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ORIGINAL PAPER

An integrated genetic linkage map of cultivated peanut (*Arachis hypogaea* L.) constructed from two RIL populations

Hongde Qin · Suping Feng · Charles Chen · Yufang Guo · Steven Knapp · Albert Culbreath · Guohao He · Ming Li Wang · Xinyou Zhang · C. Corley Holbrook · Peggy Ozias-Akins · Baozhu Guo

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Abstract Construction and improvement of a genetic map for peanut (*Arachis hypogaea* L.) continues to be an important task in order to facilitate quantitative trait locus (QTL) analysis and the development of tools for marker-assisted breeding. The objective of this study was to develop a comparative integrated map from two cultivated × cultivated recombinant inbred line (RIL) mapping populations and to apply in mapping Tomato spotted wilt virus (TSWV) resistance trait in peanut. A total of 4,576 simple sequence repeat (SSR) markers from three sources: published SSR markers, newly developed SSR markers from expressed sequence tags (EST) and from bacterial artificial chromosome end-sequences were used for screening polymorphisms. Two cleaved amplified polymorphic sequence markers were also included to differentiate *ahFAD2A* alleles and *ahFAD2B* alleles. A total of 324 markers were anchored on this integrated map covering 1,352.1 cM with 21 linkage groups (LGs). Combining information from duplicated loci between LGs and comparing with published diploid maps, seven homoeologous groups were defined and 17 LGs (A1–A10, B1–B4, B7, B8, and B9) were aligned to corresponding A-subgenome or B-subgenome of diploid progenitors. One reciprocal

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H. Qin · S. Feng · A. Culbreath
Department of Plant Pathology, University of Georgia, Tifton, GA, USA

H. Qin
Cash Crop Research Institute, Hubei Academy of Agricultural Sciences, Wuhan, China

S. Feng
College of Bioscience and Biotechnolgy, Qiongzhou University, Sanya, Hainan, China

C. Chen
National Peanut Research Laboratory, Agricultural Research Service, US Department of Agriculture, Dawson, GA, USA

Y. Guo · S. Knapp
Center for Applied Genetic Technologies, University of Georgia, Athens, GA, USA

G. He
Center for Plant Biotechnology, Tuskegee University, Tuskegee, AL, USA

M. L. Wang
Plant Genetic Resources Conservation Unit, Agricultural Research Service, US Department of Agriculture, Griffin, GA, USA

X. Zhang
Peanut Research Unit, Henan Academy of Agricultural Sciences, Zhengzhou, China

C. C. Holbrook
Crop Genetics and Breeding Research Unit, Agricultural Research Service, US Department of Agriculture, Tifton, GA, USA

P. Ozias-Akins
Department of Horticulture, University of Georgia, Tifton, GA, USA

B. Guo (✉)
Crop Protection and Management Research Unit, Agricultural Research Service, US Department of Agriculture, Tifton, GA, USA
e-mail: Baozhu.Guo@ars.usda.gov

translocation was confirmed in the tetraploid-cultivated peanut genome. Several chromosomal rearrangements were observed by comparing with published cultivated peanut maps. High consistence with cultivated peanut maps derived from different populations may support this integrated map as a reliable reference map for peanut whole genome sequencing assembling. Further two major QTLs for TSWV resistance were identified for each RILs, which illustrated the application of this map.

Introduction

Peanut (*Arachis hypogaea* L.), or groundnut, is one of the major economically important legumes that is cultivated worldwide for its adaptability to grow in semi-arid environments with relatively low inputs of chemical fertilizers. On a global basis, peanut is also a major source of protein and vegetable oil for human nutrition, containing about 25% protein and 50% oil. Cultivated peanut is an allotetraploid ($2n = 4x = 40$) originated via hybridization of two ancient diploid species, probably *A. duranensis* (A-genome) and *A. ipaensis* (B-genome) followed by a rare spontaneous duplication of chromosomes (Milla et al. 2005; Favero et al. 2006; Seijo et al. 2004, 2007). Due to origin complexity of cultivated peanut, several genetic linkage maps have been constructed either for tetraploid or for diploid species in *Arachis* family, and the limited DNA polymorphisms have impeded the application of marker-assisted breeding in peanut. A high-density genetic linkage map for all chromosomes is necessary for quantitative trait loci (QTLs) analysis and efficient marker-assisted breeding. Nevertheless, marker-assisted breeding has been applied on a limited scale (Burow et al. 1996; Jung et al. 2000; Chu et al. 2007a, b, 2009; Nagy et al. 2010).

A genetic linkage map constructed from a population segregating for a trait of interest is required for QTL identification. The first genetic map for *Arachis* species was constructed with RFLP markers from a F_2 population developed from the interspecific hybridization of two related diploid A-genome species (*A. stenosperma* × *A. cardenasii*) resulting in 11 LGs (Halward et al. 1993). Another RFLP-based linkage map was derived from a synthetic interspecific tetraploid BC_1 population {[*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)]^{4x} × *A. hypogaea* ('Florunner')} with 23 LGs (Burow et al. 2001). Because of the complex pedigree, this map is complicated and difficult to use for extraction of useful genetic information relevant to the cultivated tetraploid. The first simple sequence repeat (SSR)-based linkage map for *Arachis* was constructed from an A-genome interspecific F_2 population (*A. duranensis* and *A. stenosperm*a) with 170 SSRs and 11 LGs (Moretzsohn et al. 2005). An advanced version of this map has been published recently with 369 markers, including 188 SSRs, 80 legume anchor markers, 46 AFLPs, 32 NBS homologs, 17 SNPs, 4 RGA-RFLPs and 2 SCAR markers, mapped into 10 LGs by Leal-Bertioli et al. (2009). A diploid B-genome map was also established from an F_2 population of *A. ipaensis* × *A. magna* (Moretzsohn et al. 2009). Comparative mapping of the A and B genomes using 51 common markers revealed a high level of synteny. Fonceka et al. (2009) published an SSR-based interspecific tetraploid map using 88 individuals of the BC_1F_1 population of ['Fleur 11' × (*A. ipaënsis* × *A. duranensis*)^{4x}], and 298 loci were mapped in 21 LGs.

In addition to genetic linkage maps of diploid *Arachis* species, there are two intraspecific maps for cultivated peanuts published recently (Varshney et al. 2009; Hong et al. 2010). Varshney et al. (2009) used two distinct peanut cultivars, 'ICGV 86031' and 'TAG 24' that are drought tolerant and sensitive, respectively, for developing the mapping population. They screened 1,145 SSRs that yielded a total of 144 polymorphic markers. A linkage map was constructed with 135 of these markers which are sparsely populated in 22 LGs. Hong et al. (2010) reported an SSR-based composite genetic linkage map of cultivated peanut based on three RIL populations constructed from three crosses with one common female parent. The composite linkage map consisted of 22 composite LGs with 175 SSR markers, representing the 20 chromosomes of *A. hypogaea* (Hong et al. 2010).

Nevertheless, the application of biotechnology for improving the allotetraploid cultivated peanut has been hampered by an inability to visualize sufficient genetic variation among paired genotypes and by lack of a high-resolution linkage map (Guo et al. 2011). Improved map resolution and coverage for *Arachis* species would enhance map utilization and facilitate QTL analysis for marker-assisted selection in peanut breeding programs. Therefore, the objective of this study was to develop a comparative integrated genetic linkage map for cultivated peanut from two RIL populations with diverse genetic backgrounds (Li et al. 2011a, b), in which two runner type cultivars, one Spanish-type cultivar and one breeding line derived from a cross of Virginia type and *hirsuta* type were used as parents. Further, the utilization of this map was applied in QTL mapping of peanut resistance to Tomato spotted wilt virus (TSWV).

Materials and methods

Mapping populations

Two recombinant inbred line (RIL) populations derived from the cross 'Tifrunner' × 'GT-C20' (referred to as T

population) and the cross 'SunOleic 97R' × 'NC94022' (referred to as S population) were used in this study. 'Tifrunner' is a runner market-type peanut (*A. hypogaea* L. subsp. *hypogaea* var. *hypogaea*) cultivar with a high level of resistance to TSWV, and moderate resistance to early (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*), but it is a late maturity cultivar (Holbrook and Culbreath 2007). 'GT-C20' is a Spanish-type breeding line (*A. hypogaea* L. subsp. *fastigiata* var. *fastigiata*) and highly susceptible to TSWV and leaf spots but resistant to aflatoxin contamination (Liang et al. 2005). 'SunOleic 97R' is from a BC_4F_5 selection of a cross of 'SunOleic 95R' and 'Sunrunner' with high oleic fatty acid but susceptible to TSWV (Gorbet and Knauft 2000). The breeding line 'F NC94022-1-2-1-1-b3-B' (henceforth referred as 'NC94022') has been reported to have a high level of field resistance to TSWV (Culbreath et al. 2005), a selection from a cross between N91026E (an early maturity Virginia type) and a tan-seeded component selected from PI 576638, a *hirsuta* type line. The populations were advanced to the F_5 by single seed descent. Individual plants were harvested and progeny rows were grown to produce the $F_{5:6}$ RIL populations. The mapping populations consisted of 248 $F_{5:6}$ RILs for T population and 352 $F_{5:6}$ lines for S population. Subsets of 158 and 190 RILs of T and S populations were used to construct genetic linkage maps.

SSR markers and nomenclature

DNA markers used for linkage map construction consisted of three sources: (1) 988 primer pairs obtained from published sources (Hopkins et al. 1999; Palmieri et al. 2002, 2005; He et al. 2003; Moretzsohn et al. 2004, 2005, 2009; Ferguson et al. 2004; Proite et al. 2007) including expressed sequence tags (EST)-SSR, genomic SSR and STS markers; (2) 2,138 EST-SSR primer pairs derived from *A. hypogaea* EST sequences (Guo et al. 2008, 2009; Liang et al. 2009); (3) 1,450 primer pairs developed from bacterial artificial chromosome (BAC) end-sequences (Cook and He, personal communication) (Supplementary 6). These SSRs were screened against the parental lines of these RIL populations (Knapp and Guo, unpublished data; Cook and He, unpublished data). The polymorphic markers identified were employed to genotype individuals of T and S populations. For the first primer source, we retained original names as published except 'pPGPseq××' and 'pPGSseq××' were as abbreviated to 'Seq××' in this study, the EST-SSR primers were named as 'GM××', and BAC-SSR primers were named as 'ARS××'. Therefore, marker nomenclature consisted of the letters as described that specified the origin of the marker, followed by the primer number and the amplified polymorphic fragment sizes. In addition, two cleaved amplified polymorphic sequence (CAPS) markers were included to differentiate *ahFAD2A* alleles and *ahFAD2B* alleles (Chu et al. 2007b, 2009).

DNA extraction and PCR performance

Total genomic DNA was extracted from young leaflets of the four parents, the F_1, 158 RILs for T population and 190 RILs for S population as described by Qin et al. (2008) with minor modification. The quality and quantity of the DNA were evaluated using a Nano-Drop 1000 spectrophotometer (Nano Drop Technologies, USA). PCR reactions for all markers were carried out in a 15 µl reaction volume in a PTC-225 DNA Engine Tetrad Peltier Thermal Cycler (MJ Research, Waltham, MA, USA) and a DNA Engine Tetrad 2 Peltier Thermal Cycler (BioRad Laboratories, Hercules, CA, USA). The reaction mixture contained 0.5 µM of each primer, 25 ng genomic DNA, 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.5 U *Taq* polymerase. The PCR temperature profile was 95°C for 4 min, 35 cycles of 45 s at 94°C, 45 s at 55°C and 1 min at 72°C, and a final extension step of 7 min at 72°C. The PCR products were separated on 9 or 6% non-denaturing polyacrylamide gel (PAGE). For the 6% polyacrylamide gels, electrophoresis was performed at 160 V for 1 h 20 min, while electrophoresis of the 9% gels was performed at 180 V for 1 h 40 min in 0.5× TBE using DYCZ-30B gel rigs (Beijing, China) (Fountain et al. 2011). Amplicons were visualized by silver staining.

Map construction and integration

Segregation patterns were assigned to each marker by following JoinMap data entry notation (⟨aa × bb⟩). A χ^2 test for goodness of fit was used to assess each marker for expected 1:1 segregation ratio. Linkage analysis was performed with JoinMap 3.0 (Van Oojen and Voorips 2001) using a minimum log-of-odds (LOD) threshold of 4.0 and recombination frequency of 0.35, which analyzes cross-pollinated populations derived from homozygous parents to create an individual linkage map. The recombination frequency was converted to genetic map distance (Morgan, M) using the Kosambi mapping function (Kosambi 1944). Based on common markers between individual maps, a test for a global difference in recombination rates between the two populations was conducted. *t* Test was applied on the mean values of the differences in recombination frequency between adjacent common markers. Unilateral and bilateral tests were performed at the 1% level. An integrated map was created to merge common markers on individual maps when two LGs shared two or more common markers between T and S populations. Linkage maps were drawn using MapChart for Windows (Voorrips 2002).

LGs in the newly integrated map were assigned to chromosomes (A-subgenome or B-subgenome) based on common markers between this integrated map and published A- or B-genome linkage maps (Moretzsohn et al. 2005, 2009; Leal-Bertioli et al. 2009; Foncéka et al. 2009). The two CAPS markers (Chu et al. 2007b, 2009) designed for the two genes *ahFAD*2A and *ahFAD*2B, which are homoeologous and belong to the A- and B-subgenomes of *A. hypogaea*, respectively (Jung et al. 2000), were also used in the LG naming. When no subgenome suggestion was available, the linkage group was designated to LGJXX, where LGJ indicates joint or integrated linkage group and XX refers to its serial number.

TSWV disease evaluation and QTL analysis

A subset of 158 and 190 RILs of T and S populations were used for phenotyping TSWV disease severity in 2009 and 2010 in Costal Plain Experimental Station, Tifton, GA. The field trials were conducted using randomized complete block designs with three replications. Experiment plots were 6.0 m long, separated by 2.4-m alleys. Peanut seed was planted to 91-cm-spaced twin-row plots. Tomato spotted wilt disease rating was assessed using a scale of 1–10, where 1 indicates no disease and 10 indicates all plants severely diseased (Tillman et al. 2006) based on visual determination of presence of symptoms and estimation of the degree of stunting (reduction in plant height, width, or both) for symptomatic plants at 90 days after planting (DAP) and 120 DAP. QTL analysis was carried out using the program MapQTL according to the method of Qin et al. (2008). The implemented QTL mapping procedure is a maximum likelihood approach to the segregation of a mixture of probability distributions. QTL mapping was conducted with the disease severity of TSWV. The significance thresholds for LOD scores were calculated by permutation tests in MapQTL, with a genome-wide significance level of $\alpha < 0.05$, $n = 1{,}000$ as significant QTL and a linkage group-wide significance level of $\alpha < 0.05$, $n = 1{,}000$ as suggestive QTL. QTL position indicated location of the peak. Confidence intervals (95%) associated with QTL location is set as the map intervals corresponding to 1 LOD decline either side of the peak. Furthermore, LOD score values between 2.0 and 3.0 were used to detect suggestive QTL, as suggested by Lander and Kruglyak (1995).

Results

Marker polymorphism

Out of 4,576 primer pairs, 260 and 181 primer pairs amplified polymorphic fragments between the parents of T and S populations, respectively (Table 1) with allele sizes and PIC values (Supplementary 7). The polymorphism ratios of newly designed EST-SSRs and BAC-SSRs in this study were low (2.1–4.1%). However, the publicly available SSRs produced three times higher polymorphism (9.0 and 12.8%).

Table 1 Percentage of polymorphic markers from different resources present in the two mapping populations

Resources	Number	Polymorphic markers in T population	Polymorphic markers in S population
Public SSRs	988	127 (12.8%)	89 (9.0%)
EST-SSRs	2,138	87 (4.1%)	61 (2.9%)
BAC-SSRs	1,450	46 (3.2%)	31 (2.1%)
Total	4,576	260 (5.7%)	181 (4.0%)

Construction and comparison of two individual maps

The T linkage map consisted of 26 linkage groups (LGT1 to LGT21, including 5 linkage groups with only two markers each) with 239 loci derived from 216 primer pairs, including a CAPS marker for *ahFAD*2A, and covered 1,213.4 cM (Supplementary 1). The average distance between markers was 5.7 cM and the coverage of LGs ranged from 3.1 to 136.5 cM. Out of 77 loci showing distorted segregation, 70 were assigned on 14 linkage groups but the majority (47/70) were placed on LGT4, LGT5, LGT6, LGT9, and LGT17. The distorted loci were not evenly distributed among LGs (or chromosomes).

The S linkage map consisted of 172 loci in 22 linkage groups (LGS1–LGS22, with five LGs having two markers each), including two CAPS markers for *ahFAD*2A and *ahFAD*2B, and covering 920.7 cM (Supplementary 2). The LGs ranged from 11.2 to 127.2 cM, with an average distance of 5.7 cM between adjacent markers. Forty-six distorted loci were placed on 14 LGs excluding LGS6, LGS8, LGS9, LGS15, LGS17 and LG20. The majority (30/46) were distributed on LGS2, LGS5, LGS7, LGS11, LGS13, LGS14 and LGS19.

Genotyping data of all 181 and 260 loci were evaluated for distorted segregation with the χ^2 test. It was found that 29.6% (77/260) of loci were distorted in the T population and 28.8% (52/181) of loci were distorted in the S population.

Integration of two individual maps

Out of 87 common markers between these two populations, 68 were mapped on both T and S linkage maps. These common markers enabled integration of the two maps and identification of 15 homologous LGs when they shared at least

two common markers. Interestingly, there were 12 reversed order events occurring in 9 homologous linkage groups. We also found that there were large gaps between three adjacent marker pairs of as much as 10 cM (LGT4/LGS4, LGT10/LGS10 and LGT18/LGS18; Supplementary 3). When the three large gaps were excluded, the mean recombination frequency between pairs of common markers was not significantly different between the T ($R < 0.111$) population and the S ($R < 0.087$) population. By comparing distorted common loci between these two maps, five (Seq2A6 of LG18, Seq15C12 of LG4, ARS748 of LG 2, Seq4B11, and GM2779 of LG19) were found on both maps, and two were in the same region on LG19) (Supplementary 3).

Out of 15 homologous LGs from the T and S populations two homologous pairs (LGT6/LGS6 and LGT22/LGS22) had only one marker (TC7C06 and Seq7G2) in common, and another homologous pair (LG4) had two markers (IPAHM105 and Seq15C12) in common. Homologous linkage groups LGT6 and LGS6 and LGT22/LGS22 could not be integrated since they had only one common marker which did not allow for the determination of orientation. Therefore, 13 integrated linkage groups were generated from the homologous linkage groups between the two maps. The rest of the integrated linkage groups were directly converted from non-homologous linkage groups from either T or S population that have more markers but the linkage groups with only two markers were excluded from the integrated map except the one B9 (LGJ19) with *ahFAD*2B (Fig. 1). This new integrated map covered 1,352.1 cM with 324 loci and comprised 21 linkage groups that ranged from 14.0 to 140.0 cM. Average distance between adjacent markers was 4.5 cM (Fig. 1).

Comparison of this newly integrated map with published A- or B-genome maps using the common markers allowed assignment of 17 linkage groups in this newly integrated map to the A- or B-subgenome chromosomes (A1–A10, B1–B4, and B7–B9) (Fig. 1). No subgenome assignment could be made for LGJ15, LGJ16, LGJ20, and LGJ21 (Fig. 1).

Homoeologous relationships and nomenclature of linkage groups (LGs) on the integrated map

Comparison of the integrated map with the published diploid linkage maps of A and B genomes provided evidence for assignment of homoeologous relationships among the LGs of the integrated map, facilitating alignment of the tetraploid genome with its diploid progenitor genomes. When two LGs shared at least two common markers in order to determine the orientation, these two LGs were considered to be homoeologous, in which one represents the A-subgenome and the other represents the B-subgenome (Fig. 1). This approach allowed six pairs of homoeologous LGs (A1/B1, A2/B2, A3/B3, A4/B4, A7/B7, and A8/B8) to be designated using 58 shared common markers between the integrated map and the A and the B maps (Fig. 1). The CAPS markers for homoeologous genes, *ahFAD2A*/*ahFAD2B*, determined another pair of homoeologous LGs, A9/B9. There were 41 shared markers that are collinear with the published A- and B-genome maps; six markers (Seq8E12 on a01, PM45 on B2, RI1F06 on b02, RN13D04 on A7, PM188 on A8, and IPAHM468 on B9) located on corresponding homoeologous LGs (Moretzsohn et al. 2009; Fonceka et al. 2009), respectively; and two markers (TC9F04 and IPAHM171a-400) assigned on LGs inconsistent with diploid maps (Fig. 1). Among 41 collinear markers, 19 were mapped on five LG pairs (A1, A3, A5, A6, and A8) with more than two common markers harbored in each pair of homoeologous linkage groups with same order. Because of the collinearity with diploid maps, these common markers could be employed as anchored chromosome-specific markers (Table 2) for aligning the integrated map to diploid maps. Therefore, 17 LGs in this newly integrated map (LGJ1–LGJ10, LGJ11–LGJ14, and LGJ17–LGJ19) were named A1–A10, B1–B4, and B7–B9, respectively (Fig. 1). Three linkage groups, LGJ15, LGJ16, and LGJ21, harbored only one anchored marker each (corresponding to A5/B5, B2, and B7, respectively) (Moretzsohn et al. 2009), but were not assigned to any specific chromosome due to insufficient information. No information of common markers was available for LGJ20 to assign to specific chromosome.

Duplicated loci provide direct evidence for determining homoeologous LGs of allotetraploid species. A total of 25 primer pairs amplified markers mapped to 49 duplicated loci. Ten different homoeologous pairs were aligned based on duplicated loci generated from 20 of those 25 markers (Table 2). Most of the homoeologous relationship determined by duplicated loci was consistent with the results derived from anchored markers. LGJ1 and LGJ1-1 corresponded to LGJ11 through three duplicated loci, indicating that LGJ1 and LGJ1-1 might belong to the same chromosome. As observed in the study of Fonceka et al. (2009), we also found a quadruplet that was formed by homoeologous chromosome pairs A7/B7 and A8/B8 through shared markers in A7/B8 and A8/B7, respectively (Fig. 1). It was interesting to note that there were six markers with both alleles assigned to the same LGs, TC3H02 of A1, SC1003E10 and TC2A02 of B3, TC3A12 and GM1702 of A5, and TC3H07 of A6.

Comparison with published tetraploid linkage maps

So far, there are four tetraploid linkage maps published. Two of these are interspecific maps (Burow et al. 2001; Fonceka et al. 2009) and two are intraspecific maps (Varshney et al. 2009; Hong et al. 2010). The first map was

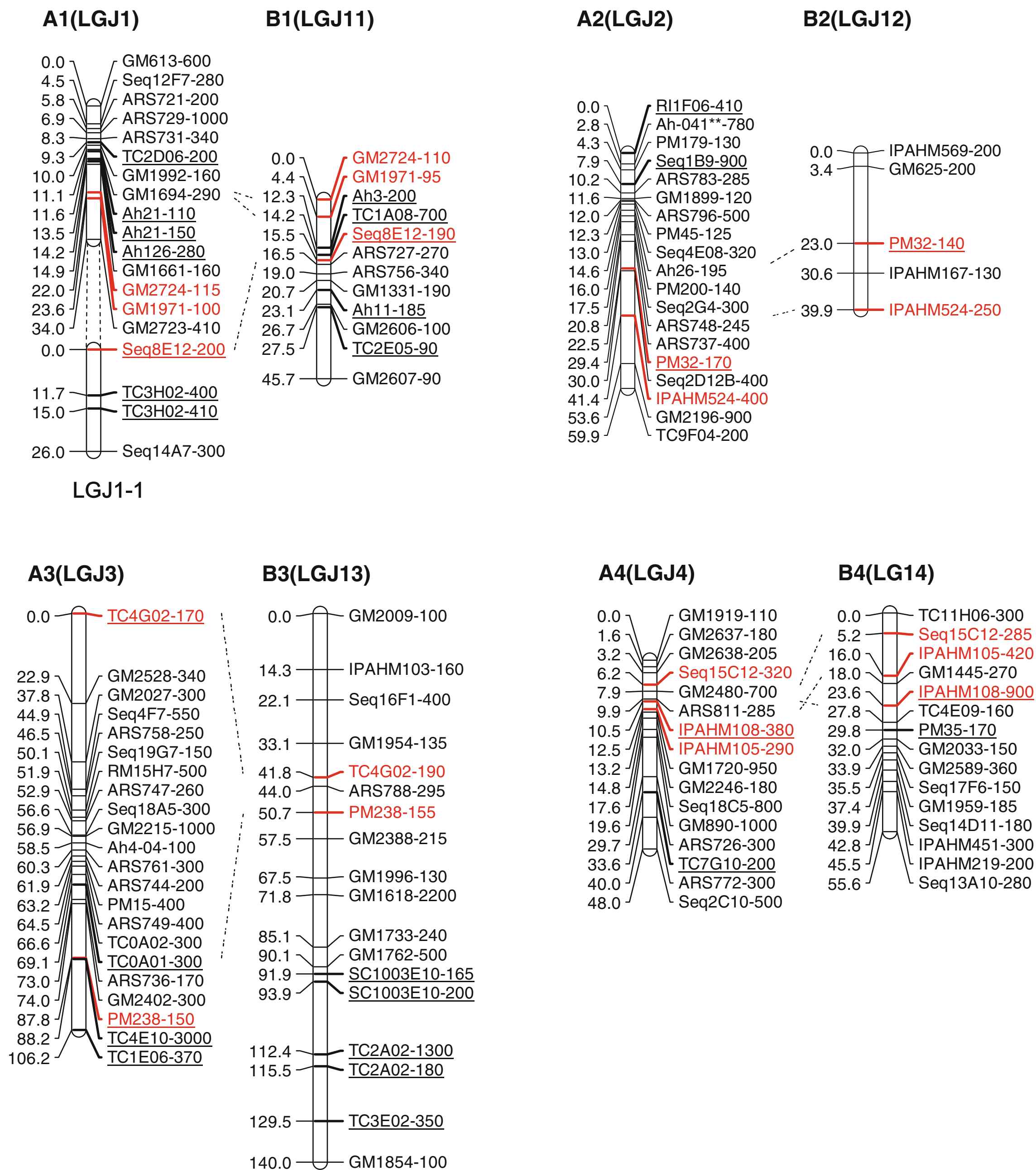

Fig. 1 Integrated genetic linkage map from two RILs population maps. Linkage groups of A-subgenome are named from A1–A10, and those of B-subgenome are named from B1–B4, and B7–B9. Four linkage groups are not assigned to chromosomes are named as LGJ15, LGJ16, LGJ20, and LGJ21. Map distances are given in Kosambi centimorgans. *Lighter-colored markers* are common markers with duplicated loci between two homoeologous linkage groups and connected with *dashed lines*. Anchor markers used to arrange linkage group to chromosome are *underlined*

a RFLP map derived from a synthetic tetraploid population (Burow et al. 2001); the second map was SSR-based and constructed from a cross of an AABB amphidiploids with a cultivated parent (Foncéka et al. 2009). Two intraspecific linkage maps were generated with SSR markers using RIL populations of cultivated peanuts (Varshney et al. 2009; Hong et al. 2010). Comparative analysis of our integrated map and each of those four tetraploid maps revealed distinct similarities. Due to differences of marker types, no synteny could be determined between our integrated map and the RFLP map (Burow et al. 2001), although an association between LG3/LG13 and LG5/LG15 was described in the RFLP map, similar to the association observed in our map described as a quadruplet including two pairs of homoeologous chromosomes (A7/B7 and A8/B8) (Fig. 1). Marker density was similar in the two studies.

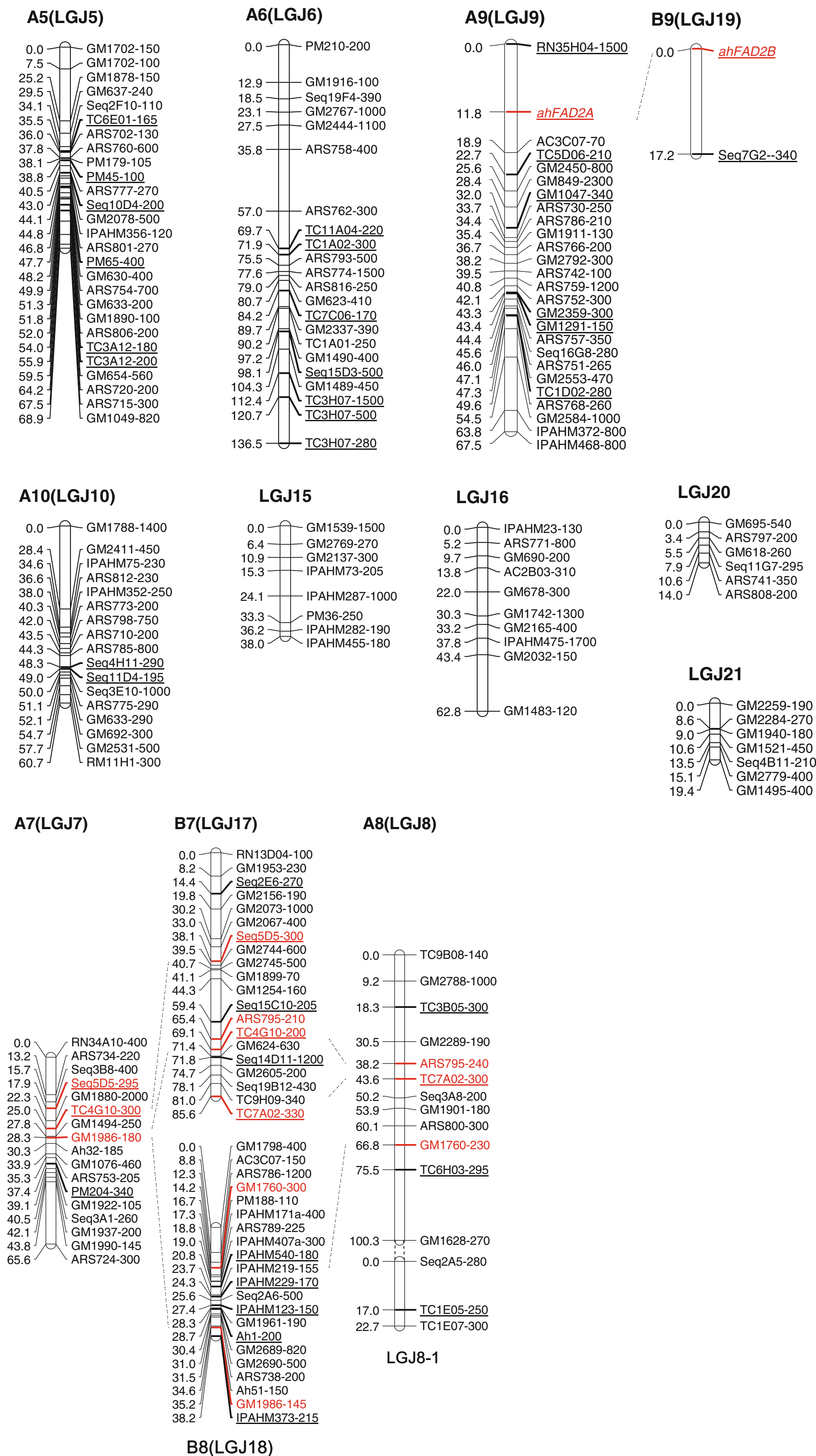

Fig. 1 continued

Table 2 The number of anchored or common markers and duplicated loci on potential homoeologous linkage group pairs

	Anchored marker	B1 (LGJ11)	B2 (LGJ12)	B3 (LGJ13)	B4 (LGJ14)	B7 (LGJ17)	B8 (LGJ18)	B9 (LGJ19)	A5 (LGJ5)
Anchored marker		5	1	5	2	6	5	1	6
A1 (LGJ1)	7	3							
A2 (LGJ2)	3		2						2
A3 (LGJ3)	5			2					
A4 (LGJ4)	2				3				
A5 (LGJ5)	6								
A6 (LGJ6)	7								
A7 (LGJ7)	3					2	1		
A8 (LGJ8)	6					2	1		
A9 (LGJ9)	6						2	1	
A10 (LGJ10)	2								

In comparison with the map developed by Foncéka et al. (2009), our integrated map was shorter with more loci (321 loci/1,335 cM vs. 298 loci/1843.7 cM). However, the ratio of loci distributed on the A-genome versus B-genome was similar in both maps (181/117 in Foncéka's map and 199/122 in our integrated map). Forty-two markers shared between the two maps aligned 15 LGs except B3, B5, B6, B9 and B10. Among them seven LGs shared more than three common markers and six LGs had the same marker order except one inversion that occurred between A7 and a07 (TC4G10 and Seq3A1). Once again, one quadruplet involving the LGs A7, B7, A8, and B8 with reciprocal translocation was also found on both maps.

There are 41 markers shared by the present integrated map and the first SSR-based map of cultivated peanut (Varshney et al. 2009). Among them, 30 common markers involving eight linkage groups were collinear (Supplementary 4). Six common markers were singly located on six different linkage groups, and five common markers (GM618, TC6H03, Seq13A10, PM129, and TC3E02) were mapped on different linkage groups relative to the first SSR map. One obvious inversion (less than 10.0 cM) was revealed between B7 (LGJ7) and LG_AhXVIII (GM624 and TC9H09), and another inverted segment was found between A3 (LGJ3) and LG_AhIII (Seq18A5 and Seq19G07) (Supplementary 4). Two flanking marker pairs, Ah26/Seq2G4 on LG_AhII and IPAHM569/GM625 on LG_AhXXII in the Varshney's map, covered a significantly greater distance (41.1 and 26.3 cM, respectively) than on the integrated map. The common marker, TC7C06, was placed in a collinear LG, A6/LG_AhVI, but was at a very distinct position (Supplementary 4).

There were 64 markers commonly shared by the present integrated map and the SSR-based composite map developed by Hong et al. (2010), where 46 common markers were distributed into 12 collinearly LGs (Supplementary 5). The remaining 18 common markers were scattered on different LGs without collinearity. Four small inverted segments were observed in four LGs (A6, A8, B4, and B7) (Supplementary 5). Five LGs had a completely consistent syntenic marker order. Three common markers (PM238, Seq15C12, and Seq3E10) were mapped on collinear LGs B3/LG4, B4/LG1, and A10/LG10 but in obviously different positions. One trio of linkage groups involving LGJ1, LGJ1-1 from the integrated map and LG8 from the Hong map was formed based on three common markers (GA1, TC2D06, and Seq14A), indicating LGJ1-1 and LGJ1 should be linked together as part of A1 (LGJ1) (Fig. 1; Supplementary 5). Similarly, another trio of linkage groups involving LGJ8, LGJ8-1 from the integrated map and LG2 from the Hong map was aligned, where LGJ8 shared five common markers with the upper part of LG2, and LGJ8-1 had two common markers with the lower part of LG2. Therefore, it is reasonable to assume that LGJ8-1 and LGJ8 should be in the same LG named as A8 (LGJ8) (Fig. 1; Supplementary 5). There were no anchored markers on LGJ20 in this integrated map, but it could be assigned to B10 because its homologous linkage group LG11 from the Hong map had two anchored markers (Seq14F4 and TC1E01) placed on the B10 of B genome map (Moretzsohn et al. 2009).

Identification of QTLs for TSWV resistance

These two RILs and the parental lines were phenotyped for TSWV disease severity in the field. The distribution of TSWV disease scores (1–10 scales) was normal for both RILs, ranged from 1 to 9 for T population and 1 to 7.5 for S population, but slightly skewed toward susceptible end in T population and toward resistant end for S population. Tifrunner scored 3.1 and GT-C20 scored 7.5; SunOleic 97R scored 7 and NC94022 scored 1.9. Some RILs in both populations showed transgression segregation for TSWV resistance.

Table 3 Summary of the locations and the effects of the QTLs

Linkage group	QTL	Position[a]	Nearest marker	aa[b]	bb[b]	Expl[c] (%)	Additive[d]
LGJ15	*qTSWV1*	24.12	IPAHM287	4.17	5.35	12.9	−0.58
A1	*qTSWV2*	4.56	Seq12F7	3.02	4.87	35.8	−0.97

[a] Position is given in centimorgan

[b] The mean value of genotype

[c] Percentage phenotypic variation explained

[d] The additive effect

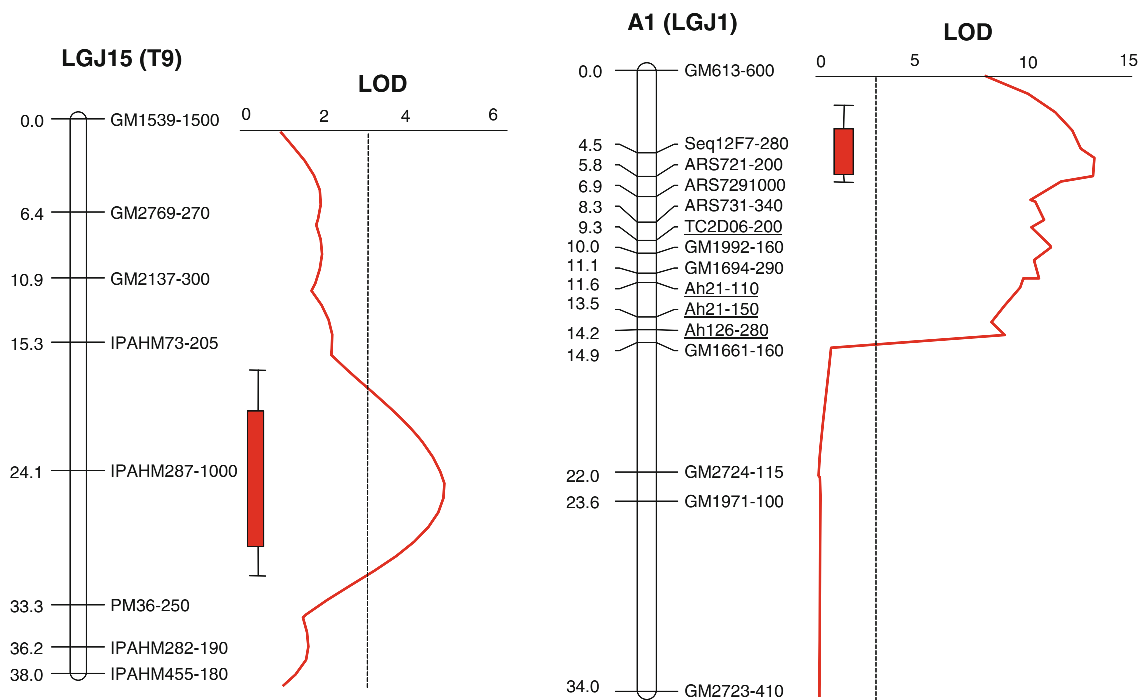

Fig. 2 Maping QTLs on chromosomes LGJ15 for T population and A1 for S population with MAPMAKER/QTL in cultivated peanut. Locations for QTLs (detected in combined analysis) are associated with disease resistance to TSWV in the populations derived from Tiftrunner/GT-C20 (T population) and SunOleic 97R/NC94022 (S population) and the distribution of SSR markers along the chromosomes and their LOD values are listed. Positions of loci are given in centimorgan

Two major different QTLs were detected in combined analysis with high LOD scores (Table 3). One QTL was detected in T population named *qtswv1* with average 12.9% phenotypic variation explained and one QTL named as *qtswv2* was detected in S population with 35.8% phenotypic variation explained. These two QTLs were located in different linkage groups, LGJ15 (T9) for *qtswv1* and A1 (LGJ1) for *qtswv2* (Fig. 2).

Discussion

The low level of SSR polymorphism within cultivated peanut detected in this study was consistent with previously published results. It has become a solid fact that cultivated peanut encompasses a low level of genetic diversity compared with its diploid peanut species and other legumes. The lack of polymorphic DNA markers poses a big challenge for construction of a high-density genetic map for cultivated peanut. It is clear that new types of DNA markers (such as SNP markers) need to be developed in cultivated peanut (Guo et al. 2011). However, regardless of DNA marker type, researchers will face the same problem for distinguishing and designating homoeologous linkage groups. Identifying homoeologous linkage groups, discriminating different subgenomes, and defining linkage groups corresponding to the chromosomes are crucial for construction of informative allotetraploid linkage maps. Therefore, the 21 LGs derived from this study have been discriminated as ten LGs for the A-subgenome (A1–A10) and seven LGs for the

B-subgenome (B1 to B4, B7 to B9). LGJ21 was named as B9 because of the homoeologous relationship of two CAPS markers for *ahFAD*2A and *ahFAD*2B (Jung et al. 2000). Although PM32 of LGJ12 in this map was present on both a02 and b02 of Foncéka map (Foncéka et al. 2009) but on B10 in Moretzsohn et al. (2009), LGJ12 was still named as B2 in our integrated map because it shared two duplicated loci with A2 (Fig. 1).

The analysis of synteny between our map and published maps with common SSR markers has provided evidence to arrange LGs. For example, a common marker, IPAHM171a, located on A6 in the A-genome map, was also found on LG_AhVI in the Varshney's map, suggesting that LGJ6 should be assigned to A6. Another common marker, TC2C07, mapped on A3 of the A-genome map, and was also placed on LG_AhIII indicating that LGJ3 was associated with A3. We also found two markers, TC1E05 and TC4E10, consistently presented in a similar position crossing over the diploid map (Moretzsohn et al. 2005), the SSR-based composite map (Hong et al. 2010), and our integrated map. These markers are valuable for constructing a consensus reference map of cultivated peanut. In comparison of the map of Hong et al. (2010) with the A diploid map of Moretzsohn et al. (2009), some markers mapped on the Hong composite map were also mapped on the Moretzsohn A-genome diploid map, such as TC4G05 placed on the A1 of the diploid map, RN0×615 on the A2, Seq2H8 and TC4E10 on the A3, and five markers (Ah-408, Ah-569, RN9C02, gi-832 and Seq18C5) on the A4, and TC1E05 and gi-716 on the A8 (Moretzsohn et al. 2009), supporting this integrated map assignment of each LG to specific chromosome through homologous relationships. Although consistent results with published maps provided confidence for aligning the 17 LGs (A1–A10, B1–B4, and B7–B9) to the diploid maps (Moretzsohn et al. 2009; Foncéka et al. 2009), there were still six common markers (Seq8E12 on a01, PM45 on B2, RI1F06 on b02, RN13D04 on A7, PM188 on A8, and IPAHM468 on B9) that were mapped on other homoeologous LGs compared with the other published maps, and two common markers (TC9F04 on A8 and IPAHM171a-400) that were mapped on contradictory LGs. If the possible duplicated loci were considered, six common markers (Seq8E12 on a01, PM45 on B2, RI1F06 on b02, RN13D04 on A7, PM188 on A8, and IPAHM468 on B9) located on corresponding homoeologous LGs did not show obvious confliction with results from collinear common markers. Two other common markers (TC9F04 and IPAHM171a-400) where positions conflicted with diploid maps might be due to experimental error or ancient genome duplication (Burow et al. 2001; Rong et al. 2004; Desai et al. 2006).

Six markers, TC3H02, SC1003E10, TC2A02, TC3A12, GM1702, and TC3H07, amplified consistently more than one locus in the same genome. However, the duplicated loci amplified by each marker were mapped on the same LG, which may be attributed to an ancient duplication predating the divergence of the A- and B-subgenomes. But in some cases, the markers producing the duplicated loci may not be assigned to homoeologous LGs (Table 2). For example, the duplicated loci of PM179 and PM45 were mapped to non-homoeologous LGs A2 (LGJ2) and A5 (LGJ5), and one locus of GM633 was mapped on A5 while another locus of GM633 was mapped on A10 (Fig. 1). Similarly, AC3C07 and ARS786 were mapped to different LGs B8 (LGJ18) and A9 (LGJ9), and the duplicated loci of other four markers, GM1899, IPAHM219, ARS758 and Seq14D11, were also mapped on non-homoeologous LGs, which suggest possible segmental duplications occurred among chromosomes. Similar scenarios have been reported by previous studies (Burow et al. 2001; Desai et al. 2006; Foncéka et al. 2009).

Interestingly, conservation of synteny between the A-genome LGs, A7 and A8, and the B-genome LGs, B7 and B8, was found in our map and Foncéka map (Fig. 1). Two markers, Seq5D5 and TC4G10, aligned homoeologous LGs A7 and B7; and another marker GM1760 linked homoeologous LGs A8 and B8. Furthermore, the association between A8 and B7 was established by sharing the markers ARS795 and TC7A02; and another association between A7 and B8 was built by marker GM1986. As a result, a quadruplet involving two LGs (A7 and A8) on A-genome and two LGs (B7 and B8) on B-genome was formed (Fig. 1). Similar feature of syntenic conservation between two linkage groups of the A-genome and two linkage groups of the B-genome have been described both in Foncéka map (Foncéka et al. 2009) and the A- and B-genome maps (Moretzsohn et al. 2005, 2009). The quadruplet found in this study further suggested that one translocation between A7 and A8 or B7 and B8 had taken place after divergence of the A- and the B-genome of peanut, but the question is that which two chromosomes were involved in this translocation occurred.

Similar to Foncéka map (Foncéka et al. 2009), in which 181 loci were mapped on the A-genome with a total size of 1,005.2 cM and 171 loci were placed on the B-genome with a total size of 762 cM, we have placed 200 markers in LGs of the A-genome with a coverage of 796.0 cM and 124 markers in LGs of the B-genome with a total of 556.1 cM coverage. When we compared common markers present in the LGs of the A- and the B-genome among different published linkage maps (Foncéka et al. 2009; Varshney et al. 2009; Hong et al. 2010) including this integrated map, we noticed that the number of common markers located on the A-genome was far more than the number located on the B-genome. The difference was nearly two folds, which indicates more conservation of the B-genome than the A-genome, or B-genome may have

arose from progenitor lines with more close relationship than did the A-genome.

The construction of a highly saturated linkage map with possible full genome coverage will enhance molecular breeding, gene discovery, understanding of the species evolution of allotetraploid peanut, and whole genome sequencing assembly. However, for peanut, it is difficult to achieve a satisfactory linkage map with a sole segregating mapping population and the existed markers due to well-known low genetic diversity and lack of DNA polymorphic markers. The low genetic diversity resulted in the failure of alignment of B5, B6, B9 and B10 to small linkage groups with only a few markers (Fig. 1).

Interestingly, the distribution pattern of polymorphic markers across the whole genome varied between these two populations. For example, the LGS4 from the S population contained only four markers, whereas the counterpart LGT4 from the T population had 14 markers (Supplementary 3). The similarity also goes to LGS6 and LGT6. The opposite pattern was observed for LGS7 having more markers (13) than LGT7 (8). Such complementary polymorphism distribution model can dramatically increase the genome coverage of this integrated linkage map. As a result, our integrated linkage map has extended coverage from 1,213.4 and 920.7 cM of S and T maps to 1,352.1 cM; and the average marker density from 5.7 to 4.5 cM. This clearly illustrated that multiple mapping populations plus newly developed DNA markers can be used to improve peanut linkage map construction.

The distribution of the phenotypic scores for TSWV disease severities in the RILs along the parents was expected and the disease scores of the parental lines are in agreement with the report of Li et al. (2011b). Two different QTLs identified for these two populations were not a surprise, because Tifrunner is selected from a Brazilian germplasm with moderate resistance to TSWV (Holbrook and Culbreath 2007) and NC94022 is selected from a Mexican highland peanut germplasm with high resistance to TSWV (Culbreath et al. 2005). It further demonstrates that Tifrunner and NC94022 have two different resistance pathways to TSWV, which needs further study. This QTL study demonstrated the utility and application of these populations and the integrated map. The full sets of 248 $F_{8:9}$ and 352 $F_{8:9}$ RILs will be re-genotyped and phenotyped for further verification and map-based cloning.

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