Genetic Diversity and Differentiation of *Alpinia Oxyphylla* Miquel as Revealed by AFLP Markers

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Abstract

In this study, we analyzed the genetic diversity and population structure of 90 *A. oxyphylla* accessions from Hainan island using amplified fragment length polymorphism (AFLP) markers. These 90 accessions were composed of 15 populations from different geographic locations and divided into 4 clusters (A, B, C, and D) using the Unweighted pair group method based on arithmetic average (UPGMA). The genetic similarity between individuals ranged from 0.47 to 1.00 (average of 0.74), and most accessions from the same geographic population were grouped together. Principal coordinate analysis (PCA) showed a clear distinction among three clusters (A, B and C). Based on the loci information, the population structure analysis results by STRUCTURE and TESS were consistent with the clustering of PCA. Nine AFLP primer combinations generated 1537 polymorphic bands displaying rich polymorphism, thus indicating high genetic diversity among these 15 populations with an average Nei's gene diversity of 0.1328 ± 0.160 . In conclusion, AFLP markers efficiently analyzed the genetic diversity in *A. oxyphylla*, demonstrating highly significant genetic variation within and among populations. However, intrapopulation genetic variance was much higher than interpopulation variability, suggesting that efforts should be made for in situ germplasm conservation and resistant varieties cultivation.

Introduction

Alpinia oxyphylla Miquel is a perennial herb belonging to the class Monocotyledoneae, genus *Alpinia*, which originates from the Hainan Island of China. It is mainly distributed throughout Hainan and Guangdong, and scattered across the provinces of Fujian and Guangxi (Wu 1964). Its capsular fruits are rich in phenolic compounds, such as nootkatone that is commonly used in traditional East Asian medicines (including China, India, Japan, Korea) for the treatment of diarrhea, intestinal disorders, diuresis, Alzheimer's disease, and enuresis (Commission 2010; But et al. 1997; Zhang et al. 2013; Wang et al. 2018; Xu et al. 2020). Yakuchinone A and B, the major diarylheptanoids present in the pericarp of its fruit exhibit anti-inflammatory, antioxidant, anti-tumorigenic activities, and found to be cardioprotective (Chen et al. 2014; Chun et al. 2002; Lin et al. 2013; Shoji et al. 2018). *A. oxyphylla* is widely distributed in the East Asian region, especially it is a Geoherb of Hainan province in China. As medicinal and flavoring properties, *A. oxyphylla* is one of the four popular traditional herbs in southern China along with *Areca catechu* Linn, *Amomum villosum* Lour, and *Morinda officinalis* How (Gao et al. 2019). Most species of Zingiberaceae exist as herbaceous undergrowth and are seriously threatened by habitat destruction due to deforestation. Since most medicinal plant species in our country are harvested from the wild, this further endangers or accelerates the extinction of some species, such as *A. oxyphylla* (Zhou et al. 2016). Meanwhile, another major problem is the declining quality of cultivated *A. oxyphylla*. Genetic diversity is an important part of biodiversity, thus, it is imperative to evaluate the genetic diversity of *A. oxyphylla*, so that we can conserve it more efficiently and guarantee its sustainable development. In addition, it is found that *A. oxyphylla* collected from different geographic regions are discrepant in the quantities of chemical constituents (Song et al. 2014; Zhai et al. 2013). In order to ensure the quality and its therapeutic efficacy, it is necessary to understand more about the genetic diversity and population structure of the species, which can serve as a guide to help characterize the chemical composition of this medicinal plant (Chen et al. 2010).

DNA markers are the most powerful tools because they are not influenced by environmental factors and the developmental stages of the plant (Zou et al. 2013; Ellegren and Galtier 2016). Amplified fragment length polymorphism (AFLP) is a powerful marker system because it can assess multiple polymorphic loci simultaneously throughout the genome, and has a high multiplex ratio allowing the generation of larger number of amplified products in a single reaction (Karimi et al. 2009). AFLP is a robust and highly informative technique that has been widely used to reveal genetic diversity, study gene flow, population structure, and high-density linkage map construction in different species (Sorkheh et al. 2007; Kafkas et al. 2006; Zhang et al. 2018; Moreira et al. 2018). Inter-simple sequence repeat (ISSR) was used for the analysis of genetic diversity in *A. oxyphylla* in a previous study (Pagliaccia et al. 2018). However, being dominant, ISSR markers are not effective in solving the problems of mating system and calculating heterozygosity. Moreover, there are few studies on genetic diversity of *A. oxyphylla* population, and it is not known whether cultivated *A. oxyphylla* populations have enough genetic diversity. Furthermore, no other comprehensive study on molecular phylogenetic analysis of this species is available. The objectives of the present study are (1) To examine the level of polymorphism of AFLP markers in the studied genotypes of *A. oxyphylla*; (2) To evaluate the genetic relationship within and among *A. oxyphylla* populations; (3) To study the gene flow among different regions.

Materials And Methods

Plant materials and DNA extraction

Fifteen populations of *A. oxyphylla* were collected respectively from 15 counties of Hainan island (Fig. 1), China. A total of 90 accessions were evaluated; each population consisted of 6 accessions. Their sampling locations and geographic coordinates are shown in Table 1. The stands were chosen to represent the ecological conditions of the area. Total genomic DNA was extracted from the young leaves using the Tiangen Plant Genomic DNA Kit (Tiangen Biotech Co., Beijing, China). The genomic DNA was assessed for quantity and quality by 0.8% agarose gel electrophoresis, NanoDropTM 2000 spectrophotometer, and Agilent 2100 bioanalyzer.

Table 1
Name locations of *A. oxyphylla* populations used in this study

Population	Size	Location	Altitude(m)	Latitude(N.)	Longitude(E.)
1	6	Tunchang (TC)	140.4	109.988441	19.204969
2	6	Qiongzong (QZ1)	291.5	109.886536	19.153849
3	6	Qiongzong (QZ2)	354.7	109.828278	19.010242
4	6	Qiongzong (QZ3)	476.9	109.762375	19.225842
5	6	Wuzhishan (WZS1)	702.1	109.553627	18.864595
6	6	Wuzhishan (WZS2)	389.3	109.576630	18.677835
7	6	Baisha (BS1)	326.1	109.553627	19.109207
8	6	Baisha (BS2)	233.1	109.437569	19.19680
9	6	Wanning (WN1)	32.4	110.188637	18.716948
10	6	Wanning (WN2)	88.4	110.076866	18.638124
11	6	Lingshui (LS1)	32.5	110.570373	18.601246
12	6	Lingshui (LS2)	64.8	109.954460	18.648668
13	6	Baoting (BT1)	155.4	109.732185	18.731859
14	6	Baoting (BT2)	251.1	109.743408	18.735134
15	6	Baoting (BT3)	390.3	109.579536	18.608482

AFLP analysis

AFLP assays, including PCR amplification and product electrophoresis, were performed as described by Vos et al. (Vos P et al. 1995). Pre-selective amplification was performed with (EcoRI + 1: 5'> GAC TGC GTA CCA ATT CA < 3') / (MseI + 1: 5'> GAT GAG TCC TGA GTA A C < 3') primers. Nine informative and reliable combinations of selective primers (EcoRI + 3)/(MseI + 3) were selected from a set of 64 primer pairs. All the primers were fluorescently labeled (Table 2).

Table 2
Sequences of AFLP primers and their diversity analysis.

Primers	<i>Na</i>	<i>Ne</i>	<i>H</i>	<i>I</i>	PB	PPL(%)
E-AAC/M-CAG	1.807 ± 0.398	1.204 ± 0.295	0.131 ± 0.160	0.217 ± 0.226	174	80.56
E-AAC/M-CTA	1.722 ± 0.448	1.206 ± 0.302	0.129 ± 0.166	0.210 ± 0.238	156	72.22
E-AAC/M-CTT	1.773 ± 0.419	1.201 ± 0.271	0.134 ± 0.151	0.223 ± 0.218	167	77.31
E-AAG/M-CTC	1.662 ± 0.474	1.260 ± 0.337	0.157 ± 0.184	0.245 ± 0.262	143	66.20
E-ACT/M-CAG	1.856 ± 0.351	1.189 ± 0.280	0.124 ± 0.151	0.211 ± 0.212	185	85.65
E-AGG/M-CAC	1.671 ± 0.471	1.155 ± 0.262	0.101 ± 0.149	0.168 ± 0.217	145	67.13
E-AGG/M-CAG	1.842 ± 0.365	1.188 ± 0.269	0.124 ± 0.151	0.209 ± 0.216	182	84.26
E-AGG/M-CTC	1.884 ± 0.321	1.215 ± 0.305	0.136 ± 0.164	0.225 ± 0.229	191	88.43
E-AGG/M-CTT	1.898 ± 0.303	1.236 ± 0.291	0.154 ± 0.160	0.255 ± 0.224	194	89.81
Average	1.791 ± 0.394	1.206 ± 0.290	0.133 ± 0.159	0.218 ± 0.227		79.06
Total					1537	
Note: E, EcoRI; M, MseI; PB, polymorphic band; PPL, percentage of polymorphic loci; <i>Na</i> = Observed number of alleles; <i>Ne</i> = Effective number of alleles [Kimura and Crow (1964)]; <i>H</i> = Nei's (1973) gene diversity; <i>I</i> = Shannon's Information index [Lewontin (1972)].						

Selective amplification reactions were carried out in a PCR volume of 25 µL, containing 2 µL of pre-amplification products, 2.5 µL PCR buffer, 0.5 µL dNTP, 0.5 µL Taq DNA polymerase (Tiangen Biotech Co., Beijing, China), and 1 µL of EcoRI + 3 and MseI + 3 primer, respectively. Amplification was conducted in a Gene Amp PCR System 9600 (Perkin Elmer, USA), and the action program was set as follows: initial denaturation at 94°C for 30 s, 30 s annealing at 65°C, and 80 s extension at 72 °C, followed by 12 cycles in which annealing temperature was decreased progressively by

0.7°C per cycle. It was followed by 23 cycles of 30 s denaturation at 94°C, 30 s annealing at 55°C, and 80 s extension at 72°C, 4°C storage. The amplification products were resolved by electrophoresis through 4% polyacrylamide gel at 6V/cm for 1.0 h in a large horizontal tank electrophoresis unit (Ding Guo Chang Sheng, Beijing). The gels were stained with SYBR Goldview™ (Invitrogen, USA) DNA fluorescent dye and visualized using a Tanon 4100 imaging system.

Data analysis

The gels were scanned by ABI 377 DNA Sequencer; bands were scored using software GENESCAN version 3.1 as present (1) or absent (0) to create a binary matrix, and scores were recorded using Microsoft Excel software. The DNA ladder ROX500 (ABI, USA) with 70–500 bp were used to estimate the size of each DNA fragment. A dendrogram of genetic relationship was plotted through Nei's genetic distance (Nei 1973) and produced by clustering the data using unweighted pair group method with arithmetic average (UPGMA). Principal coordinate analysis (PCA) was also based on similarity matrices, and all were performed using the NTSYS-pc2.10 software. Markov chain monte carlo algorithm was used to infer genetic structure and to define the number of clusters in the data set using STRUCTURE v2.3.4 (Falush et al. 2000) software. For further assessing the genetic variation of populations, a combination of matrix factorization and spatial statistical method was used to estimates of ancestry coefficients by TESS Version 2.3.1 (Caye et al. 2016).

The percentage of polymorphic loci (PPL), observed number of alleles (N_a), effective number of alleles (N_e), Nei's genetic diversity (H), Shannon's information index (I), total genetic diversity (H_t), and genetic diversity within a population (H_s) were calculated in each primer. The genetic differentiation coefficient (G_{st}), gene flow (N_m), and the genetic distance were calculated among populations. All calculations were performed using POPGENE version 1.31 (Yeh et al. 1999). Analysis of molecular variance (AMOVA) was performed using Genalex6.2 software (Peakall and Smouse 2006).

Results

Genetic diversity

The prescreening analysis showed robust and reproducible amplification products, all of which displayed polymorphism among the accession of species. As highly polymorphic primers were used for analysis, a relatively large number of polymorphic AFLP markers were detected by these primer combinations. A total of 1537 polymorphic bands (PB) were found, and the number of PB per AFLP primer combination ranged from 143 to 196 for EcoRI + 3/MseI + 3 with an overall average of 170.78. For each of 9 primer combinations, the percentage of polymorphic loci (PPL) value ranged between 66.2% and 89.81%, and the average comprised of 79.06% of total PPL (Table 2). It shows that there is a higher genetic diversity for these 15 populations with an average Nei's gene diversity of 0.1328 ± 0.1598 and an average genetic diversity index of 0.2185 ± 0.2273 . The genetic diversity index ranged from 0.1684 ± 0.2171 to 0.2553 ± 0.2245 with most of the primers exhibiting a genetic diversity index over 0.2, demonstrating high polymorphism in this study.

Clusters of *A. oxyphylla* accessions collected from different locations

On the basis of UPGMA clustering of the genetic similarity matrix, 88 *A. oxyphylla* accessions (15 populations) were divided into two main groups (group I and II) with a similarity coefficient about 0.50. Accessions from the same geographic location were grouped together with several crosses. Group I consisted of cluster A and B, while group II had only cluster C. Clusters A and B with a similarity coefficient of approximately 0.522, were composed of populations 1, 2, 3, 4, 5 from TC, QZ1, QZ2, QZ3, WZS1 and populations 6, 7, 8, 9, 10 from WZS2, BS1, BS2, WN1, and WN2, respectively. Cluster C was composed of accessions from populations 11, 12, 13, 14 and 15 from LS1, LS2, BT1, BT2 and BT3, respectively (Fig. 2). Two accessions 7 – 6 and 9 – 1 from BS1 and WN1, respectively, occurred in cluster D, which were remote relative to the others, with the coefficient value 0.47. In order to provide an objective comparison, matrices comparison and correlation test were generated from UPGMA data using the Mantel test that revealed not significant and quite low correlation between the dendrograms. After doing 1000 random permutations with Max comp procedure, cophenetic values were obtained ($r = 0.77$, $P = 1.00$) from NTSYS program, which proved the clustering result is credible. According to Bayesian clustering analysis using STRUCTURE software, based on the most likely value of K (Evanno et al. 2005) was three the division of variation into three clusters. we observed Cluster A and B was similar in structure (blue color), Cluster C (red color) was distinct with them. These results confirmed the two grouping that in UPGMA dendrogram. According to the loci information, the two accession 7 – 6 and 9 – 1 were similar with accessions of Cluster B. The results indicate that the accessions of *A. oxyphylla* that originated from different regions were genetically distinct from each other. The genetic diversity was primarily high within *A. oxyphylla* populations while the gene flow between populations was not high.

PCA of all *A. oxyphylla* accessions

The Principal coordinates analysis (PCA) based on a bi-dimensional space of genetic structure revealed genetic differences between populations. The PCA plot (Fig. 3) shows that the accessions from Clusters A, B, and C were grouped together, Cluster A and B are closer than with C, and without unique accessions (Cluster D consist with 7 – 6 and 9 – 1, which can't be distinguished from Cluster B in PCA plot), all of them were grouped into corresponding centralized clusters, which was consistent with the results from least-squares method using TESS 3 software. Three

clusters were divided (blue, purple and green), indicating the most appropriate three main clusters within *A. oxyphylla* species populations. This observation confirmed that these populations evolved independently in separate locations within Hainan island, with limited germplasm exchange.

Estimation of genetic diversity parameters and differentiation of populations

The total genetic diversity (H_t) of *A. oxyphylla* was 0.133, and the genetic diversity within a population (H_s) was 0.094. The coefficient of gene differentiation (G_{st}) of 0.284 indicated that 28.4% of the total genetic variation of *A. oxyphylla* occurred within populations, while the majority (approximately 71.6%) occurred between populations. There were low levels of gene flow with the Shannon's information index (I) between populations being 1.381 (Table 3).

Table 3
Genetic differentiation coefficient of 15 populations.

	<i>Ht</i>	<i>Hs</i>	<i>Gst</i>	<i>Nm</i>
Mean	0.133	0.094	0.283	1.381
St. Dev.	0.026	0.012		
<i>*Ht</i> , total genetic diversity; <i>Hs</i> , genetic diversity within populations; <i>Gst</i> , coefficient of genetic differentiation; $Gst = Dst/Ht$; <i>Nm</i> , gene flow, where $Nm = 0.5 (1-Gst)/Gst$.				

Using Poptools version 1.31, we calculated Nei's genetic distance and genetic identity between each pair of populations. It was found that Nei's genetic distance ranged from 0.024 (for population 7 and population 9) to 0.062 (for population 3 and population 13), and the genetic identity ranged from 0.932 (for population 1 and population 13) to 0.976 (for population 7 and population 9) (Table 4). This indicated that the genetic distance between the populations of Qiongzong and Baoting was the longest, while that between Baisha and Wanning populations was the shortest. The molecular variance obtained in this study indicates a highly significant genetic differentiation ($P = 0.001$) within and among populations of *A. oxyphylla* (Table 5). However, intrapopulation genetic variance was much higher than interpopulation genetic variance. These results suggest means that for cultivated populations of *A. oxyphylla*, most (81%) of the genetic variation occurs within populations and far less (18%) occurs among populations.

Table 4
Nei's Original Measures of genetic identity (above diagonal) and genetic distance (below diagonal) among populations

Pop.ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	****	0.969	0.960	0.963	0.955	0.937	0.945	0.937	0.941	0.938	0.937	0.936	0.931	0.937	0.937
2	0.031	****	0.974	0.976	0.969	0.946	0.957	0.953	0.956	0.953	0.948	0.947	0.943	0.948	0.946
3	0.040	0.025	****	0.975	0.969	0.943	0.956	0.952	0.951	0.956	0.944	0.943	0.940	0.943	0.940
4	0.037	0.024	0.024	****	0.974	0.947	0.956	0.949	0.954	0.953	0.948	0.948	0.945	0.948	0.947
5	0.045	0.031	0.030	0.026	****	0.950	0.957	0.951	0.952	0.956	0.948	0.948	0.947	0.945	0.943
6	0.064	0.055	0.059	0.054	0.051	****	0.968	0.954	0.966	0.967	0.945	0.949	0.947	0.950	0.947
7	0.056	0.043	0.045	0.044	0.043	0.032	****	0.974	0.976	0.972	0.954	0.955	0.955	0.957	0.954
8	0.064	0.048	0.048	0.052	0.050	0.046	0.026	****	0.964	0.966	0.946	0.946	0.946	0.946	0.941
9	0.061	0.045	0.049	0.046	0.049	0.034	0.024	0.035	****	0.973	0.949	0.951	0.951	0.954	0.952
10	0.064	0.048	0.045	0.048	0.045	0.033	0.027	0.034	0.027	****	0.951	0.953	0.951	0.952	0.948
11	0.064	0.053	0.057	0.053	0.053	0.056	0.046	0.055	0.052	0.050	****	0.973	0.968	0.968	0.964
12	0.065	0.054	0.059	0.053	0.054	0.052	0.045	0.055	0.050	0.048	0.026	****	0.974	0.975	0.970
13	0.071	0.058	0.062	0.056	0.055	0.054	0.045	0.055	0.050	0.050	0.032	0.025	****	0.974	0.969
14	0.064	0.053	0.058	0.052	0.056	0.051	0.043	0.055	0.047	0.049	0.031	0.025	0.025	****	0.974
15	0.065	0.056	0.061	0.054	0.059	0.054	0.046	0.061	0.049	0.053	0.036	0.029	0.031	0.025	****

Table 5
Results of analysis of molecular variance of 15 populations.

SV	DF	MSD	VC(%)	P*
Interpopulations	14	308.779	19	0.001
Intrapopulations	75	128.998	81	0.001
SV, source of variance; DF, degree of freedom; MSD, mean squared deviation; VC, variance component. *After 1000 random permutations.				

Discussion

Genetic diversity values obtained with molecular markers are difficult to compare between studies, because the statistical methods used to process the data often vary, particularly with dominant and co-dominant markers. However, parameters such as intrapopulation genetic markers may serve to establish comparisons between species (Mueller and Wolfenbarger 1999). The genetic diversity of *A. oxyphylla* using ISSR molecular markers has been studied partially with 163 accessions collected from 8 populations in a previous study (Wang et al. 2012). The Observed number of alleles (N_a) was 1.9080, the effective number of alleles (N_e) was 1.3418, Nei's (1973) gene diversity (H) was 0.2129, and the Shannon's Information index (I) was 0.3373. Looking at the values obtained in our study ($N_a = 1.7906$, $N_e = 1.2046$, $H = 0.1328$, $I = 0.2185$), the level of genetic diversity was less compared to previous studies. This may be attributable to our accessions that were collected from relatively concentrated populations in the central part of Hainan Island and all of them were cultivated populations. Moreover, the sample size of present study (90) is smaller than that of ISSR (163), which may be another reason for low genetic diversity index. Lastly, since AFLP overestimates numbers of loci and underestimates numbers of alleles, it can detect many fragments that occur at low frequency and the low diversity estimates obtained may be due to the markers technology used rather than the low genetic diversity in the species (Nybom 2004; Caicedo 1999).

The variation of population genetic structure is an important manifestation of genetic diversity (Hamrich 1989). A species' evolutionary potential and ability to resist adverse environment depend not only on the size of intraspecific genetic variation but also on the genetic structure of the population (Millar et al. 1991). With limited lifespan of organisms, it becomes necessary for the population to evolve and become better suited to the environment. Therefore, a genetic mechanism that affects the genetic structure of the population is required. The genetic structure is mainly reflected by the genetic differentiation within and between populations. The amount of gene flow can reflect the extent of population genetic differentiation. If the gene flow is smooth, the genetic differentiation between populations is small, whereas if there is restricted gene flow, the genetic differentiation between populations is large (Collevatti et al. 2001). But if the gene flow is greater than 1, there is no problem in the communication among populations. As shown in the UPGMA tree (Fig. 1), accessions from the same geographic population were grouped together. This was consistent with a previous report, wherein WZS populations with rich genetic diversity were older than the other populations studied (Wang et al. 2012). However, WZS populations in our study were clustered with TC, QZ, WN and BS populations, whereas BT and LS populations were clustered together more remote than them. The lower gene flow ($N_m = 0.9074$) exposed by Wang (2012) may be due to the large sampling range and lack of continuity; many intermediate populations were not collected (Wang et al. 2012). In addition, the gene flow among populations in our study ($N_m = 1.3814$) was higher compared with previous study ($N_m = 0.9074$) because of geographical proximity affecting germplasm conservation and gene exchange among these populations.

Genetic diversity is the primary objective in nature conservation, whereby the evolutionary potential of species is preserved. Thus, it is crucial to investigate the genetic variation among and within populations in order to formulate appropriate strategies of plant species' conservation (Milligan et al. 1994). Our results reveal that the genetic diversity of cultivated *A. oxyphylla* populations is limited; gene flow exists among populations, and variation mainly exists within populations. Due to the frequent human activities in the past few decades in Hainan Island, the habitat of *A. oxyphylla* has been seriously damaged. With rapid deforestation, wild *A. oxyphylla* and associated species have also disappeared. The degeneration of cultivated germplasm is not optimistic, especially in recent years, the diseases and insect pests become more and more serious. In general, the collection and propagation of seeds using seedling propagation technology to improve germplasm diversity and cultivate resistant varieties is required urgently.

Conclusion

This study demonstrates that the AFLP technique is a reliable tool for differentiating *A. oxyphylla* accessions and determining the genetic relationship among accessions. Nine AFLP primer combinations generated 1537 polymorphic loci. PPL value ranged between 66.2% and 89.81%, and the average comprised of 79.06% of total PPL, which displayed rich polymorphism. The population structure and phylogenetic analysis revealed that the genetic diversity of cultivated *A. oxyphylla* populations is limited, gene flow exists among populations, and variation mainly exists within populations. Hence, the genetic diversity and population structure analysis reported here will facilitate genetic improvement and cultivar development with desired traits in further *A. oxyphylla* resistant varieties breeding programs.

Declarations

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Author contribution Conceived and designed the experiments: BG. Performed the experiments: KP, WS and JH. Analyzed the data: KP, BY and BG. Wrote the original draft preparation: KP. Wrote and review and editing: BG, KP, WS and BY.

Data availability All data generated or analyzed during this study are included in this published article.

Conflict of interest The authors declare no competing interests.

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Figures

Figure 1

Geographical locations for 15 *A. oxyphylla* populations on Hainan Island. ☆ Tunchang(TC) ○ Qiongzhong(QZ1) ● Qiongzhong(QZ2) ● Qiongzhong(QZ3) □ Wuzhishan(WZS1) ■ Wuzhishan(WZS2) ▲ Baisha(BS1) △ Baisha(BS2) ⊙ Wanning(WN1) ☆ Wanning(WN2) ◇ Lingshui(LS1) ⊙ Lingshui(LS2) ⊖ Baoting(BT1) ⊖ Baoting(BT2) ≡ Baoting(BT3).

Figure 2

Dendrogram of 90 *A. oxyphylla* accessions based on Jaccard's similarity coefficient through unweighted paired-group method with arithmetic average clustering analysis. Bar graphs showing genetic diversity structure for the 90 accessions of *A. oxyphylla* species as assessed using STRUCTURE and TESS software. Each cluster is represented by a different color (blue, red, purple and green).

Figure 3

Principal coordinates analysis plot of 90 *A. oxyphylla* Accessions based on AFLP binary data.