RESEARCH ARTICLE

Quantitative Trait Locus Analysis of Late Leaf Spot Resistance and Plant-Type-Related Traits in Cultivated Peanut (*Arachis hypogaea* L.) under Multi-Environments

Xiaojing Zhou[1], Youlin Xia[2], Junhua Liao[2], Kede Liu[3], Qiang Li[4], Yang Dong[1], Xiaoping Ren[1], Yuning Chen[1], Li Huang[1], Boshou Liao[1], Yong Lei[1], Liying Yan[1], Huifang Jiang[1]*

1 Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan, Hubei, China, 2 Nanchong Academy of Agricultural Sciences, Nanchong, Sichuan, China, 3 National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei, China, 4 Department of Plant Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

* peanutlab@oilcrops.cn

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Late leaf spot (LLS) is one of the most serious foliar diseases affecting peanut worldwide leading to huge yield loss. To understand the genetic basis of LLS and assist breeding in the future, we conducted quantitative trait locus (QTL) analysis for LLS and three plant-type-related traits including height of main stem (HMS), length of the longest branch (LLB) and total number of branches (TNB). Significant negative correlations were observed between LLS and the plant-type-related traits in multi-environments of a RIL population from the cross Zhonghua 5 and ICGV 86699. A total of 20 QTLs were identified for LLS, of which two QTLs were identified in multi-environments and six QTLs with phenotypic variation explained (PVE) more than 10%. Ten, seven, fifteen QTLs were identified for HMS, LLB and TNB, respectively. Of these, one, one, two consensus QTLs and three, two, three major QTLs were detected for HMS, LLB and TNB, respectively. Of all 52 unconditional QTLs for LLS and plant-type-related traits, 10 QTLs were clustered in five genetic regions, of which three clusters including five robust major QTLs overlapped between LLS and one of the plant-type-related traits, providing evidence that the correlation could be genetically constrained. On the other hand, conditional mapping revealed different numbers and different extent of additive effects of QTLs for LLS conditioned on three plant-type-related traits (HMS, LLB and TNB), which improved our understanding of interrelationship between LLS and plant-type-related traits at the QTL level. Furthermore, two QTLs, *qLLSB6-7* and *qLLSB1* for LLS resistance, were identified residing in two clusters of NB-LRR—encoding genes. This study not only provided new favorable QTLs for fine-mapping, but also suggested that the relationship between LLS and plant-type-related traits of HMS, LLB and TNB should be considered while breeding for improved LLS resistance in peanut.

Introduction

Peanut or groundnut (*Arachis hypogaea* L.) is an important source of edible plant oil and protein and is cultivated in more than 100 countries. The total global production was 42.4 million tonnes and average yield was 1,653 kg ha^{-1} in 2014 (FAO, 2016) [1]. There is always a big gap between genetic potential of modern cultivars and their actual yield in the field. Yield losses are generally substantial when the crop is attacked by peanut diseases [2,3]. Late leaf spot (LLS) is one of the most serious foliar diseases caused by fungi [*Phaeoisariopsis personata*(Berk & M. A. Curtis)Arx] affecting peanut worldwide [4,5]. Peanut plants infected with this disease exhibit defoliating nature and result in over 50% yield loss [6]. Traditionally, LLS disease is controlled by fungicides, which are costly and toxic to the environment, therefore, the development of host-plant resistant cultivars has been considered as the primary strategy for controlling LLS disease.

Many important traits in agriculture are complex genetic behavior [7–9]. Genetic studies on LLS resistance suggest that resistance to this fungal disease is complex and polygenic in nature and sensitive to environments [10–15]. It has been long known that environment factors, such as temperature and humidity, are important affecting infection and development of *Phaeoisariopsis personata* [16–18]. In the field, a microclimate in which LLS may develop was directly influenced by plant-type-related traits. Several studies indicated associations between disease resistance and plant-type-related traits [19–21]. However, the genetic basis of LLS and relationship between LLS and plant-type-related traits in peanut are poorly understood.

QTL mapping is essential for identifying the genomic regions that control traits of interest before deploying linked markers in the breeding using marker-assisted selection (MAS) and also facilitate gene mining [22–25]. In recent years, a number of QTLs for LLS resistance have been identified in peanut. Khedikar et al. (2010) identified 11 minor QTLs for LLS using a partial linkage map comprising 56 SSR loci [14]. Sujay et al. (2012) identified 13 QTLs including five major QTLs for LLS in TAG 24 × GPBD 4 (RIL-4) population, and 15 QTLs including eight major QTLs for LLS in TG 26 × GPBD 4 (RIL-5) population, using the linkage maps with 188 and 181 loci in RIL-4 and RIL-5, respectively [15]. Based on a consensus map of these two populations, two candidate genomic regions conferring resistance to LLS were reported [15]. In the case of plant-type-related traits of height of the main stem (HMS), length of the longest branch (LLB) and total number of branches (TNB), only a few QTLs have been identified so far. Huang et al. (2014) identified three minor QTLs for HMS and two minor QTLs for TNB by using a genetic linkage map containing 470 simple sequence repeat (SSR) loci for cultivated peanut [26]. Shirasawa et al. (2012) identified three QTLs for HMS; two QTLs for LLB and one QTL for TNB with a genetic map containing 1,114 loci [27]. A major limitation of these studies was that the analyses were based on linkage maps mainly using simple sequence repeat (SSR) markers in which many regions were sparsely represented, thus it was not possible to obtain precise information about the numbers and locations of the QTLs. On the other hand, although the genetic linkage maps based on abundant single nucleotide polymorphism (SNP) markers could greatly increase marker density, there were rare cases of completeness and the maps have not been applied in QTL research in peanut yet [28,29].

Our previous study reported development of a SNP-based genetic linkage map consisting of 1,685 loci using the RIL population of Zhonghua 5 × ICGV 86699 [29]. In present study, we conducted QTL identification for resistance to LLS and the plant-type-related traits of HMS, LLB and TNB in multi-environments using this linkage map to investigate the genetic basis of LLS and the interrelationship between LLS and the plant-type-related traits.

Materials and Methods

Ethics statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Plant material and field design

A RIL population including 166 lines derived from a cross between maternal parent of Zhonghua 5 and paternal parent of ICGV 86699 was employed in this study for mapping and trait analysis. Zhonghua 5 is a popular cultivar in China that is susceptible to LLS [29,30]. ICGV 86699 is a breeding variety which is highly resistant to LLS. ICGV 86699 has wild-derived source of LLS resistance and its pedigree is [*Arachis batizocoi*/*A. duranensis*//*A. hypogaea* cv. NC 2]—CS 29 [31]. In addition, the two parents have significant differences in HMS, LLB, TNB and other traits such as insect resistance and other disease resistances [31].

The RIL lines were grown using a randomized complete block design with two replications at two experimental stations. One is located in the experimental farm at Oil Crops Research Institute (OCRI) of Chinese Academy of Agricultural Sciences (CAAS), Wuhan (WH), Hubei, China (30˚35'N, 114˚33'E). The other experimental station is located at Nanchong Academy of Agricultural Sciences, Nanchong (NC), Sichuan, China (30˚80'N, 106˚06'E). The seeds were sown at the end of April of each experimental year. Each accession was planted in a single row, with 8–10 plants in each row and 10 cm intervals between plants within each row and 30 cm intervals between rows. The parental genotypes were also sown after every 50 rows as controls.

Phenotypic observation

The parents and 166 RIL lines were used to assess LLS resistance and plant-type-related traits of HMS, LLB and TNB. For LLS, phenotyping was carried out in NC in year 2011 (NC11), year 2012 (NC12), year 2013 (NC13), and in WH in year 2012 (WH12) and year 2013 (WH13). The traits of HMS, TNB and LLB were investigated in two consecutive years (2012 and 2013) in NC and WH. Each location and year combination was considered as individual experimental environment.

The investigation of LLS in NC was under natural environment as this region belongs to the special basin terrain and is easy for the occurrence and epidemic of LLS disease. The average lowest ~ highest temperature, average day length, and average humidity of experiment periods of 2011–2013 in NC presented as following: 22.4~30.2˚C/138.9h/88% (June, 2011), 24.3~31.6˚C/185.4h/86% (July, 2011), 25.8~34.3˚C/197.0h /83% (August, 2011); 21.6~28.5˚C/ 129.7h/85% (June, 2012), 24.3~31.1˚C/136.9h/83% (July, 2012), 25.3~33.7˚C/183.0/82% (August, 2012); 23.3~32.1˚C/135.5h/80% (June, 2013), 26.1~33.6˚C/187.3h/79% (July, 2013), 25.6~33.5˚C/191.3h/80% (August, 2013). While LLS in WH was created using artificial disease epiphytotic since the region is not conducive to the epidemic of LLS disease. Methods for isolating LLS conidia and inoculating field plants were conducted as described in Khedikar et al. (2010) [14]. The average lowest ~ highest temperature, average day length, and average humidity of experiment periods of 2012–2013 in WH were as following: 22.8~31.4˚C/192h/81% (June, 2012), 26.9~34.4˚C/196h/77% (July, 2012), 25.1~32.3˚C/198h/69% (August, 2012); 22.5~30.8˚C/200.5h/81% (June, 2013), 27.4~34.6˚C/287.5h/72% (July, 2013), 26.9~35.1˚C/ 281.7h/69% (August, 2013). After 120 days of sowing, severity of LLS was assessed using a 1 to 9 severity scale in both places [32].

Height of main stem, length of the longest branch and total number of branches were measured at the harvesting stage (after 130 days of sowing). The unit of measured values of HMS

and LLB was centimeter (cm). TNB was counted by the number of branches. A schematic diagram of HMS and LLB was shown in S1 Fig.

Statistical analysis

The broad-sense heritability was calculated according to Hallauer and Miranda (1998) [33] as $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{ge} / n + \sigma^2_e / nr)$, where σ^2_g was the genetic variance, σ^2_{ge} was the interaction variance of the genotype with environment, σ^2_e was the variance of residual error, n was the number of environments and r was the number of replications. The estimation of the variance components was obtained using the SAS software by general linear model (GLM) procedure [34]. Correlation coefficients between traits across environments were estimated using the PROC CORR procedure of SAS.

Unconditional QTL mapping

Detection of QTL in each environment was performed by using the composite interval mapping (CIM) program of the Windows QTL Cartographer 2.5 [35]. The forward regression method model 6 (default model) was selected to obtain covariates. The number of control markers, window size and walk space were set to 5, 10 and 2 cM, respectively. A LOD threshold of 2.5 was chosen to declare a putative QTL as significant [36]. QTLs repeatedly identified with clearly similar positions (overlapping 1-LOD confidence intervals) for the same trait in different environments were integrated into a consensus QTL [37]. QTLs detected for different traits with overlapped confidence intervals and common marker(s), or couples of overlapped QTLs with distance less than 2 cM was defined as a QTL cluster in which at least one major QTL with PVE >10% was included [37]. Average values of two replicates for each trait in a single environment were used as phenotypic data for single environment QTL analysis.

Joint QTL was analyzed by mixed linear model-based composite interval mapping (MCIM) of QTLNetwork software version 2.0 [38]. Phenotypic values of each trait in all environments were averaged for joint QTL analysis. The LOD thresholds were determined by a 1,000 permutation test at a 95% confidence level. The nomenclature of QTL was similar to that described by McCouch et al. (1997) [39]. QTL was designated with initial letter "q" followed by trait name, linkage groups and serial number (if more than one QTL of the same trait exist in the same chromosome), respectively.

Conditional QTL mapping

The phenotypic values for conditional QTL analysis was showed as y (T1|T2), where T1|T2 indicated that trait 1 conditioned on trait 2. y (T1|T2) was obtained by the mixed model approach using software QGAStation 2.0 (http://ibi.zju.edu.cn/software/qga/index.htm) [40,41]. In this study, the correlation analysis based on the phenotypic data showed significant negative correlation between LLS and plant-type-related traits. The QTL-clusters showed that the QTLs controlled different traits were mapped on the same genomic region. To further investigate the genetic interrelationship between LLS resistance and plant-type-related traits, we performed conditional QTL mapping with correlated traits as proposed by Zhao et al. (2006) [42]. Conditional phenotypic values including LLS|HMS, LLS|LLB and LLS|TNB in the four environments (NC12, NC13, WH12 and WH13) were calculated. Conditional QTL mapping was performed by the composite interval mapping method as described above for unconditional QTLs.

Fig 1. Phenotypes of two parental inbred lines used for QTL mapping in this study. Plants were photographed at 120 days after sown on soil. Left two lines were Zhonghua 5, right two lines were ICGV 86699. The picture of top left corner showed infected leaves by late leaf spot.

doi:10.1371/journal.pone.0166873.g001

Results

Trait performance

The two parents, Zhonghua 5 and ICGV 86699, showed significant differences (P < 0.001) for LLS and plant-type-related traits of HMS, LLB and TNB. ICGV 86699 consistently exhibited lower disease score of LLS and higher values of the three plant-type-related traits than Zhonghua 5 (Fig 1, Table 1). The RIL individuals showed a continuous distribution for all traits across different environments (S2 Fig), which suggested a quantitative inheritance pattern for the investigated traits.

ANOVA of all four traits across environments indicated that genotype (G), environment (E) and genotype-environment interactions (G×E) had significant effects on the traits. The four traits (LLS, HMS, LLB and TNB) showed high broad-sense heritability of 86.9%, 75.0%, 77.5% and 82.2%, respectively (Table 2), which suggested that genetic factors played a major role in the expression of these traits with significant environmental variation affecting these traits. To pinpoint their relationships, correlation coefficients (*r*) between the traits across environments were calculated (Table 3). The disease score of LLS of the RIL population exhibited significant (P < 0.05) negative correlations with three plant-type-related traits in all environmental cases, except in NC13 which had no significant phenotypic correlation between LLS and HMS. Significant positive relationships have been found between different plant-type-related traits, especially for HMS and LLB which showed the strongest correlation.

QTL analysis for LLS and plant-type-related traits

To identify QTLs for LLS, HMS, LLB and TNB, phenotypic data were analyzed together with genetic mapping data of 1,685 markers [29] (S1 Table). A total of 52 significant QTLs were identified for all four traits through single-environment QTL analysis (Table 4, S3 Fig). The QTLs were distributed on 15 chromosomes and explained 3.41% to 19.12% phenotypic variation. Six QTLs (qLLSA1-3, qLLSB6-2, qHMSA9-2, qLLBA9-2, qTNBA8 and qTNBB6-3) were identified in multiple environments. Joint QTL analysis detected 18 putative QTLs associated with the four traits (Table 4, S2 Table) and 12 of these QTLs were coincident with the results of single-environment mapping (Table 4).

Late leaf spot resistance. A total of 20 QTLs controlling LLS were identified in all investigated environments (Table 4, S3 Fig) and were mapped on chromosomes A1, A4, A5, A6, A8,

Table 1. Phenotypic performance of the four traits in parents and RIL lines under multi-environments.

Trait	Env.	Zhonghua 5	ICGV 86699	Population					
				Mean	SD	Min	Max	Skew	Kurt
LLS	NC11	8.3	1.0	5.1	1.8	1.0	9.0	0.01	-0.81
	NC12	9.0	2.7	5.3	1.9	1.3	9.0	0.41	-0.78
	NC13	8.0	1.3	5.8	1.9	1.3	9.0	-0.42	-0.66
	WH12	8.1	2.3	5.3	2.0	1.7	9.0	-0.06	-0.94
	WH13	8.0	2.7	5.0	2.5	1.0	9.0	0.08	-0.89
HMS	NC12	27.3	51.6	38.9	8.1	21.2	68.4	0.69	0.96
	NC13	50.4	82.3	62.6	11.2	36.8	105.7	0.98	1.39
	WH12	47.2	80.4	56.9	12.0	23.8	89.6	0.11	-0.55
	WH13	42.3	72.2	59.7	13.6	35.0	97.9	0.26	-0.71
LLB	NC12	46.3	70.1	53.6	9.7	35.8	90.4	0.77	0.87
	NC13	56.2	94.3	73.9	10.7	48.5	106.7	0.6	0.11
	WH12	42.2	91.2	65.3	13.9	35.0	102.0	0.05	-0.58
	WH13	50.4	80.4	69.6	15.5	41.5	112.8	0.33	-0.61
TNB	NC12	11.6	45.6	18.7	9.1	8.2	51.8	0.98	0.96
	NC13	13.2	33.6	18.7	6.5	8.7	45.4	0.80	0.97
	WH12	10.0	28.2	18.0	6.6	6.8	42.7	0.98	0.99
	WH13	11.5	29.4	18.2	9.9	6.0	55.7	1.05	1.12

Zhonghua 5: maternal parent; ICGV86699: paternal parent.

LLS (late leaf spot disease) was assessed by 1–9 scale disease score; HMS (height of main stem) and LLB (length of the longest branch) were measured in the unit of centimeter (cm); TNB (total number of branches) was counted by the number of branches.

Env. represents environment; NC11, NC12, NC13, WH12 and WH13 represent Nanchong in 2011, 2012, 2013respectively; WH12 and WH13 represent Wuhan in 2012 and 2013, respectively.

SD: standard deviation.

doi:10.1371/journal.pone.0166873.t001

Table 2. Variance (ANOVA) Analysis and the broad-sense heritability (h^2) for the four traits of RIL lines in multi-environments.

Trait	Source	DF	Sum of square	Mean square	F value	P	h^2
LLS	Genotype	165	4155.82	25.19	49.3	< .0001	86.86
	Environment	4	180.05	45.01	88.1	< .0001	
	Genotype×Environment	654	2164.65	3.31	6.48	< .0001	
	Error	1154	589.62	0.51			
HMS	Genotype	165	133791.44	810.86	22.05	< .0001	74.95
	Environment	3	73007.51	24335.84	661.87	< .0001	
	Genotype×Environment	493	100121.08	203.09	5.52	< .0001	
	Error	982	36106.39	36.77			
LLB	Genotype	165	172190.66	1043.58	17.56	< .0001	77.5
	Environment	3	56379.56	18793.19	316.2	< .0001	
	Genotype×Environment	493	115751.2	234.79	3.95	< .0001	
	Error	983	58424.8	59.46			
TNB	Genotype	165	66507.18	403.07	18.31	< .0001	82.2
	Environment	3	253.55	84.52	21.84	< .0001	
	Genotype×Environment	492	35278.45	71.7	3.26	< .0001	
	Error	974		22.01			

LLS: late leaf spot disease; HMS: height of main stem; LLB: length of the longest branch; TNB: total number of branches.

DF indicates degree freedom.

h^2 indicates the broad-sense heritability.

doi:10.1371/journal.pone.0166873.t002

Table 3. Correlation analysis for the four traits across multi-environments.

Trait	Env.	LLS	HMS	LLB
HMS	NC12	-0.22**		
	NC13	-0.14^{ns}		
	WH12	-0.56**		
	WH13	-0.19*		
LLB	NC12	-0.31**	0.84**	
	NC13	-0.19*	0.9**	
	WH12	-0.58**	0.96**	
	WH13	-0.21**	0.94**	
TNB	NC12	-0.55**	0.24**	0.42**
	NC13	-0.47**	0.23**	0.15*
	WH12	-0.22**	0.21**	0.23**
	WH13	-0.23**	0.24**	0.28**

Env. represents environments; NC12 and NC13 represent Nanchong in 2012 and 2013 respectively; WH12 and WH13 represent Wuhan in 2012 and 2013, respectively.

*, ** and ns indicate significance at $P < 0.05$, $P < 0.01$ and non-significant effect, respectively.

doi:10.1371/journal.pone.0166873.t003

A10, B1, B4, B6 and B10. Chromosome B6 had seven QTLs, the most number of QTLs of all chromosomes. Phenotypic variation explained by individual QTL ranged from 3.41% to 19.12% and by QTL together in single environment ranged from 25.58% to 62.38%. Among them, six QTLs (*qLLSA10*, *qLLSB6-1*, *qLLSB6-2*, *qLLSB6-4*, *qLLSB6-6* and *qLLSB6-7*) with over 10% PVE were considered as major QTLs. Two QTLs (*qLLSA1-3* and *qLLSB6-2*) were identified in multiple environments, including the major QTL *qLLSB6-2* which was accounted for 11.66–12.90% PVE with LOD value 7.81–8.92. In addition, LLS resistance alleles at all QTLs were contributed by ICGV 86699.

Height of main stem. For HMS, 10 QTLs distributed on six chromosomes (A5, A9, A10, B2, B3 and B6) were identified. They individually accounted for 4.76–15.28% of the phenotypic variation and totally explained 16.79–45.61% of the HMS variation in each environment (Table 4, S3 Fig). Three major QTLs (*qHMSA5-1*, *qHMSA5-2* and *qHMSA9-1*) were identified. The *qHMSA9-2* was a consensus QTL detected in WH12 and WH13. The *qHMSA9-2* together with two environment-specific QTLs (*qHMSB3-2* and *qHMSB6*) was also identified by joint analysis. The alleles for increasing HMS value at nine QTL regions were contributed by ICGV 86699, while the allele for increasing trait value at one QTL region was contributed by Zhonghua 5.

Length of the longest branch. Seven QTLs influencing LLB were identified on chromosomes A9, B1, B3, B6 and B8. They individually displayed 4.00% to 11.45% PVE and totally displayed 6.86–24.90% cumulative PVE for LLB in each environment (Table 4, S3 Fig). Two major QTLs (*qLLBA9-1* and *qLLBA9-2*) were identified. One of the major QTL *qLLBA9-2* explained 5.55% and 11.45% of the phenotypic variation in the WH12 and WH13 environments, respectively. Six QTLs were environment-specific. The alleles for increasing LLB value at all QTL regions were contributed by ICGV 86699.

Total number of branches. A total of 15 QTLs for TNB were identified on chromosomes A3, A5, A6, A8, A10, B1, B3, B4, B6 and B8 (Table 4, S3 Fig). They individually displayed 4.59–13.44% PVE with cumulative PVE of 11.96 to 61.84% for TNB across environments. Two QTLs (*qTNBA8* and *qTNBB6-3*) were identified across multi-environments, and they were also identified in correspondence with joint analysis. Three major QTLs, *qTNBB4-3*, *qTNBB6-*

Table 4. QTLs identified for LLS and plant-type-related traits through single-environment QTL mapping using high density SNP-based map.

Trait	QTL[a]	Chr.[b]	Marker interval	Range (cM)	Env.[c]	Position	Additive[d]	PVE[e] (%)	LOD
LLS	qLLSA1-1	A1	Ahsnp978-Ahsnp275	30.3–33.5	NC13	32.01	0.46	5.12	3.47
	qLLSA1-2	A1	Ahsnp517-Ahsnp422	38–43.4	NC13	39.11	0.48	5.63	3.89
	qLLSA1-3	A1	Ahsnp1339-Ahsnp819	45.3–52	WH13	46.51	0.64	5.07	2.58
					NC13	48.51	0.49	5.11	3.32
	qLLSA4-1	A4	Ahsnp1681-Ahsnp1897	50.4–52.7	NC13	51.51	0.52	5.29	3.66
	qLLSA5	A5	Ahsnp1180-Ahsnp78	20.6–28.1	NC13	25.81	0.38	3.64	2.56
	qLLSA6-1*	A6	Ahsnp629-Ahsnp1883	11.4–14.6	NC12	13.71	0.39	3.41	2.85
	qLLSA6-2	A6	Ahsnp1843-Ahsnp1521	19.2–24.2	WH12	21.61	0.47	4.31	2.57
	qLLSA6-3	A6	Ahsnp419-Ahsnp418	31–35.8	WH12	33.61	0.67	7.6	3.96
	qLLSA8	A8	Ahsnp320-Ahsnp919	57–76.1	NC12	66.41	0.39	3.69	3.09
	qLLSA10	A10	Ahsnp1559-Ahsnp809	27.5–31.1	NC11	31.01	0.69	10.26	6.37
	qLLSB1*	B1	GM1331-Ahsnp1805	0–8.8	WH12	4.41	0.64	8.22	5.06
	qLLSB4-1*	B4	Ahsnp505-Ahsnp471	11.4–32.9	NC11	22.54	0.52	5.75	3.38
	qLLSB6-1*	B6	Ahsnp295-AHGS1422	31.8–40.1	NC11	35.36	0.74	13.89	8.17
	qLLSB6-2	B6	Ahsnp539-Ahsnp136	44.5–47	NC12	45.81	0.75	11.66	8.92
					NC11	45.81	0.74	12.9	7.81
	qLLSB6-3	B6	Ahsnp1070-Ahsnp1665	56–57.6	NC12	57.01	0.6	7.16	4.23
	qLLSB6-4	B6	Ahsnp676-Ahsnp231	62.18–64.62	NC13	63.61	0.89	18.47	10.79
	qLLSB6-5	B6	Ahsnp231-AHGS1431	64.9–67.8	WH13	65.31	0.69	6.33	2.9
	qLLSB6-6	B6	AHGS1431-Ahsnp163	68.4–71.5	NC13	71.01	0.91	19.12	11.25
	qLLSB6-7	B6	Ahsnp1166-Ahsnp996	73.3–78	WH13	74.51	1.03	14.18	6.85
	qLLSB10	B10	Ahsnp1458-Ahsnp299	5.3–8.6	NC11	7.51	0.44	4.1	2.73
HMS	qHMSA5-1	A5	Ahsnp1180-Ahsnp1133	21.6–25.7	NC13	23.71	-5.64	15.28	4.88
	qHMSA5-2	A5	Ahsnp1338-Ahsnp213	33.6–38	NC13	35.61	-4.81	10.66	3.46
	qHMSA9-1	A9	Ahsnp1103-Ahsnp1059	0–2.2	NC13	0.01	-3.91	10.73	4.98
	qHMSA9-2*	A9	Ahsnp902-Ahsnp1167	71.6–82.2	WH12	73.11	-3.08	5.39	3.06
					WH13	75.11	-3.36	5.22	2.68
	qHMSA10-1	A10	Ahsnp77-Ahsnp787	7.8–8.8	WH12	7.91	-3.22	5.26	2.98
	qHMSA10-2	A10	Ahsnp712-GM692	14.4–19.9	WH12	17.81	-3.31	6.14	3.49
	qHMSB2	B2	Ahsnp1331-Ahsnp1308	1.6–12.1	NC13	5.01	3.97	8.94	4.24
	qHMSB3-1	B3	Ahsnp586-Ahsnp1194	48–52.2	WH13	50.21	-3.39	5.39	2.93
	qHMSB3-2*	B3	Ahsnp1236-Ahsnp1549	59.7–61.8	WH13	60.81	-4.02	7.99	4.63
	qHMSB6*	B6	Ahsnp227-Ahsnp295	12.1–32.3	WH13	24.11	-3.22	4.76	2.78
LLB	qLLBA9-1	A9	Ahsnp1103-Ahsnp1059	0–2.4	NC13	0.01	-3.7	10.59	5.09
	qLLBA9-2	A9	Ahsnp902-Ahsnp1167	72.2–82.2	WH13	77.11	-4.05	5.55	2.75
					WH12	77.11	-5	11.45	5.8
	qLLBB1	B1	Ahsnp966-Ahsnp1373	28.2–31.1	WH12	30.91	-3.61	5.17	3.02
	qLLBB3	B3	Ahsnp1236-Ahsnp1549	59.3–61.8	WH13	60.81	-3.61	4.73	2.68
	qLLBB6*	B6	Ahsnp227-Ahsnp295	16.6–31.1	WH12	27.81	-2.93	4	2.55
	qLLBB8-1	B8	AHGS1388-Ahsnp1644	11.5–16	NC12	14.71	-2.86	6.86	3.25
	qLLBB8-2*	B8	Ahsnp779-Ahsnp732	62.6–73.4	WH12	66.31	-3.25	4.28	2.71
TNB	qTNBA3	A3	Ahsnp965-Ahsnp1014	35.7–39.2	WH12	36.81	-1.78	6.19	3.25
	qTNBA5	A5	Ahsnp926-Ahsnp236	15.7–17.8	WH12	17.51	-1.78	5.22	2.87
	qTNBA6-1	A6	Ahsnp418-AHGS0153	37.2–43.6	WH12	42.11	1.8	4.59	2.66
	qTNBA6-2	A6	Ahsnp19-Ahsnp1889	51.5–57.5	WH12	55.51	1.97	6.05	2.87
	qTNBA8*	A8	Ahsnp1305-Ahsnp1244	23.4–41.9	NC12	32.71	-2.32	5	2.76
					NC13	28.41	-1.63	5.34	3.06
					NC13	40.71	-1.6	4.96	2.7

(*Continued*)

Table 4. (*Continued*)

Trait	QTL[a]	Chr.[b]	Marker interval	Range (cM)	Env.[c]	Position	Additive[d]	PVE[e] (%)	LOD
	qTNBA10	A10	ARS775-Ahsnp1387	0–6.8	NC12	0.01	-2.61	6.96	3.81
	*qTNBB1**	B1	GM1331-Ahsnp642	0–2	WH13	0.01	-2.67	5.58	3.3
	qTNBB3	B3	AHGS1201-AHGS2526	12.6–25.9	WH13	17.51	3.98	5.32	3.56
	qTNBB4-1	B4	Ahsnp781-Ahsnp1160	46.8–47.8	WH13	46.81	-2.88	5.76	3.4
	qTNBB4-2	B4	Ahsnp207-Ahsnp232	54.7–56.6	WH13	55.61	-3.29	9.54	5.82
	qTNBB4-3	B4	Ahsnp282-Ahsnp1066	64.9–68.3	WH13	67.81	-3.67	11.85	6.48
	qTNBB6-1	B6	Ahsnp425-Ahsnp349	48.9–62.2	NC13	54.51	-2.32	9.8523	4.77
	qTNBB6-2	B6	Ahsnp1309-Ahsnp64	62.6–65	WH13	63.61	-3.58	10.35	5.92
	*qTNBB6-3**	B6	AHGS1431-Ahsnp690	69.5–71.5	NC13	71.01	-2.05	8.1	4.74
					WH13	71.01	-4.05	13.44	7.95
	qTNBB8	B8	Ahsnp1700-Ahsnp1055	10.1–14.2	WH12	10.81	-2.05	7.55	4.16

[a] The nomination of QTL is comprised of four parts. The first part "q" stands for QTL. The second part is the abbreviation of traits. The third part, the number stands for chromosome. The fourth part is the serial number of QTL on the same chromosome. The names followed by * were QTLs simultaneously detected by joint QTL analysis among all environments.

[b] Chr. indicates chromosome.

[c] Env. indicates environment. NC11, NC12, NC13 WH12 and WH13 represent Nanchong in 2011, Nanchong in 2012, Nanchong in 2013, Wuhan in 2012 and Wuhan 2013, respectively.

[d].Additive effect of the QTL.

[e] The phenotypic variation explained of corresponding QTL.

doi:10.1371/journal.pone.0166873.t004

2 and *qTNBB6-3* were identified, in which *qTNBB6-3* explained 8.10% and 13.44% of the phenotypic variation in NC13 and WH13, respectively. The alleles for increasing TNB value at twelve QTL regions were contributed by ICGV 86699, while the allele for increasing the trait value at three QTL regions were contributed by Zhonghua 5.

Clusters with co-located QTLs

Five QTL clusters were identified on chromosomes A5, A9 and B6 (Table 5, S3 Fig). Among them, three clusters overlapped between LLS and one of plant-typed traits. Clusters IV and V, both located on chromosome B6, included QTL for LLS co-located with QTL for TNB. It is worth mentioning that four QTLs (*qLLSB6-4* and *qTNBB6-2*, *qLLSB6-6* and *qTNBB6-3*) in the two clusters were all displayed PVE >10%, in which one QTL (*qTNBB6-3*) was detected in multi-environments. QTL for LLS overlapped with QTL for HMS located on A5. These results indicated that obviously genetic correlation between LLS and plant-type-related traits. In addition, clusters II and III showed co-located QTLs for HMS and LLB on chromosomes A9.

Conditional analysis

Conditional analysis was performed to determine genetic relationship between LLS and plant-type-related traits. We identified 21 conditional QTLs for LLS distributed on chromosomes A1, A2, A3, A4, A6, B2, B3, B4 and B6 in four environments (NC12, NC13, WH12 and WH13). Of the 21 conditional QTLs, 10 were also detected as unconditional QTLs and 11 were detected as new QTLs (Table 6). Six unconditional QTLs were not recaptured under conditional analysis (Table 6).

Conditional QTL mapping of LLS|HMS revealed 12 QTLs. Nine marker intervals encompassed both unconditional and conditional QTLs, of which five (*qLLSA1-2*, *qLLSA1-3*, *qLLSB6-4*, *qLLSB6-6* and *qLLSB6-7*) were detected in multiple environments. Furthermore, the

Table 5. QTL clusters for four traits examined in this study.

QTL cluster No.	Chr.	Trait included	QTL	Marker interval	Range (cM)	Env.	Pos.	PVE (%)	A
I	A5	LLS+HMS	qLLSA5	Ahsnp1180-Ahsnp78	20.6–28.1	NC13	25.81	3.64	-0.38
			qHMSA5-1	Ahsnp1180-Ahsnp1133	21.6–25.7	NC13	23.71	15.28	5.64
II	A9	HMS+LLB	qHMSA9-1	Ahsnp1103-Ahsnp1059	0–2.2	NC13	0.01	10.73	3.91
			qLLBA9-1	Ahsnp1103-Ahsnp1059	0–2.4	NC13	0.01	10.59	3.70
III	A9	HMS+LLB	qHMSA9-2	Ahsnp902-Ahsnp1167	71.6–82.2	WH12	73.11	5.39	3.08
						WH13	75.11	5.22	3.36
			qLLBA9-2	Ahsnp902-Ahsnp1167	72.2–82.2	WH12	77.11	11.45	5
						WH13	77.11	5.55	4.05
IV	B6	LLS+TNB	qLLSB6-4	Ahsnp676-Ahsnp231	62.18–64.62	NC13	63.61	18.47	-0.89
			qTNBB6-2	Ahsnp1309-Ahsnp64	62.6–65	WH13	63.61	10.35	3.58
V	B6	LLS+TNB	qLLSB6-6	AHGS1431-Ahsnp163	68.4–71.5	NC13	71.01	19.12	-0.91
			qTNBB6-3	AHGS1431-Ahsnp690	69.5–71.5	NC13	71.01	8.1	2.05
						WH13	71.01	13.44	4.05

Chr. represents chromosome.

PVE indicates the percentage of phenotypic variation explained by corresponding QTL.

A represents the additive effect.

doi:10.1371/journal.pone.0166873.t005

three QTLs (*qLLSB6-4*, *qLLSB6-6* and *qLLSB6-7*) were also detected with PVE over 10%. Of the nine QTLs detected in both strategies, five conditional QTLs changed additive effects significantly and four conditional QTLs showed similar additive effects compared to the corresponding unconditional QTLs. Three new QTLs for LLS|HMS were detected and seven unconditional QTLs were not identified again.

Conditional QTL mapping of LLS|LLB identified 11 QTLs. Nine were detected with the same marker intervals as unconditional QTLs, and four of them (*qLLSA1-2*, *qLLSB6-4*, *qLLSB6-6* and *qLLSB6-7*) were detected in multiple environments. When LLS was conditioned on LLB, *qLLSB6-4* displayed 10.85% and 19.41% PVE in NC12 and NC13, respectively. The *qLLSB6-6* was also identified at both NC12 and NC13 and displayed 14.16% and 22.55% PVE, respectively. These two QTLs were also detected as major QTLs under the unconditional QTL mapping. Of the nine QTLs detected in both strategies, five conditional QTLs changed additive effects significantly and the other four showed similar effects to the unconditional ones. Two extra conditional QTLs identified for LLS and seven QTLs were detected only under unconditional situation.

A total of 13 conditional QTLs of LLS|TNB were detected. Six conditional QTLs were also detected by unconditional mapping. Among them, two QTLs (*qLLSA1-3* and *qLLSB6-6*) were detected in multiple environments, but none of them were detected with PVE more than 10%. Of the six QTLs detected in both strategies, five conditional QTLs changed additive effects obviously and one conditional QTL showed similar additive effect to their corresponding unconditional QTLs. Conditioned on TNB, however, 10 unconditional QTLs were not identified again, but seven extra QTLs were detected.

Discussion

QTL analysis has been well documented to dissect the genetic basis of important, complex traits in cultivated peanut [43–49]. However, due to the nature of low genetic diversity and polyploidy in peanut, precisely locating the QTLs controlling the traits of interest is still challenging. In this study, we conducted QTL mapping using our previously constructed genetic

Table 6. The unconditional and conditional QTLs for LLS in the four environments (NC12, NC13, WH12, WH13).

QTL	Marker interval	LLS			LLS|HMS			LLS|LLB			LLS|TNB		
		Env.	A	PVE	Env.	A	PVE	Env.	A	PVE	Env.	A	PVE
qLLSA1-1	Ahsnp978-Ahsnp275	NC13	0.46	5.12	NC13	0.47	6.12	NC13	0.55	8.05			
qLLSA1-2	Ahsnp517-Ahsnp422	NC13	0.48	5.63	NC13	0.5	6.91	NC13	0.63	9.99	NC13	0.48	6.24
					NC12	0.46	5.67	NC12	0.4	4.57			
qLLSA1-3	Ahsnp1339-Ahsnp819	NC13	0.49	5.11	NC13	0.45	4.99	NC13	0.57	7.21	NC13	0.63	9.46
		WH13	0.64	5.07	NC12	0.45	4.98				NC12	0.46	5.93
qLLSA1-4	Ahsnp89-Ahsnp1913										WH13	0.8	7.7
qLLSA2	AHGS1495-Ahsnp1397							WH13	0.82	7.52			
qLLSA3	Ahsnp1321-Ahsnp988				NC12	0.41	3.8				WH12	0.52	4.57
qLLSA4-1	Ahsnp1681-Ahsnp1897	NC13	0.52	5.29				NC13	0.51	6.05	NC13	0.56	7.92
qLLSA4-2	Ahsnp237-Ahsnp667							NC12	0.4	4.01			
qLLSA5	Ahsnp1180-Ahsnp78	NC13	0.38	3.64									
qLLSA6-1	Ahsnp629-Ahsnp1883	NC12	0.39	3.41									
qLLSA6-2	Ahsnp1843-Ahsnp1521	WH12	0.47	4.31	WH12	0.49	4.5				WH12	0.4	5.2
qLLSA6-3	Ahsnp419-Ahsnp418	WH12	0.67	7.6	WH12	0.67	8	WH12	0.65	7.8	WH12	0.68	8.0
qLLSA6-4	Ahsnp1731-Ahsnp1889										NC12	0.42	4.86
qLLSA8	Ahsnp320-Ahsnp919	NC12	0.39	3.69									
qLLSB1	GM1331-Ahsnp1805	WH12	0.64	8.22									
qLLSB2-1	Ahsnp1281-Ahsnp1308				WH13	1.01	12.34						
qLLSB2-2	Ahsnp784-Ahsnp1260				WH13	0.81	8.39						
qLLSB2-3	Ahsnp784-Ahsnp1694										WH13	0.77	8.4
qLLSB3	AHGS1201-AHGS1661										WH13	0.87	8.72
qLLSB4-2	Ahsnp108-Ahsnp789										WH12	0.54	6.29
qLLSB4-3	Ahsnp239-Ahsnp929										WH12	0.57	7.26
qLLSB6-2	Ahsnp539-Ahsnp136	NC12	0.75	11.66									
qLLSB6-3	Ahsnp1070-Ahsnp1665	NC12	0.6	7.16									
qLLSB6-4	Ahsnp676-Ahsnp231	NC13	0.89	18.47	NC13	0.71	11.87	NC13	0.85	19.41			
					NC12	0.7	11.32	NC12	0.67	10.85			
qLLSB6-5	Ahsnp231-AHGS1431	WH13	0.69	6.33	WH13	0.52	5.74	WH13	0.47	4.68			
qLLSB6-6	AHGS1431-Ahsnp163	NC13	0.91	19.12	NC13	0.76	13.89	NC13	0.92	22.55	NC13	0.44	5.42
					NC12	0.77	14.42	NC12	0.74	14.16	NC12	0.44	5.2
qLLSB6-7	Ahsnp1166-Ahsnp996	WH13	1.03	14.18	WH13	1.66	11.15	WH13	0.91	8.98			
					WH12	0.55	6.86	WH12	0.53	6.5			

A indicates the additive effect of the QTL.

PVE: percentage of phenotypic variance explained by the QTL.

LLS|HMS: late leaf spot conditioned on height of main stem.

LLS|LLB: late leaf spot conditioned on length of the longest branch.

LLS|TNB: late leaf spot conditioned on total number of branches.

doi:10.1371/journal.pone.0166873.t006

map with the highest number of markers (1,685 marker loci) among so far published population-specific linkage maps for tetraploid peanut [29]. Totally, we detected 20 QTLs for LLS and 32 QTLs for three plant-type-related traits. The number of QTLs was much more than the published QTL mapping in a single population of peanut [26,44], suggesting the detection power of QTL mapping was significantly improved.

QTL analysis in single environment is limited in predicting QTL positions and the stability of QTLs as well as the magnitude of genetic effects on target traits [50,51]. In addition, QTL

that has obvious genetic effect could be preferentially identified in different environments. In this study, we performed QTL analysis based on phenotypic data from multiple environments. Although most of detected loci were environment-specific, six QTLs were identified in multiple environments and hence were stable in phenotypic expression. For example, a QTL for LLS resistance, namely *qLLSB6-2*, flanked by Ahsnp539 and Ahsnp136, was expressed with 11.66% PVE in NC12 and with 12.9% PVE in NC11. And a QTL for TBN, namely *qTNBB6-3*, flanked by AHGS1431 and Ahsnp690, was expressed with 8.1% PVE in NC13 and with 13.44% PVE in WH13. Due to the limitation of experimental material in the present study, the identified major QTLs and the markers linked the studied traits are needed further validation before they are deployed in MAS.

For LLS resistance, we identified 20 QTLs. Of which, six QTLs showed PVE over 10%, and other QTLs were basically significant loci with relatively lower phenotypic contributions. These results indicated that the resistance to LLS in cultivated peanut was controlled by several major QTLs and many minor QTLs, consistent with previous research results [15,46]. When comparing the QTLs for LLS in this study with previous studies (S3 Table) [14,15,46,52,53,54], six major and 10 minor QTLs for LLS in our study were shown to be novel QTLs. This could be due to different genetic backgrounds of the used materials. We also found that the physical region of *qLLSA1-2* was involved in a previously identified QTL *qF2LS3* [46], and *qLLSA4-1* was involved in QTL$_{LLS}$09 which reported by Khedikar et al. (2010) [14]. The physical region of *qLLSB6-3* was overlapped with QTL$_{R5-LLS}$13 in other study [15], *qLLSB10* overlapped with reported QTLs of QTL$_{LLS}$11 and *qF2LS2* [14,46]. Therefore, these four QTLs were repeatedly detected in different populations. With respect to the QTLs for plant-type-related traits, no similar segment was found between our study and previously studies [26,27,55,56].

Nucleotide-binding—leucine-rich repeat (NB-LRR)-encoding genes usually confer resistance against pests and diseases. Bertioli et al. (2016) [57] identified 345 and 397 of these genes in the *A. duranensis* and *A. ipaensis* genotypes, which serve as proxies for the A and B subgenomes of peanut, respectively. We therefore compared the physical locations of QTLs for LLS resistance in our study with the NB-LRR—encoding genes and found five QTLs for LLS resistance were associated with these genes (S4 Table). A major QTL *qLLSB6-7* for resistance to LLS identified on B6 of ICGV 86699 [31] resides in a cluster of six NB-LRR—encoding genes covering 3.9 Mb. Another QTL *qLLSB1* was identified on a segment that covering 8.9 Mb of chromosome B1 which contains five NB-LRR—encoding genes. The genes harbored on these genome segments provide good disease resistance and warrant further investigation.

In the RIL population of Zhonghua 5 × ICGV 86699, the LLS resistance alleles at 20 QTL regions were contributed by ICGV 86699. This level of resistance to LLS has never been identified in "pure" cultivated peanut and in order to widen primary genetic pool, wild species were used [58–62]. From the pedigree of ICGV 86699, we knew that the resistance to LLS was introgressed from wild species of *A. duranensis* and *A. batizocoi* [31]. Due to that genome fragments from wild peanut were transferred into cultivated peanuts, this population therefore had high genetic variation and the map had so many polymorphic markers [29]. In the previous studies of QTL mapping for resistance to LLS, GPBD 4 was usually used as a resistant parent [14,15,53]. The source of resistance of GPBD 4 can be traced back to wild species of *A. cardenasii* [63], which is different from this study.

QTL cluster is an important central concept in genetical genomics [64] and associative traits tend to share regions with QTLs [65–67]. Several studies in plants have mapped QTLs for disease resistances which overlap with those for plant-type-related traits [20,21,68,69]. In an overview of QTL distribution in this study, 10 QTLs including eight major QTLs were clustered in five genetic regions on chromosomes A5, A9 and B6. The genomic regions of QTL cluster IV and V harbored co-localized QTLs for LLS and TNB while cluster I harbored co-

localized QTL for LLS and HMS. These results provided evidence that correlation of LLS and plant-type-related traits could be genetically constrained. In peanut, Shoba et al. (2013) [50] found that markers PM 384, pPGPseq5D5, PM 3, PMc 588 and PM 343 associated with number of branches and LLS severity score through single marker analysis, also suggesting genetic correlation existed between LLS and plant type. The genetic regions with clustered robust QTLs of this study are worth of further investigation due to the importance of genetic control of LLS as well as plant type.

The conditional analysis provides an efficient method for dissecting the genetic interrelationship between traits at individual QTL level [9]. Comparing the QTLs of LLS analyzed by unconditional and conditional mappings, the following four possible implications existed in this study: (1) the QTLs, such as *qLLSB6-2* and *qLLSA5*, were detected by unconditional mapping and not be recaptured when LLS was conditioned on HMS, LLB or TNB, suggesting that these QTLs for LLS were completely depended on the plant-type-related traits; (2) the loci, such as *qLLSA2* and *qLLSB2-1*, were identified by conditional, but not unconditional mapping, indicating that such QTLs were completely suppressed by conditional plant-type-related traits; (3) the QTLs, such as *qLLSA6-3*, identified by two mapping methods had similar effects, indicating that the QTLs for LLS were independent of conditional traits; (4) the QTLs, such as *qLLSB6-4* and *qLLSB6-6*, identified by both unconditional and conditional mapping methods showed significantly changed effects, indicating that these QTLs for LLS were partially influenced by plant-type-related traits. Of the 16 unconditional QTLs for LLS, seven, seven and 10 QTLs were entirely determined by HMS, LLB and TNB respectively; five QTLs each were partial contributions from HMS, LLB and TNB, respectively; four, four and one QTLs were independent of HMS, LLB and TNB, respectively. These results suggested that TNB contributed the strongest influence on LLS.

This study improved our understanding of genetic basis of LLS and provided several QTLs for LLS, HMS, LLB and TNB. From the QTL cluster and conditional analysis, we found that interrelationship existed between LLS and plant-type-related traits. However, challenging questions remain such as how to fast-expanding the population to meet the needs of fine-mapping in peanut. Further validation of major QTLs and markers lined the studied traits will be proceeded before applied in MAS. Besides, support from other omics researches, like transcriptomics, should also be considered to promote genetic analysis and the isolation of favorable alleles based on this study.

Supporting Information

S1 Fig. The schematic diagram of HMS and LLB investigated in this study. HMS and LLB were measured in the unit of centimeter (cm). HMS: height of main stem; LLB: length of the longest branch.
(EPS)

S2 Fig. Frequency distribution of RIL populations for LLS, HMS, LLB and TNB traits in multi-environments.
(EPS)

S3 Fig. Distribution of identified QTLs for LLS, HMS, LLB and TNB on genetic linkage maps. Scale bars on the left side describe map distance in centimorgans. Markers were shown on left side of the linkage groups. The QTLs for LLS, HMS, LLB and TNB were shown by boxes filled with red, green, blue and purple, respectively. The regions for QTL clusters were designed as yellow box on chromosome bars. Each cluster includes at least one major QTL. LLS: late leaf spot disease; HMS: height of main stem; LLB: length of the longest branch; TNB:

total number of branches.
(PDF)

S1 Table. The marker sequences of the linkage map used for QTL mapping.
(XLSX)

S2 Table. QTLs of LLS and plant-type-related traits identified only in joint analysis.
(XLSX)

S3 Table. The identified QTLs (or nearest marker) for LLS (or LS) of peanut in previous and this studies.
(XLSX)

S4 Table. (A) Information of QTLs for LLS resistance in this study in which genomic regions harbored NB-LRR—encoding genes. Physical locations of each marker on the genome were determined by searching SNP markers via blast and SSR markers using ePCR to align to AA and BB genome sequence. **(B) Genes with NBS domains in the QTL covering segments of pseudomolecule.**
(XLSX)

Acknowledgments

Author Contributions

Data curation: XZ YX JL.

Formal analysis: XZ YD LH.

Funding acquisition: XZ HJ XR.

Investigation: XZ YX JL XR YC.

Methodology: XZ HJ.

Project administration: XZ HJ.

Resources: BL YL LY.

Software: XZ YD.

Writing – original draft: XZ.

Writing – review & editing: KL QL.

References

1. FAO. FAO statistical database. FAO, Rome, Italy. Available: http://faostat.fao.org/. 2016. Accessed 23 October 2014.

2. Subrahmanyam P, Williams JH, McDonald D, Gibbons RW. The influence of foliar diseases and their control by selective fungicides on a range of groundnut (*Arachis hypogaea* L.) genotypes. Annals of Applied Biology. 1984; 104: 467–476.

3. Nigam SN, PrasadaRao RDVJ, Bhatnagar-Mathur P, Sharma KK. Genetic management of virus diseases in peanut. Plant Breed. 2012; 36:293–356.

4. McDonald D, Subrahmanyam P, Gibbons RW, Smith DH. Early and late leaf spot of groundnut. Information Bulletin no.21. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 24 pp; 1985.

5. Kokalis-Burette N, Porter DM, Rodríguez-Kábana R, Smith DH, Subrahmanyam P. Compendium of peanut diseases. American Phytopathology Society, St. Paul, MN. USA, pp. 94; 1997.

6. Waliyar F. Evaluation of yield losses due to groundnut leaf diseases in West Africa. In: Nduguru BJ, Waliyar F and Ntare BR eds. Summary proc. of the second ICRISAT regional groundnut meeting for West Africa. ICRISAT Sahelian Centre, Niamey, Niger, pp.32–33. 1991.

7. Wu R, Lin M. Functional mapping: how to map and study the genetic architecture of dynamic complex traits. Nat Rev Gent. 2006; 7: 229–237.

8. Zhu J. Analysis of conditional genetic effects and variance components in developmental genetics. Genetics. 1995; 141: 1633–1639. PMID: 8601500

9. Zhang Z, Liu Z, Hu Y, Li W, Fu Z, Ding D, et al. QTL analysis of kernel-related rraits in maize using an immortalized F_2 population. PLoS ONE, 2014; 9(2): e89645. doi: 10.1371/journal.pone.0089645 PMID: 24586932

10. Nevill DJ. Inheritance of resistance to *Cercosporidium personatum* in groundnuts: a genetic model and its implications for selection. Oleagineux. 1982; 37: 355–362.

11. Green CC, Wynne JC. Diallel and generation means analyses for the components of resistance to *Cercospora arachidicola* in peanut. Theor Appl Genet. 1986; 73:228–235. doi: 10.1007/BF00289279 PMID: 24240855

12. Motagi. Genetic analysis of resistance to late leaf spot and rust vis-à-vis productivity in groundnut (*Arachis hypogaea* L.). Dissertation, University of Agriculture Sciences, Dharwad, India; 2001.

13. Dwivedi SL, Pande S, Rao JN, Nigam SN. Components of resistance to late leaf spot and rust among interspecific derivatives and their significance in a foliar disease resistance breeding in groundnut (*Arachis hypogaea* L.). Euphytica. 2002; 125:81–88.

14. Khedikar YP, Gowda MVC, Sarvamangala C, Patgar KV, Upadhyaya HD, Varshney RK. A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor Appl Genet. 2010; 121:971–984. doi: 10.1007/s00122-010-1366-x PMID: 20526757

15. Sujay V, Gowda MVC, Pandey MK, Bhat RS, Khedikar YP, Nadaf HL, et al. Quantitative trait locus analysis and construction of consensus genetic map for foliar disease resistance based on two recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). Mol Breeding. 2012; 30:773–788.

16. Butler DR, Wadia KDR, Reddy RK. Effects of humidity, leaf wetness, temperature and light on conidial production by *Phaeoisariopsis personata* on groundnut. Plant Pathology.1995; 44(4):662–674.

17. Butler DR, Reddy RK, Wadia KDR. Single plant chambers to control humidity for pathological studies. Plant pathology.1995b; 44:1–9.

18. Nath BC,Sarma BK,Vaish SS, Chand R,Pal C. Temperature-growth relationship of *Phaeoisariopsis personata*. Asian Journal of Bio Science. 2013; 8:32–35.

19. Yi H, Richards EJ. Phenotypic instability of *Arabidopsis* alleles affecting a disease Resistance gene cluster. BMC Plant Biology 2008, 8:36 doi: 10.1186/1471-2229-8-36 PMID: 18410684

20. Li Z, Pinson SRM, Marchetti MA, Stansel JW, Park WD. Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theor Appl Genet. 1995; 91:382–388. doi: 10.1007/BF00220903 PMID: 24169789

21. Sato H, Ideta O, Audo I, Kunihiro Y, Hirabayashi H, Iwano M, et al. Mapping QTLs for sheath blight resistance in the rice line WSS2. Breeding Sci. 2004; 54 (3): 265–271.

22. Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, et al. Molecular mapping of QTL for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet. 2003; 107:503–508. doi: 10.1007/s00122-003-1272-6 PMID: 12768240

23. Ruge-Wehling B, Linz A, Habekus A, Wehling P. Mapping of *Rym16^{Hb}*, the second soil-borne virus-resistance gene introgressed from *Hordeum bulbosum*. Theor Appl Genet. 2006; 113:867–873. doi: 10.1007/s00122-006-0345-8 PMID: 16838136

24. Scheurer KS, Friedt W, Huth W, Waugh R, Ordon F. QTL analysis of tolerance to a German strain of BYDV-PAV in barley (*Hordeum vulgare* L.). Theor Appl Genet. 2001; 103:1074–1083.

25. Su Y, Liu Y, Li Z, Fang Z, Yang L, Zhuang M, et al. QTL analysis of head splitting resistance in cabbage (*Brassica oleracea* L. var. *capitata*) using SSR and InDel makers based on whole-genome re-sequencing. PLoS ONE. 2015; 10(9): e0138073. doi: 10.1371/journal.pone.0138073 PMID: 26406606

26. Huang L, He H, Chen W, Ren X, Chen Y, Zhou X, et al. Quantitative trait locus analysis of agronomic and quality-related traits in cultivated peanut (*Arachis hypogaea* L.). Theor Appl Genet. 2015; 128:1103–1115. doi: 10.1007/s00122-015-2493-1 PMID: 25805315

27. Shirasawa K, Koilkonda P, Aoki K, Hirakawa H, Tabata S, Watanabe M, et al. *In silico* polymorphism analysis for the development of simple sequence repeat and transposon markers and construction of linkage map in cultivated peanut. BMC Plant Biology. 2012; 12:80. doi: 10.1186/1471-2229-12-80 PMID: 22672714

28. Nagy ED, Guo Y, Tang S, Bowers JE, Okashah RA, Taylor CA, et al. A high-density genetic map of *Arachis duranensis*, a diploid ancestor of cultivated peanut. BMC Genomics. 2012; 13:469. doi: 10.1186/1471-2164-13-469 PMID: 22967170

29. Zhou X, Xia Y, Ren X, Chen Y, Huang L, Huang S, et al. Construction of a SNP-based genetic linkage map in cultivated peanut based on large scale marker development using next-generation double-digest restriction-site-associated DNA sequencing (ddRADseq). BMC Genomics. 2014; 15:351. doi: 10.1186/1471-2164-15-351 PMID: 24885639

30. Xia Y, Jing Y, Mao J, Ren X, Qi Y, Liao J, et al. Segregation analysis of resistance to late leaf spot in peanut. Chinese Journal of Oil Crop Science. 2015; 37(2):134–140.

31. Reddy LJ, Nigam SN, Moss JP, Singh AK, Subrahmanyam P, Mcdonald D, et al. Registration of ICGV 86699 Peanut Germplasm Line with Multiple Disease and Insect Resistance. Crop Sci. 1996; 36:821.

32. Subbarao PV, Subrahmanyam P, Reddy PM. A modified nine point disease scale for assessment of rust and late leaf spot of groundnut. In: Second international congress of French phytopathological society. 28–30 November 1990, Montpellier, France, pp. 25, 1990.

33. Hallauer AR, Miranda JB. Quantitative genetics in maize breeding, 2nd edn. Iowa State Univ Press, Ames; 1998.

34. Sas Institute. SAS/STAT User's Guide, Version 8. SAS Institute, Cary, NC; 2000.

35. Wang S, Basten C, Zeng Z. Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC; 2007.

36. Lander ES, Botstein D. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics. 1989; 121(1):185–199. PMID: 2563713

37. Liu Y, Wang L, Sun C, Zhang Z, Zheng Y, Qiu F. Genetic analysis and major QTL detection for maize kernel size and weight in multi-environments. Theor Appl Genet. 2014; 127:1019–1037. doi: 10.1007/s00122-014-2276-0 PMID: 24553962

38. Yang J, Zhu J, Williams RW. Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics. 2007; 23:1527–1536. doi: 10.1093/bioinformatics/btm143 PMID: 17459962

39. McCouch SR, Cho YG, Yano M, Paul E, Blinstrub M. Report on QTL nomenclature. Rice Genet Newlett. 1997; 14:11–13.

40. Zhu J. Mixed model approaches for estimating genetic variances and covariances. J Biomath. 1992; 7: 1–11.

41. Chen GB, Zhu J. Software for the classical quantitative genetics. Institute of Bioinformatics, Zhejiang University, Hangzhou, China; 2003. Available: http://ibi.zju.edu.cn/software/qga/index.htm

42. Zhao JY, Becker HC, Zhang DQ, Zhang YF, Ecke W. Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theor Appl Genet. 2006; 113: 33–38. doi: 10.1007/s00122-006-0267-5 PMID: 16614833

43. Sarvamangala C, Gowda MVC, Varshney RK. Identification of quantitative trait loci for protein content, oil content and oil quality for groundnut (*Arachis hypogaea* L.) Field Crops Research. 2011; 122: 49–59.

44. Chen W, Jiao Y, Cheng L, Huang L, Liao B, Tang M, et al. Quantitative trait locus analysis for pod- and kernel-related traits in the cultivated peanut (*Arachis hypogaea* L.). BMC Genetics. 2016; 17:25. doi: 10.1186/s12863-016-0337-x PMID: 26810040

45. Gautami B, Pandey MK, Vadez V, Nigam SN, Ratnakumar P, Krishnamurthy L, et al. Quantitative trait locus analysis and construction of consensus genetic map for drought tolerance traits based on three recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.) Mol Breeding. 2012; 30:757–772.

46. Wang H, Pandey MK, Qiao L, Qin H, Culbreath AK, He G, et al. Genetic mapping and quantitative trait loci analysis for disease resistance using F_2 and F_5 generation-based genetic maps derived from 'Tifrunner' × 'GT-C20' in peanut. Plant Genome. 2013; 6(3): doi: 10.3835/plantgenome2013.05.0018

47. Ravi K, Vadez V, Isobe S, Mir RR, Guo Y, Nigam SN, et al. Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theor Appl Genet. 2011; 122:1119–1132. doi: 10.1007/s00122-010-1517-0 PMID: 21191568

48. Varshney RK, Bertioli DJ, Moretzsohn MC, Vadez V, Krishnamurthy L, Aruna R, et al. The first SSR-based genetic linkage map for cultivated groundnut (*Arachis hypogaea* L.). Theor Appl Genet. 2009; 118:729–739. doi: 10.1007/s00122-008-0933-x PMID: 19048225

49. Wang ML, Khera P, Pandey MK, Wang H, Qiao L, Feng S, et al. Genetic mapping of QTLs controlling fatty acids provided insights into the genetic control of fatty acid synthesis pathway in peanut (*Arachis hypogaea* L.). PLoS ONE. 2015; 10(4): e0119454. doi: 10.1371/journal.pone.0119454 PMID: 25849082

50. Malosetti M, Ribaut JM, Vargas M, Crossa J, van Eeuwijk FA. A multi-trait multi-environment QTL mixed model with an application to drought and nitrogen stress trials in maize (*Zea mays* L.). Euphytica. 2008; 161:241–257.

51. Messmer R, Fracheboud Y, Bänziger M, Vargas M, Stamp P, Ribaut J-M. Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. Theorl Appl Genet. 2009; 119:913–930

52. Shoba D, Manivannan N, Vindhiyavarman P, Nigam SN. Identification of quatitative trait loci (QTL) for late leaf spot disease resistance in groundnut (*Arachis hypogaea* L.). Legume Res. 2013; 36 (5): 467–472.

53. Kolekar RM, Sujay V, Shirasawa K, Sukruth M, Khedikar YP, Gowdan MVC. QTL mapping for late leaf spot and rust resistance using an improved genetic map and extensive phenotypic data on a recombinant inbred line population in peanut (*Arachis hypogaea* L.). Euphytica. 2016; 209:147–156.

54. Leal-Bertioli SCM, AC VF José, Alves-Freitas DMT, Moretzsohn MC, Guimarães PM, Nielen S, et al. Identification of candidate genome regions controlling disease resistance in *Arachis*. BMC Plant Biology. 2009; 9:112 doi: 10.1186/1471-2229-9-112 PMID: 19698131

55. Fonceka D, Tossim H-A, Rivallan R, Vignes H, Lacut E, de Bellis F, et al. Construction of chromosome segment substitution lines in peanut (*Arachis hypogaea* L.) using a wild synthetic and QTL mapping for plant morphology. PLoS ONE. 2012; 7(11): e48642. doi: 10.1371/journal.pone.0048642 PMID: 23185268

56. Fonceka D, Tossim H-A, Rivallan R, Vignes H, Faye I, Ndoye O, et al. Fostered and left behind alleles in peanut: interspecific QTL mapping reveals footprints of domestication and useful natural variation for breeding. BMC Plant Biology. 2012; 12:26. doi: 10.1186/1471-2229-12-26 PMID: 22340522

57. Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EKS, et al. The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. Nature Genetics. 2016; 48:438–446. doi: 10.1038/ng.3517 PMID: 26901068

58. Burow MD, Simpson CE, Starr JL, Paterson AH. Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (*Arachis hypogaea* L.): broadening the gene pool of a monophyletic polyploid species. Genetics. 2001; 159: 823–837. PMID: 11606556

59. Burow MD, Starr JL, Simpson CE, Paterson AH. Identification of RAPD markers in peanut (*Arachis hypogaea*) associated with root-knot nematode resistance derived from A. cardenasii. Mol Breed. 1996; 2: 307–319.

60. Choi K, Burow MD, Church G, Burow G, Paterson AH, Simpson CE, et al. Genetics and mechanism of resistance to Meloidogyne arenaria in peanut germplasm. J. Nematol. 1999; 31: 283–290. PMID: 19270898

61. Simpson CE, Starr JL. Registration of 'COAN' peanut. Crop Sci. 2001; 41: 918.

62. Kochert G, Stalker HT, Gimenes M, Galgaro L, Lopes CR, Moore K. RFLP and cytological evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (*Leguminosae*). Am J Bot. 1996; 83: 1282–1291.

63. Gowda MVC, Motagi BN, Naidu GK, Diddimani SB, Sheshagiri R. GPBD 4: A Spanish bunch groundnut genotype resistant to rust and late leaf spot. Int Arachis Newsl. 2002; 22:29–32.

64. Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, Colinayo V, et al. Genetics of gene expression surveyed in maize, mouse and man. Nature. 2003; 422:297–302. doi: 10.1038/nature01434 PMID: 12646919

65. Austin D, Lee M. Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in maize. Theor Appl Genet. 1996; 92:817–826. doi: 10.1007/BF00221893 PMID: 24166546

66. Austin DF, Lee M. Detection of quantitative trait loci for grain yield and yield components in maize across generations in stress and nonstress environments. Crop Sci. 1998; 38:1296–1308.

67. Li Y, Niu S, Dong Y, Cui D, Wang Y, Liu Y, et al. Identification of trait-improving quantitative trait loci for grain yield components from a dent corn inbred line in an advanced backcross BC2F2 population and comparison with its $F_{2:3}$ population in popcorn. Theor Appl Genet. 2007; 115:129–140. doi: 10.1007/s00122-007-0549-6 PMID: 17492267

68.   Zou JH, Pan XB, Chen ZX, Xu JY, Lu JF, Zhai WX, et al. Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). Theo Appl Genet. 2000; 101: 569–575.

69.   Kunihiro Y, Qian Q, Sato H, Teng S, Zeng D-L, Fujimoto K, et al. QTL analysis of sheath blight resistance in rice (*Oryza sativa* L.). Acta Genetica Sinica. 2002; 29(1):50–55. PMID: 11838375