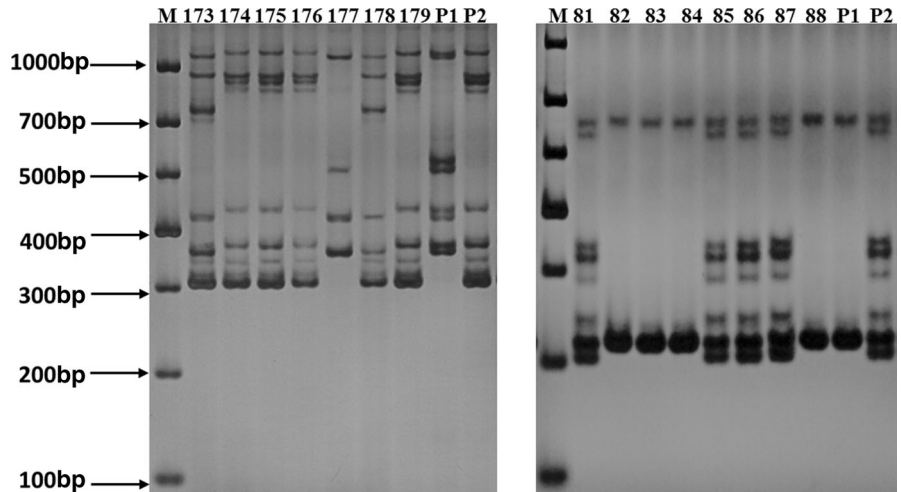

Fig. 6 The alignment markers of the linkage map (LG01–LG21) in this study and the integrated consensus map (A01–A10 and B01–B10) developed by Shirasawa et al. (2013)

parents was relatively close, so with the exception of the detected site in the experiment, the other genetic loci had high similarity. In the germplasm population with complex genetic background, there may be other main-effect genetic loci with effects on the phenotypic values of MSH and LBL.

Conclusions

A genetic map of cultivated peanut was constructed, consisting of 231 markers with total length of 905.18 cM and average marker interval of 3.92 cM. A total of 27 QTLs were detected in six environments: 11 QTLs were related to MSH and 16 QTLs were related to LBL, with PVE of 5.89–22.53%. A total of 7 QTLs were detected in two or more environments, including 3 QTLs for MSH and 4 QTLs for LBL. Two main-effect QTLs with stable heredity and environment insensitivity, Qmsh-14-3/Qllb-14-4 and Qllb-11-1, were identified. The physical map positions corresponding to the marker regions of Qmsh-14-3/Qllb-14-4 and Qllb-11-1 were predicted using the genome physical map of *A. duranensis*. The effects of flanking

markers of main-effect QTLs, Qmsh-14-3/Qllb-14-4 and Qllb-11-1, on MSH and LBL were validated in the germplasm population of peanut. The results provide references for fine mapping of QTLs for MSH and LBL, as well as breeding optimum plant-types in peanut.

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