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Research Article

Comparative Assessment of Genetic Diversity of Peanut (*Arachis hypogaea* L.) Genotypes with Various Levels of Resistance to Bacterial Wilt Through SSR and AFLP Analyses

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Abstract: Bacterial wilt (BW) caused by *Ralstonia solanacearum* is an important constraint to peanut (*Arachis hypogaea* L.) production in several Asian and African countries, and planting BW-resistant cultivars is the most feasible method for controlling the disease. Although several BW-resistant peanut germplasm accessions have been identified, the genetic diversity among these has not been properly investigated, which has impeded efficient utilization. In this study, the genetic relationships of 31 peanut genotypes with various levels of resistance to BW were assessed based on SSR and AFLP analyses. Twenty-nine of 78 SSR primers and 32 of 126 AFLP primer combinations employed in this study were polymorphic amongst the peanut genotypes tested. The SSR primers amplified 91 polymorphic loci in total with an average of 3.14 alleles per primer, and the AFLP primers amplified 72 polymorphic loci in total with an average of 2.25 alleles per primer. Four SSR primers (14H06, 7G02, 3A8, 16C6) and one AFLP primer (P1M62) were found to be most efficient in detecting diversity. The genetic distance between pairs of genotypes ranged from 0.12 to 0.94 with an average of 0.53 in the SSR data and from 0.06 to 0.57 with an average of 0.25 in the AFLP data. The SSR-based estimates of the genetic distance were generally larger than that based on the AFLP data. The genotypes belonging to subsp. *fastigiata* possessed wider diversity than that of subsp. *hypogaea*. The clustering of genotypes based on the SSR and AFLP data were similar but the SSR clustering was more consistent with morphological classification of *A. hypogaea*. Optimum diverse genotypes of both subsp. *hypogaea* and subsp. *fastigiata* can be recommended based on this analysis for developing mapping populations and breeding for high yielding and resistant cultivars.

Keywords: peanut; bacterial wilt resistance; genetic diversity; SSR; AFLP

Bacterial wilt (BW) caused by *Ralstonia solanacearum* E. F. Smith has been the most important bacterial disease affecting peanut or groundnut (*Arachis hypogaea* L.) production in several countries in Asia including China, Indonesia, and Vietnam and Uganda in Africa[1]. It has been estimated that 10% of peanut fields are infested with the BW pathogen in China, with higher incidencein south and central regions [2, 3]. With the expansion of peanut production, the disease is expected to become more widespread. As a soil-borne bacterial disease, BW has proven to be difficult to control. Unlike most other diseases affecting peanut, no chemical is available for BW control. Although some cultural approaches such as long-term rotation and soil

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solarization have been regarded as effective for reducing BW incidence to some extent, these are less applicable in most developing countries where arable land per capita is generally limited. Conversely, host plant resistance has been regarded as the most important component for any integrated approach to control the disease in the farmer's field and BW-resistant cultivars are essential for peanut production in heavily infested fields [1].

New sources of resistance are crucial for developing resistant cultivars in crops. In peanut, there have been several BW resistance screening and breeding activities in China since the 1970s[1,3,4]. Worldwide, more than 170 accessions across four botanical varieties of cultivated peanut and its related wild species have been identified as BW-resistant[4–6]. Among the 5,700 peanut germplasm accessions screened in China, 112 were found to be highly resistant[6, 7], about 60% of which belonged to subsp. *hypogaea* var. *hirsuta*, 30% belonged to subsp. *fastigiata* var. *vulgaris*, and 10% belonged to subsp. *hypogaea* var. *hypogaea* and subsp. *fastigiata* var. *fastigiata*.

In China, extensive efforts have been made in peanut breeding for BW-resistance and several resistant cultivars have been released[3,5,6]. However, these cultivars have relatively low yields with poor resistance or tolerance to other constraints, such as foliar diseases and drought[2–6]. Only a few sources of BW-resistance have been successfully used in breeding programs in China even though several resistant genotypes are available[5, 6]. Most BW-resistant cultivars released in China are based on just three sources of resistance ('Xiekangqing', 'Taishan Sanlirou', or 'Taishan Zhenzhu'), all of which belong to subsp. *fastigiata*[6]. Obviously, the genetic background of parents in breeding programs is still narrow, which may have impeded the progress of breeding. Hence, a better understanding of the genetic diversity amongst the available BW-resistant germplasm is a prerequisite for further efficient improvement of BW resistance.

Several approaches including molecular[8–17] and morphological characterization[18] have been used in assessing the genetic diversity of peanut germplasm. However, this is the first report focusing on peanut germplasm with BW resistance. In the present study, 31 peanut genotypes with various levels of BW resistance were used for a comparative diversity assessment based on SSR (simple sequence repeats) and AFLP (amplified fragment length polymorphism) analyses.

1 Materials and Methods

1.1 Plant materials

Thirty-one peanut accessions comprising 15 genotypes belonging to subsp. *hypogaea* var. *hirsuta*, 2 belonging to var. *hypogaea*, 13 belonging to subsp. *fastigiata* var. *vulgaris*, and 1 belonging to var. *fastigiata*, were used in this study. Among these, 27 were BW-resistant and 4 were highly susceptible to BW (Table 1).

1.2 DNA extraction

Genomic DNA was isolated from young leaves of 15-day-old seedlings. Each sample consisted of leaves pooled from 3−5 seedlings (approximately 2 g), and DNA was extracted according to the procedure described by Kochert *et al.* (1991)[19]. The DNA quality was visually checked using 0.8% agarose gel electrophoresis and quantified at a wavelength of 260 nm using a spectrophotometer.

1.3 SSR analysis

Seventy-eight SSR primer pairs (sequence provided by Applied Genomics Laboratory, International Crops Research Institute for the Semi-Arid Tropics, India. Table 2) were used to amplify the genomic DNAs. Polymerase chain reaction (PCR) was carried out in 10 μL reaction volume containing 10 ng genomic DNA, 1 × PCR reaction buffer, 2 mmol/L Mg^{2+}, 0.15 mmol/L dNTPs, 10×10^{-6} μmol/L primers, and

Table 1 Peanut genotypes used in SSR and AFLP analyses

Entry No.	Genotype	Botanical type	Reaction to BW [a)]	Pedigree	Origin [b)]
1	Zhonghua 6	*vulgaris*	R	Breeding line	Hubei
2	Goulezhong	*hypogaea*	R	Landrace	Fujian
3	Qingmiaodou	*hirsuta*	R	Landrace	Guangxi
4	Zao 18	*vulgaris*	S	Breeding line	Hubei
5	Daye Bentianzi	*hirsuta*	S	Landrace	Jiangxi
6	Yuanza 9102	*vulgaris*	R	Breeding line	Henan
7	Yueyou 200	*vulgaris*	R	Breeding line	Guangdong
8	Xiekangqing	*vulgaris*	R	Landrace	Guangdong
9	Luoao Wanhuasheng	*hirsuta*	R	Landrace	Jiangxi
10	Wuxuan Laohuasheng	*hirsuta*	R	Landrace	Guangxi
11	Zhonghua 2	*vulgaris*	R	Breeding line	Hubei
12	Changsha Tuzi	*hirsuta*	R	Landrace	Hunan
13	Feilongxiang	*hirsuta*	R	Landrace	Guangxi
14	Shitang Dahuasheng	*hirsuta*	R	Landrace	Guangxi
15	Zhonghua 212	*vulgaris*	R	Breeding line	Hubei
16	Taishan Zhenzhu	*vulgaris*	R	Landrace	Guangdong
17	Jiangtianzhong	*hypogaea*	R	Landrace	Fujian
18	Qidong Dahusheng	*hirsuta*	R	Landrace	Hunan
19	Nanning Sanjindou	*hirsuta*	R	Landrace	Guangxi
20	Lingui Make	*hirsuta*	R	Landrace	Guangxi
21	Chico	*vulgaris*	S	Landrace	ICRISAT
22	Bobai Dahuasheng	*hirsuta*	R	Landrace	Guangxi
23	Mashan Guling	*hirsuta*	R	Landrace	Guangxi
24	ShengxianXiaohongmao	*hirsuta*	R	Landrace	Zhejiang
25	Ehua 5	*vulgaris*	R	Breeding line	Hubei
26	Zhongxingchi	*hirsuta*	R	Landrace	Fujian
27	Bobai Shiyaodou	*hirsuta*	R	Landrace	Guangxi
28	Taishan Sanlirou	*fastigiata*	R	Landrace	Guangdong
29	91-074	*vulgaris*	R	Breeding line	Hubei
30	89-15048	*vulgaris*	R	Breeding line	Hubei
31	Zhonghua 5	*vulgaris*	S	Breeding line	Hubei

[a)] R=population survival ratio over 80%, and S=population survival ratio less than 50%;
[b)] All origin places are the province name in China except for ICRISAT.

1.2 U *Taq* DNA polymerase. The reaction was performed at 94℃ for 2 min; 35 cycles of 94℃ for 45 s, empirically defined annealing temperature (55–60℃, Table 2) for 1 min, 72℃ for 90 s, then a final extension of 10 min with 72℃. The amplified products were visualized on non-denaturing 6% 29:1 (w/w) polyacrylamide/bisacrylamide gels followed by silver staining as described by Ferguson *et al.* (2004) [9].

1.4 AFLP analysis

AFLP fingerprints were generated based on the protocol of Vos *et al.*[20] with minor modifications. Genomic DNA (0.6 μg) of each sample was digested for 3 h with *Mse* I and *Pst* I followed by overnight ligation at 15℃ with corresponding adaptors in a total volume of 5 μL.

A 5 μL mixture of 1:20 dilution of the adaptor-ligated fragments was pre-amplified using corresponding pre-amplification primers in a volume of 20 μL for 1 min at 94℃, then 25 cycles of 30 s at 94℃, 30 s at 56 ℃ and 1 min at 72℃, followed by an elongation of 5 min at 72℃. Selective amplification reactions were performed in a total of 20 μL volume containing 5 μL of 1:50 diluted pre-amplification product, 2 μL 10 × polymerase buffer with Mg^{2+}, 0.2 μL dNTPs with 25 mmol/L, 1 μL *Mse* Ⅰ-primer + 3 (50 ng/μL), 1 μL *Pst* Ⅰ-primer + 3 (50 ng/μL), and 1 U *Taq* DNA polymerase (Table 3). The cycling program used for selective amplification was carried out at 94℃ for 1 min followed by 12 touch down cycles of 30 s at 94℃, 30 s at 65℃, and 1 min at 72℃. In which, the annealing temperature was lowered by 0.7℃ per cycle. The mixture then underwent 22 cycles at 94℃ for 30 s, 56℃ for 30 s, and 72℃ for 1 min followed by an elongation of 5 min at 72℃. The AFLP products were separ- ated on 6% denaturing polyacrylamide gels (29:1 acryl- amide: bis-acrylamide with 210 g of urea) follow ed by silver staining as described by Ferguson *et al.* (2004)[9].

Table 2 Sequences and alleles number amplified of the polymorphic primers used in SSR analysis

Primer	5′ Left / Forward / Sense Seq 3′	5′ Right / Reverse / Anti-Sense Seq 3′	Optimum annealing temperature (℃)	Number of alleles
2G3	ATTCACAAGGGGACAGTTGC	ATTCAAGCCTGGGAAACAGA	60	3
2G4	TTCTTGGTTCCTTTGGCTTC	TGCTCAAGTGTCCTTATTGGTG	60	3
2A5	GGGAATAGCGAGATACATGTCAG	CAGGAGAGAAGGATTGTGCC	60	3
2A6	GCTTCTTCGTTGTTGCCTTC	TGCCAGTTGTTCATAGCTTCA	60	4
2F5	TGACCAAAGTGATGAAGGGA	AAGTTGTTTGTACATCTGTCATCG	58	4
2E6	TACAGCATTGCCTTCTGGTG	CCTGGGCTGGGGTATTATTT	60	2
2C11	TGACCTCAATTTTGGGGAAG	GCCACTATTCATCGCGGTA	60	3
3A8	ATACGTGACTTGGGCCAGAC	AGTGAAAAATACACCCAACGAA	60	4
5D5	AAAAGAAAGACCTTCCCCGA	GCAGGTAATCTGCCGTGATT	60	3
8D9	TGAGTTTCCCCAAAAGGAGA	CAACAACAATACGGCCAACA	60	3
7G2	ACTCCCGATGCACTTGAAAT	AACCTCTGTGCACTGTCCCT	60	4
8E12	TCTGTTGAGAACCACCAGCA	GTGCTAGTTGCTTGACGCAC	60	3
11H1	TTTGTGTTTAAGAAGGGGTGC	GCGGTCCAACATCCTTTTT	60	3
12F7	TGTCGTTGTAAGACCTCGGA	TTGGTTTCCTTAAGGCTTCG	55	2
13A10	AACTCGCTTGTACCGGCTAA	AGGAATAATAACAATACCAACAGCA	60	3
13E9	GGAGGAGGACGACGATGATA	TGTCGTCTCATCCAAAGGAA	60	2
14A7	GTACGCTTTTAATTTGCGGG	CTGGAAAGCCTTGAGAGCAT	60	2
14E10	ACCTAGTGGGACAAGGCTTTA	TTGACAAAATAACCTCACTTCGAT	55	2
14F4	ACGTTTAGTTGCTTGCGTGA	TGAATTCAAAGGAAAATGAAAAA	60	3
14H6	GCAACTAGGGTGTATGCCGT	CAACCCTATACACCGAGGGA	60	8
15C12	ACAATGCAATGACCGTTGTT	TTGTTGCATGAGAACGTGAA	60	4
16C6	TTGCTACTAAGCCGAAAATGAAG	CTTGAAATTAACACATATGCACACA	60	4
16F10	TGGAGGGAAAAACATTTTGG	CCTGGAGGGGTGAGAGGT	60	2
17E1	TTCGTTGACGTGAGCGTTAC	TTAGGATTGTTCCAAGGCCA	60	3
17F6	CGTCGGATTTATCTGCCAGT	AGTAGGGGCAAGGGTTGATG	58	3
18C5	GGACAGCCGGATGCTATTTA	ACATGAGTCCCTTTTCCCTT	60	3
19A5	ATTCGTCTCCTTCTTTTGGC	TTTTGCTTCCAAATGGCTTC	60	2
2D12B	AAGCTGAACGAACTCAAGGC	TGCAATGGGTACAATGCTAGA	60	4
10H1A	TGACAATGGGGTGTTCTTCA	GTAAACAGACGCCGTTCCAT	60	2

Table 3 Sequences of the polymorphic primers used in AFLP analysis

Series (P)	Sequences	Series (P)	Sequences	Series (M)	Sequences	Series (M)	Sequences
P1	P+AGA	P9	P+GGA	M47	M+CAA	M56	M+CGC
P2	P+AGT	P13	P+TGT	M48	M+CAC	M57	M+CGG
P3	P+CAC			M51	M+CCA	M58	M+CGT
P4	P+CAG			M52	M+CCC	M59	M+CTA
P5	P+CCA			M53	M+CCG	M60	M+CTC
P7	P+GCA			M54	M+CCT	M61	M+CTG
P8	P+GCT			M55	M+CGA	M62	M+CTT

P: gac tgc gta cat gca g; M: gat gag tcc tga gta a.

1.5 Data analysis

For SSR and AFLP analyses, the amplified bands were scored as present "1" and absent "0". Genetic similarity (GS) was calculated as described by Nei and Li (1979)[21]. Genetic distance (GD) was calculated as -ln(GS). Cluster analysis was conducted using the unweighted pair-group method of arithmetic means (UPGMA) based on the GDs.

2 Results

2.1 Diversity based on SSR analysis

Diversity assessment of the 31 peanut genotypes was performed using 78 SSR primer pairs, of which 29 primers amplified polymorphic bands. A total of 91 polymorphic loci were recorded among the peanut genotypes tested (Table 4). The 29 polymorphic SSR primers each amplified 2 to 8 microsatellite loci, with an average of 3.14 loci per primer. Several primers including 7G02, 14H6, 3A8, and 16C6 were more efficient than the rest in detecting the diversity among peanut genotypes since each amplified 4 to 8 loci.

Based on SSR analysis, the average genetic pairwise distance among the 31 genotypes was 0.53. The largest distance was 0.94 between 'Wuxuan Laohuasheng' and 'Taishan Zhenzhu' and the shortest distance was 0.12 between 'Feilongxiang' and 'Shitang Dahuasheng'. All these four lines were BW-resistant. Moreover, the 17 most diverse genotype pairs (with genetic distances over 0.8) were all BW-resistant. The distances among the susceptible accessions were relatively smaller, ranging from 0.47 (between 'Zhonghua 5' and 'Zao 18') to 0.55 (between 'Zhonghua 5' and 'Daye Bentianzi'). The average distance between the resistant and susceptible genotypes was 0.54 with the greatest diversity between 'Chico' and 'Zhongxinchi' (0.78). A BW-susceptible line, 'Zhonghua 5' with high yield and high oil content, had an average distance of 0.52 from the resistant genotypes with a range from 0.32 (between 'Zhonghua 5' and 'Shenxian Xiaohongmao') to 0.64 (between 'Zhonghua 5' and 'Lingui Make'). Six genotypes including 'Taishan Zhenzhu', 'Xiekangqing', 'Feilongxiang', 'Shitang Dahuasheng', 'Qidong Dahuasheng', and 'Lingui Make' had distances of over 0.6 in comparison with 'Zhonghua 5'. These results indicate that there is a considerable amount of genetic variation among the peanut genotypes involved, particularly the BW-resistant germplasm.

The cluster analysis using UPGMA based on genetic distances from SSR marker analysis revealed that the 31 genotypes can be divided into 2 groups at a genetic distance of 0.86 (Fig. 1). All genotypes belonging to subsp *hypogaea* (including var. *hirsuta* and var. *hypogaea*) except 'Shengxian Xiaohongmao' (belonging to var *hirsuta*) were grouped together (referred as 'subsp. *hypogaea* group'), and all genotypes of subsp. *fastigiata* including var. *fastigiata* and var. *vulgaris* were clustered in another group (referred as 'subsp. *fastigiata* group'). The genotypes of the subsp. *hypogaea* group were all landraces including 15 resistant genotypes and one susceptible genotype, and could be further divided into 3 sub-

Table 4 Polymorphic information among 31 peanut genotypes as revealed by AFLP analysis

Primer	Polymorphic loci	Primer	Polymorphic loci	Primer	Polymorphic loci	Primer	Polymorphic loci
P1M58	2	P3M59	1	P8M52	4	P9M52	2
P1M61	3	P3M60	2	P8M53	3	P9M57	1
P1M47	4	P3M61	1	P8M54	3	P9M61	1
P1M48	1	P4M47	3	P8M55	1	P13M50	2
P1M62	5	P4M55	2	P8M56	3	P13M51	1
P2M47	2	P5M48	2	P8M57	1	Total	72
P2M48	2	P5M53	1	P8M60	1		
P3M52	3	P7M55	1	P9M48	2		
P3M58	3	P8M47	3	P9M51	6		

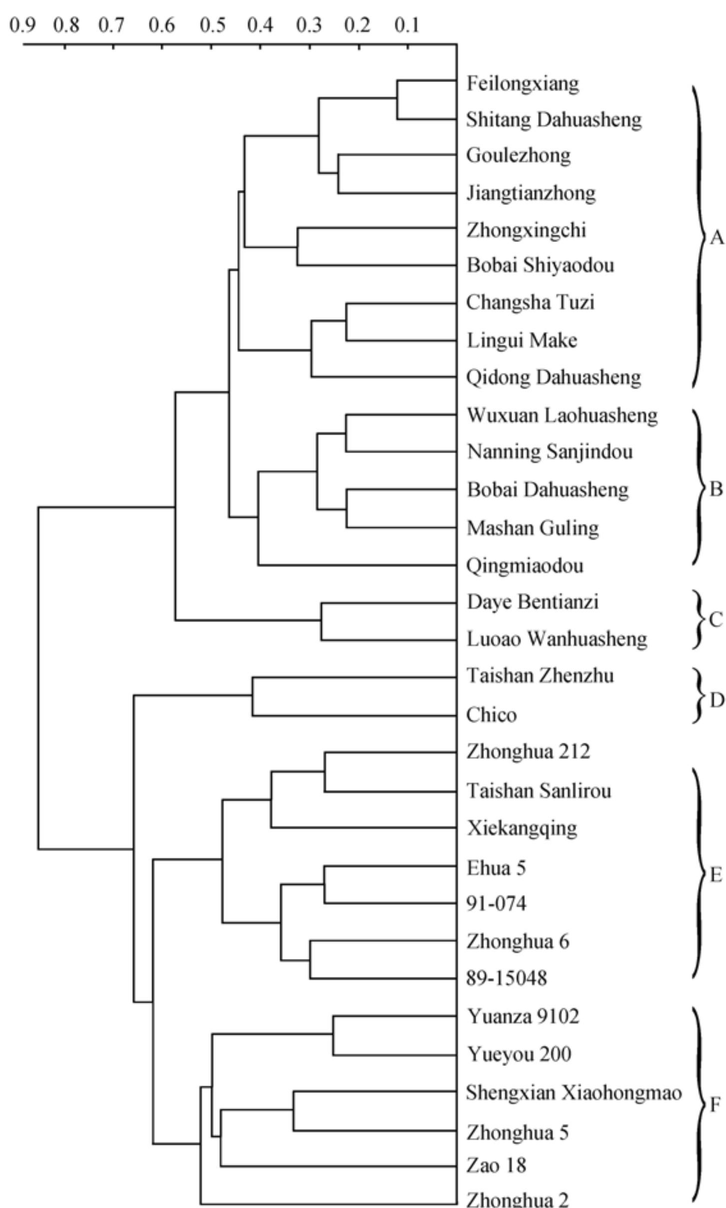

Fig. 1 Dendrogram of 31 peanut genotypes by SSR data analysis

groups at the genetic distance of 0.47 (subgroup A, B, and C). Subgroup A consist of 9 resistant genotypes including 7 *hirsuta* genotypes and 2 *hypogaea* genotypes. Two var. *hypogaea* genotypes, ‘Goulezhong’ and ‘Jiangtianzhong’, were grouped into the same sub-sub-group. Two var *hirsuta* genotypes from Hunan Province, ‘Changsha Tuzi’ and ‘Qidong Dahuasheng’, were also grouped into a sub-sub-group. Sub-group B included 5 var *hirsuta* genotypes with BW resistance. Sub-group C consisted of 2 var. *hirsuta* genotypes from Jiangxi Province, the BW-susceptible ‘Daye Bentianzi’ and the BW-resistant ‘Luoao Wanhuasheng’. The genetic variation in sub-group A was greater than in sub-group B and sub-group C.

The subsp. *fastigiata* group, included all the genotypes belonging to subsp. *fastigiata* except a var *hirsuta* landrace, ‘Shengxian Xiaohongmao’. This group consisting of 12 resistant and 3 susceptible genotypes could be further divided into 3 sub-groups (sub-group D, E, and F) at the genetic distance of 0.61. Therefore, the variation in this group was wider than that in the subsp. *hypogaea* group. Sub-group D included 2 var. *vulgairs* genotypes, the resistant landrace ‘Taishan Zhenzhu’ and the susceptible line ‘Chico’. Sub-group E consisted of 7 resistant genotypes; Zhonghua 212, 89-15048, and their common resistant parent ‘Taishan Sanlirou’; ‘Ehua 5’ and its reistant donor ‘Xiekangqing’ were grouped into this sub-group. Sub-group F consisted of ‘Shengxian Xiaohongmao’ and 5 improved breeding lines belonging to var *vulgaris*, of which, 3 were resistant and 2 susceptible to BW. In this sub-group, BW-susceptible ‘Zhonghua 5’ and BW-resistant ‘Zhonghua 2’ had a common susceptible parent ‘Ehua 4’. ‘Zhonghua 2’ however had another resistant parent ‘Xiekangqing’.

2.2 Genetic diversity based on AFLP analysis

One hundred and twenty-six randomly selected *Pst* Ⅰ/*Mse* Ⅰ primer combinations were used to amplify the DNA of the 31 peanut genotypes. Thirty-two combinations detected polymorphic DNA bands, each of these amplified 1 to 6 polymorphic loci, generating a total of 72 polymorphic bands with an average of 2.25 loci per primer (Table 4). Primer P9M51 amplified 6 polymorphic loci, and could group the 31 genotypes into 11 clusters and identify 5 unique genotypes among the 31 accessions. Primer P1M62 could amplify 5 polymorphic loci, and could classify the 31 genotypes into 11 clusters and identify 9 unique genotypes.

Based on AFLP analysis, the highest and the lowest pairwise distances among the 31 genotypes were 0.57 and 0.06, respectively, with an average distance of 0.25. There were 6 genotype pairs with distances over 0.5. In the dendrogram based on AFLP data (Fig. 2), the BW-susceptible var. *vulgaris* genotype, ‘Chico’ introduced from ICRISAT, was highly different from all other genotypes tested and was as such placed in a unique group. The other 30 genotypes, all of which originated from China, were divided into two groups at the genetic distance of 0.41, with all subsp. *hypogaea* genotypes (except ‘Shengxian Xiaohongmao’) in the first group (referred as ‘subsp. *hypogaea* group’) and most subsp. *fastigiata* genotypes in the second group (referred as ‘subsp. *fastigiata* group’). The breeding lines ‘Zao 18’, ‘Yueyou 200’, and ‘Ehua 5’ belonging to var. *vulgaris* were in the subsp. *hypogaea* group. The first group consisting of 14 var. *hirsuta*, 2 var. *hypogaea*, and 3 var. *vulgaris* genotypes was further divided into 3 sub-groups (A, B, and C). In sub-group A, all five genotypes were BW-resistant landraces belonging to var. *hirsuta*. Sub-group B consisted of 2 var. *vulgaris*, 1 var. *hypogaea*, and 8 var. *hirsuta* genotypes. Among the 11 genotypes, 9 were resistant and 2 were susceptible to BW (‘Zao 18’ and ‘Daye Bentianzi’). Sub-group C consisted of 1 var. *hypogaea*, 1 var. *hirsuta* landrace, and 1 advanced breeding line, ‘Ehua 5’. All these genotypes were BW-resistant.

The second group was comprised of 11 genotypes including a susceptible line. Among these, 1 was the var. *hirsuta* genotype and the other 10 were subsp. *fastigiata*. The genetic diversity in this group was greater than that in the first group. Two resistant

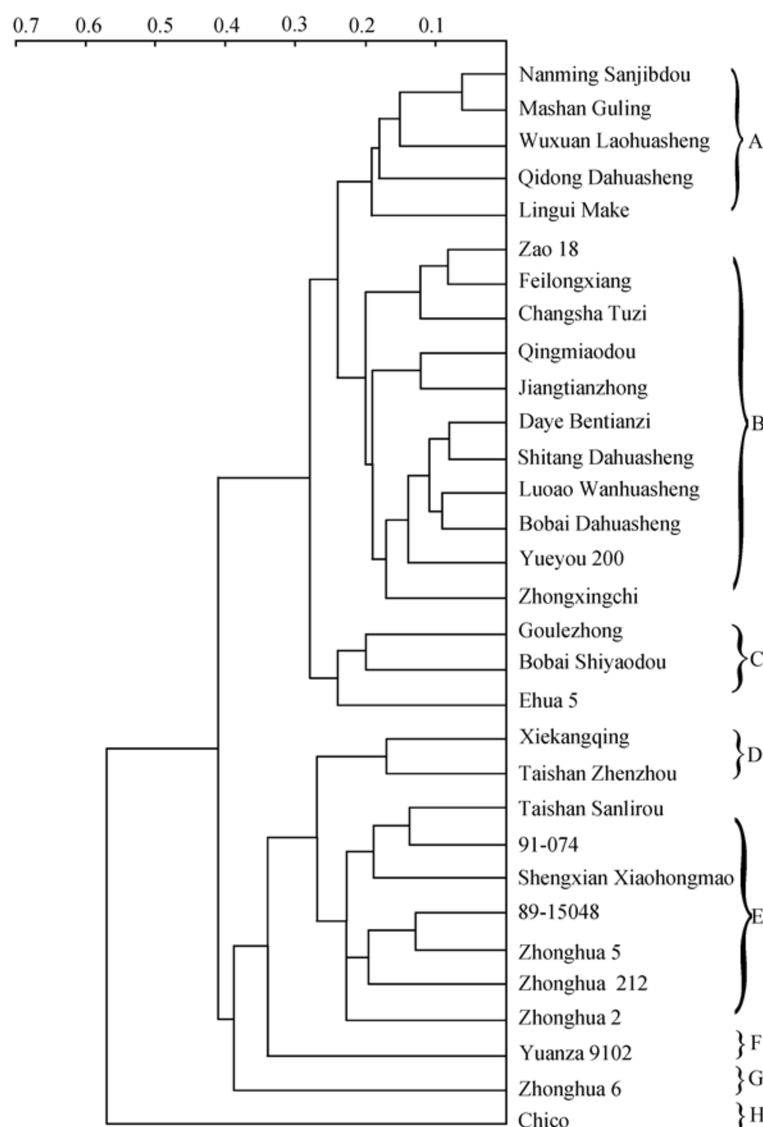

Fig. 2 Dendrogram of 31 peanut genotypes by AFLP data analysis

breeding lines, 'Yuanza 9102' and 'Zhonghua 6', were different from the other 9 genotypes and were grouped into two unique sub-groups (F and G), respectively. The remaining 9 genotypes were divided into two sub-groups (D and E). Sub-group D consisted of 2 var. *vulgaris* resistant landraces ('Xiekangqing' and 'Taishan Zhenzhu'), which have been extensively used in the previous breeding programs in China. In sub-group E, 'Shengxian Xiaohongmao' with BW resistance is a var. *hirsuta* line and 'Zhonghua 5' is a BW-susceptible breeding line belonging to var. *vulgaris*. The other four BW-resistant breeding lines, '91-074', '89-15048', 'Zhonghua 212', and 'Zhonghua 2', all having 'Taishan Sanlirou' as the resistant donor in their pedigrees, were all clustered in the same sub-group.

2.3 Relationship and diversity of peanut based on SSR together with AFLP analysis

Based on a combined analysis of SSR and AFLP data, the 31 peanut genotypes with bacterial wilt resistance were divided into two main groups (dendrogram

not shown). All subsp. *hypogaea* genotypes, except the *hirsuta* variety 'Shengxian Xiaohongmao', were clustered together in a group, which also contained 3 *vulgaris* varieties. The remaining 11 subsp. *fastigiata* varieties and one *hirsuta* genotype 'Shengxian Xiaohongmao' were placed in another group. The subsp. *hypogaea* group could be divided into four sub-groups and the two *hypogaea* genotypes, 'Goulezhong' and 'Jiangtianzhong' were divided into different subgroups. The subsp. *fastigiata* group could be divided into five subgroups. 'Chico' and 'Zhonghua 6' were different from the other genotypes in this group and formed two unique subgroups. The clustering based on the combined analysis of SSR and AFLP data was broadly similar to that generated based on either individual dataset.

3 Discussion

Bacterial wilt is a serious disease for peanut in certain regions in the world and genetic enhancement for resistance is important for sustainable development of peanut production in these disease-prone areas. More efforts are required to improve peanut cultivars by integration of BW resistance with other desirable agronomic characteristics. Better understanding of the genetic diversity of BW-resistant peanut germplasm is crucial for various reasons. Although the cultivated peanut germplasm exhibits a high level of morphological variation, the detectable level of DNA polymorphism in this species is relatively low when compared to other crops[19, 22–24]. Since He and Prakash (1997)[11] reported that there was considerable DNA polymorphism in *A. hypogaea* as revealed by the AFLP approach, this assay has been used for molecular diversity studies in peanut by several researchers [8-18, 25-28]. In the present study, both SSR and AFLP approaches detected acceptable levels of molecular diversity among the peanut lines with various levels of resistance to bacterial wilt, although some primers were more efficient than others.

Based on the SSR and AFLP analyses, genetic diversity among the peanut genotypes was verified at the molecular level. The diversity detected by SSR markers was greater than that observed through AFLP profiles. In the dendrogram based on SSR analysis (Fig. 1), all genotypes belonging to subsp. *fastigiata* including var. *vulgaris* and var. *fastigiata* were grouped together, and those belonging to subsp. *hypogaea* including var. *hypogaea* and var. *hirsuta* were classified into another group with the exception of 'Shengxian Xiaohongmao'.

The clustering of peanut genotypes based on SSR data and AFLP data were similar (Figs. 1 and 2), although the SSR clustering was more comparable to the current classification system based on morphological characteristics. Thus, the SSR approach may be more efficient for peanut. In the dendrogram based on AFLP analysis, several breeding lines, i.e. Zao 18, Yueyou 200, and Ehua 5, were grouped into the subsp *hypogaea* group (Fig. 2) even though these belonged to subsp *fastigiata*. It is interesting to note that these three breeding lines had direct or indirect parents belonging to subsp *hypogaea* [29], and there was no landrace of subsp *fastigiata* in the subsp *hypogaea* group (Fig. 2). Thus, it is possible that the AFLP approach can trace the genomic introgression among the subspecies or botanic varieties. Another var. *hirsuta* genotype 'Shengxian Xiaohongmao' was in the subsp *fastigiata* group in both SSR and AFLP based dendrograms. This genotype is morphologically different from most other var. *hirsuta* landraces particularly in a shorter growth period and less reticulated pods. Natural genomic introgression from subsp. *fastigiata* may have occurred during the evolution of this genotype, and its classification will be re-considered.

The frequency of BW-resistant germplasm accessions in different botanic types of *A. hypogaea* is different. The var. *hirsuta* (also known as dragon type) widely cultivated in China for hundreds of years before other botanical varieties were introduced [29], has the highest frequency of BW-resistant accessions. Among the 315 var. *hirsuta* lines collected, 68 have been identified as highly BW-resistant [6, 7], indicating

that var. *hirsuta* is an important source of BW resistance. One objective of investigating the genetic diversity of BW-resistant germplasm lines is to identify suitable genotypes for developing mapping population(s) and new cultivars. Based on the study, var. *hirsuta* resistant lines such as 'Lingui Make' and 'Feilongxiang' and var. *vulgaris* line 'Taishan Zhenzhu' are recommended as parents in crosses with the BW-susceptible breeding such as 'Zhonghua 5' with high yield, high seed oil content, and early maturity.

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用SSR和AFLP技术分析花生抗青枯病种质遗传多样性的比较

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摘 要: 由 *Ralstonia solanacearum* E. F. Smith 引起的青枯病是若干亚洲和非洲国家花生生产的重要限制因子，利用抗病品种是防治这一病害最好的措施。虽然一大批抗青枯病花生种质资源材料已被鉴定出来，但对其遗传多样性没有足够的研究，限制了在育种中的有效利用。本研究以 31 份对青枯病具有不同抗性的栽培种花生种质为材料，通过简单序列重复(SSR)和扩增片段长度多态性(AFLP)技术分析了它们的遗传多样性。通过 78 对 SSR 引物和 126 对 AFLP 引物的鉴定，筛选出能显示抗青枯病种质多态性的 SSR 引物 29 对和 AFLP 引物 32 对。所选用的 29 对多态性 SSR 引物共扩增 91 条多态性带，平均每对引物扩增 3.14 条多态性带; 32 对多态性 AFLP 引物共扩增 72 条多态性带，平均扩增 2.25 条多态性带。在所筛选引物中, 4 对 SSR 引物(14H06, 7G02, 3A8, 16C6)和 1 对 AFLP 引物(P1M62)检测花生多态性的效果优于其他引物。SSR 分析获得的 31 个花生种质的遗传距离为 0.12−0.94，平均为 0.53，而 AFLP 分析获得的遗传距离为 0.06~0.57，平均为 0.25，基于 SSR 分析的遗传距离大于基于 AFLP 分析的遗传距离，疏枝亚种组的遗传分化相对大于密枝亚种组。基于两种分析方法所获得的聚类结果基本一致，但 SSR 数据聚类结果与栽培种花生的形态分类系统更为吻合。根据分析结果，对构建青枯病抗性遗传图谱群体的核心亲本和抗性育种策略提出了建议。

关键词: 花生；抗青枯病种质；遗传多样性；SSR；AFLP

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