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Population genetic diversity and genetic structure of *Amorphophallus yunnanensis* in Southwestern China and its conservation implication

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ABSTRACT: *Amorphophallus yunnanensis* is a perennial herb distributed throughout southwestern China. The degradation and reduction of habitats due to human activities caused declines in *A. yunnanensis*. We developed simple sequence repeat (SSR) markers with restriction site-associated DNA sequencing (RAD-seq) to estimate the genetic diversity and evaluate population differentiation of *A. yunnanensis* in southwestern China. We found that the genetic diversity of *A. yunnanensis* was relatively low compared to the widely distributed *Amorphophallus konjac*. Population structure analyses revealed three genetic groups that corresponded to geographic distributions. One of these genetic groups (group A) that consisted of two populations in the relatively southern part exhibited the highest genetic diversity and more rare alleles, suggesting a hotspot of diversification in *A. yunnanensis*. These results indicated that the three genetic groups should be treated as distinct evolutionarily significant units and a higher conservation priority should be placed on group A.

KEY WORDS: *Amorphophallus yunnanensis*, Genetics diversity, Genetic monitoring, Population structure, RAD-seq, SSR.

INTRODUCTION

Amorphophallus Bl. (Araceae) is a genus of perennial herbs with a solitary leaf and an underground stem. This genus contains nearly 200 species distributed across Southeast Asia, Africa, and Australia (Chua *et al.*, 2010; Tomlinson *et al.*, 1998). Southwestern China is recognized as one of the origin centers of *Amorphophallus* species, and over 20 species are found in China (Liu, 2003). As the only plant producing large amounts of glucomannan, the *Amorphophallus* corm is considered low-calorie health food (Srzednicki and Borompichaichartkul, 2020; Yin *et al.*, 2019). *Amorphophallus* species have been historically used as sources for foods and traditional medicine in China (Liu, 2003). However, many *Amorphophallus* species are threatened by habitat loss and degradation in China (Gao *et al.*, 2017; Long *et al.*, 2003).

Amorphophallus yunnanensis is a diploid plant (2n = 26) distributed across Southwestern China (Yunnan, Guizhou, and Guangxi provinces), Laos, Thailand, and Vietnam (Li *et al.*, 2010; Liu, 2003). Due to the wide application of Konjac glucomannan (KGM) in many industrial fields, the production of KGM can hardly meet the high market demand. Therefore, the natural resources of *Amorphophallus* species are over-exploited, which severely affects the sustention of wild populations (Jiang *et al.*, 2019; Tang *et al.*, 2020). In addition, *A. yunnanensis* favors shady, fertile, and moist environments. It is usually distributed in forest margins, such as roadsides, valleys, and shaded mountain slopes (Li *et al.*, 2010). These habitats are very vulnerable because of human disturbance. The wild populations are strongly threatened by human activities such as land opening for agriculture, road building, and tourism development in their natural habitats (Long *et al.*, 2003). Based on our field investigations, *A. yunnanensis* can no longer be found in some places previously reported. Although *A. yunnanensis* is not listed in the red list of threatened species in China, the conservation of this species is needed. Genetic diversity is one of the three levels of biodiversity. It is also the basis for natural selection. Therefore, maintaining genetic diversity is critical to the persistence of the species (Faith, 1994; Rao and Hodgkin, 2002). However, no population genetic study has been conducted for *A. yunnanensis.* To make reliable conservation suggestions, it is necessary to assess the genetic diversity and population genetic structure of this species.

Simple sequence repeats (SSRs) are one of the most informative genetic markers, and have been widely used in population genetic studies in numerous taxa, such as *Triticum aestivum*, *Pandanus boninensis*, and *Blumeria graminis* (Chae *et al.*, 2014; Ekué *et al.*, 2009; Gupta *et al.*, 2003; Santosa *et al.*, 2007; Setsuko *et al.*, 2020; Wu *et al.*, 2019). Additionally, next-generation sequencing technologies, such as restriction site-associated DNA sequencing (RAD-seq), provide an affordable opportunity for characterizing thousands of SSRs from organisms without genome sequences (Thiel *et al.*, 2003; Zalapa *et al.*, 2012). SSR markers for several *Amorphophallus* species (*A. paeoniifolius* and *A. albus*) have been developed using RAD-seq (Gao *et al.*, 2018a; Yin *et al.*, 2019). However, no SSR markers are

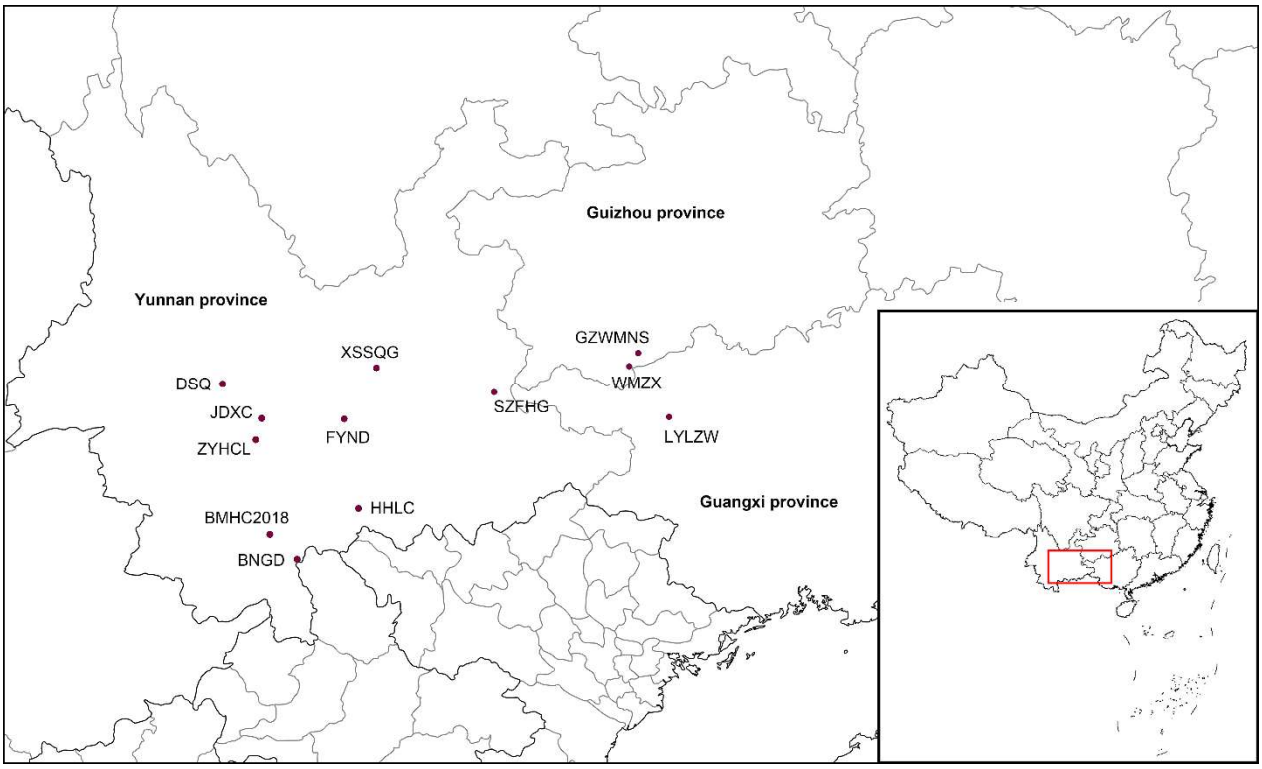

Fig. 1. Sampling locations of ***Amorphophallus yunnanensis*** populations in China.

available for *A. yunnanensis* to date, which hindered population genetic researches of this species.

This study sequenced three individuals of *A. yunnanensis* with RAD-seq and developed genomic SSRs from the sequencing data. These SSR markers were then used to estimate the genetic diversity and evaluate the population genetic structure of *A. yunnanensis* in southwestern China.

MATERIALS AND METHODS

Sample collection and DNA isolation

Twelve populations were sampled during 2017 and 2018, which covered the entire geographic range of *A. yunnanensis* in China (Table 1; Fig. 1). According to our observation, the population size of *A. yunnanensis* is relatively small (usually 5 to 30 individuals), and 4-12 individuals per population were sampled for population genetic studies (98 individuals in total). Approximately 5 g of leaves were collected per plant, and the genomic DNA was then isolated using a commercial DNA extraction kit (DP305; Tiangen, Beijing, China).

SSR Mining and Primer Design

Three samples (one individual from each population: SZFHG, GZWMNS and WMZX; Table 1) were used for genome sequencing. DNA samples were digested with EcoRI, randomly fragmented, and prepared as multiplexed RAD libraries following the established methods (Baird *et al.*, 2008) and then paired-end sequenced with a read length of 150 bp (PE150) on a NovaSeq 6000 platform (Illumina, USA). Sequence assembly and SSR mining procedures were adopted from previous studies (Gao *et al.*, 2018a; Tian *et al.*, 2016). Primers for each locus were designed using Primer 3 (Rozen and Skaletsky, 2000).

SSR genotyping

One hundred SSR primers were randomly selected and assessed in a pilot experiment with 24 individuals from four populations (GZWMNS, SZWJSD, BMHC2018, and ZYHCL; six samples each population; Table 1). Twelve polymorphic markers were chosen to genotype for all samples. The reagent of polymerase chain reactions (PCRs) (20 μL) comprised 1× PCR Mix (GeneStar, Beijing, China), 0.025 μM forward primer with an M13 tail (5′-CACGACGTTGTAAA ACGAC-3′), 0.5 μM reverse primer, 0.5 μM M13 primer fluorescently labeled with 5′ 6-FAM or HEX, and 30 ng of template DNA. A touchdown PCR procedure was employed, which included five amplification cycles with the annealing temperature starting from 62°C and decreasing 1°C per cycle, and additional 30 cycles with the annealing temperature set to 56°C. The duration of each annealing, denature, and extension step of PCR was 30s, 30s, and 45s, respectively. PCR products were electrophoretically separated on an ABI 3730 sequencer and analyzed using GeneMarker 2.3 (Applied Biosystems).

Table 1. Summary of population locations and genetic diversity for 12 populations of ***Amorphophallus yunnanensis*** in China.

	Population	Latitude	Longitude	Altitude	*N*	N_A	N_E	A_P	*I*	H_O	H_E	F_{IS}
Group A					16	4.333	2.459	19	0.934	0.261	0.452	**0.289**
	BMHC2018	22°39'25.2"	101°9'28.8"	1680 m	8	2.583	1.816	6	0.634	0.234	0.371	**0.329**
	ZYHCL	23°57'38.27"	100°57'48.24"	1897 m	8	3.083	2.197	11	0.734	0.264	0.379	**0.178**
Group B					58	3.083	1.450	4	0.470	0.352	0.263	-0.189
	SZFHG	24°36'46.8"	104°15'3.6"	1249 m	10	1.917	1.470	1	0.395	0.317	0.242	-0.265
	XSSQG	24°57'32"	102°37'50.16"	2216 m	8	1.583	1.341	1	0.282	0.313	0.189	-0.459
	HHLC	23°0'56.3"	102°22'58.8"	1715 m	8	1.333	1.184	1	0.211	0.250	0.143	-0.538
	FYNG	24°14'53.02"	102°11'8.16"	1249 m	12	2.167	1.409	1	0.411	0.363	0.247	-0.275
	BNGD	22°18'54"	101°32'9.6"	1016 m	4	-	-	-	-	-	-	-
	DSQ	24°44'5.21"	100°30'19.08"	2160 m	8	2.000	1.534	0	0.443	0.410	0.276	-0.424
	JDXC	24°15'24.44"	101°2'50.28"	1754 m	8	2.000	1.513	0	0.453	0.437	0.289	-0.396
Group C					24	3.750	1.715	12	0.693	0.404	0.372	-0.044
	GZWMNS	25°9'51.26"	106°14'44.16"	1075 m	8	2.500	2.006	7	0.661	0.334	0.389	0.033
	WMZX	24°58'48.79"	106°7'14.52"	432 m	8	2.167	1.488	2	0.444	0.358	0.264	-0.304
	LYLZW	24°16'30.97"	106°40'14.52"	396 m	8	2.167	1.628	3	0.541	0.521	0.350	-0.413

N: number of individuals; N_A: mean number of different alleles per population; N_E: mean effective number of alleles; A_P: number of private alleles; *I*: Shannon's Information Index; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: fixation index, bold values indicate significant departures from Hardy–Weinberg equilibrium (HWE) after sequential Bonferroni correction.

Data analysis

Micro-Checker 2.2.3 (van Oosterhoutet *et al.*, 2004) was used to check for large allele drop-outs and scoring errors within each population (1000 randomizations). The test for deviation from Hardy-Weinberg equilibrium (HWE) was computed with FSTAT 2.9.3 (Goudet, 1995). Genetic diversity statistics including the total number of alleles (N_A), effective number of alleles (N_E), private alleles (A_P), Shannon's information index (*I*), observed heterozygosity (H_O), expected heterozygosity (H_E) and the fixation index (F_{IS}) were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012). One population (BNGD) was excluded from the genetic diversity analysis because of the small sample size (n<5 individuals). Genetic differentiation between populations (F_{ST}) was calculated using GenAlEx 6.5 (Peakall and Smouse, 2012), and the significance was tested with 999 permutations by Arlequin 3.5.1 (Excoffier and Lischer, 2010). The gene flow (*N*m) between populations was estimated from *F*-statistics with the formula $Nm = (1-F_{ST})/4F_{ST}$ (Hedrick, 1983).

Bayesian clustering analysis of the twelve *A. yunnanensis* populations was performed using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). The STRUCTURE analysis was conducted with the admixture model and the option of correlated allele frequencies among populations. Simulations were conducted with the number of clusters (*K*) ranging from one to ten, and were repeated ten times. Each simulation included a burn-in of 50,000 iterations, followed by 100,000 Markov Chain Monte Carlo chains. The most likely population structure was determined by calculating Δ*K* (Evanno *et al.*, 2005) in STRUCTURE HARVESTER 0.6 (Earl and Vonholdt, 2012). A neighbour-joining (NJ) tree was constructed with Nei's D_A distance (Nei *et al.*, 1983) using POPTREE 2 and branch support was assessed with 1000 bootstraps (Takezaki *et al.*, 2010). In addition, principal component analysis (PCA) was used to assess population genetic structure. The PCA plot of *A. yunnanensis* populations was drawn using the R package 'adegenet' (Jombart, 2008).

To quantify the genetic variation among hierarchical levels of populations (between genetic groups, among populations within groups, and within populations), an analysis of molecular variance (AMOVA) with predefined genetic clusters of STRUCTURE was conducted using Arlequin 3.5.1 (Excoffier and Lischer, 2010) with 9999 permutations. Geographic distance (km) among populations was calculated with geographic coordinates of populations with the modified Haversine formula (Sinnott, 1984) using GenAlEx 6.5 (Peakall and Smouse, 2012). The pattern of isolation by distance (IBD) was tested using the Mantel test based on the matrices of pairwise genetic distance ($F_{ST}/(1-F_{ST})$) and geographic distance with 1,000 permutations (Smouse and Long, 1992). The Mantel test for isolation by environment (IBE) was also conducted to assess the environmental contribution in genetic differentiation of *A. yunnanensis*. Climate variables (annual mean temperature, temperature annual range, and annual precipitation) were downloaded from the WorldClim website (https://www.worldclim.org/) based on GPS coordinates of sampled populations. The Euclidean distance of climatic variables between populations was calculated using the R package 'ecodist' to create a pairwise environmental distance matrix for all populations (Goslee and Urban, 2007). Finally, the genetic and environmental correlations were tested using GenAlex 6.5 with 1,000 permutations (Smouse and Long, 1992).

Table 2. Characteristics of the 12 SSRs developed for ***Amorphophallus yunnanensis***.

Locus	Primer sequence	Repeat motif	Annealing temperature*	Allele range (bp)	*A*	N_A	N_E	H_O	H_E	F_{IS}
SSR2	F: CCCGAGCTGAGCCTACTTCT R: AAAATACACCCCGGGAAAAG	(AAGAG)5	62-56 °C	140-170	5	2.833	2.330	0.717	0.534	-0.342
SSR66	F: ACCACGAGGTCGAATCAAAC R: GATTTGATCCACGGTCCAAC	(GAA)5	62-56 °C	232-253	6	2.250	1.855	0.774	0.446	-0.733
SSR8	F: GATGTCAGGCCGACTAGCAG R: TCTGCCAAACCCTTCGATAC	(TTC)6	62-56 °C	139-172	7	1.833	1.172	0.126	0.127	0.009
SSR13	F: ACAGGCCCTACAACAAGTGG R: CTCGTTCCATTTCCTTCAGC	(TTC)5	62-56 °C	150-186	11	2.500	1.613	0.183	0.251	0.269
SSR14	F: CCTTGCTCTGACCAGGTGAT R: ATCCTCCCGAGGAAGAAGAC	(TGA)5	62-56 °C	155-221	10	2.333	1.846	0.269	0.323	0.166
SSR71	F: GGCCTTGGGTGTGTATTGAT R: AATGCCTTTGCTGCATGTTT	(TTC)6	62-56 °C	245-299	9	1.917	1.656	0.038	0.198	0.807
SSR27	F: TTTTGTGCAAGTCAGGCAAG R: GCCAGGCATCATGTAGTGGT	(CA)8	62-56 °C	180-186	2	1.417	1.256	0.215	0.149	-0.449
SSR37	F: TTCTTCTGCTGATCCCTCGT R: AACCAAGTCGGGTAAAGCCT	(GCC)5	62-56 °C	187-223	6	1.833	1.295	0.132	0.191	0.310
SSR41	F: TCACTCGACGACTCGACATC R: CCAAGGTTTGACTCTTGGGT	(TAT)6	62-56 °C	191-212	6	2.000	1.406	0.319	0.230	-0.384
SSR51	F: GGAGGGGCGTGAATTTATTT R: ACCACCCTAAGGAAATGTGC	(CT)6	62-56 °C	218-226	4	1.833	1.567	0.510	0.305	-0.672
SSR85	F: GTGCCAAGACCACCAAACTT R: TGCATTCATTCTGCCATCAT	(CGC)5	62-56 °C	289-304	6	2.667	1.617	0.474	0.362	-0.312
SSR96	F: ATGCACGGGATAGCCTACAC R: GAGGCGTCAATTGGTGAAAT	(TG)6	62-56 °C	318-324	2	1.583	1.354	0.318	0.203	-0.563

A: number of alleles; N_A: mean number of different alleles; N_E: mean effective number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: fixation index; *, Touchdown PCR.

RESULTS

Sequencing assembly and SSR development

Raw data of RAD-seq of the three samples (SZFHG_49, GZWMNS_4, and WMZX_13) was 6.14 Gb, 7.35 Gb, and 5.18 Gb, respectively. After filtering, a total of 17.64 Gb clean data was used to assemble genome sequences. The final assembled sequence comprised 532,962 contigs (184 Mb in total), with an average length of 345.71 bp and a contig N50 size of 343 bp. A total of 18,336 SSR loci were detected in the assembled genome. The numbers of di-, tri-, tetra-, penta-, and hexa-nucleotide repeats were 14,803, 3,115, 259, 151, and 8, respectively.

Primer pairs were successfully designed for 9,005 loci out of 18,336 SSRs. Among 100 primer pairs selected for validation, 73 were successfully amplified, 12 of which showed polymorphisms. The 12 polymorphic SSR markers were then used for genotyping all the samples (Table 2). The Micro-Checker analysis suggested no evidence of systematic allelic dropouts, null alleles, or scoring errors in the SSR data. In total, 74 alleles were detected among the 98 individuals from 12 populations, ranging from 2 (SSR27 and SSR96) to 11 (SSR13) alleles with an average number of 6.17 (Table 2). The observed and expected heterozygosity (H_O and H_E) varied from 0.038 to 0.774 and from 0.127 to 0.534, respectively. The mean number of effective alleles (N_E) of each locus ranged from 1.172 to 2.330 (Table 2).

Genetic diversity and genetic differentiation of A. yunnanensis

The mean allele numbers (N_A) for each population ranged from 1.333 (HHLC) to 3.083 (ZYHCL). Nine populations had private alleles ranging from 1 (SZFHG, XSSQG, HHLC, and FYNG) to 11 (ZYHCL). The H_O and H_E values varied from 0.234 (BMHC2018) to 0.521 (LYLZW) and from 0.143 (HHLC) to 0.389 (GZWMNS), respectively. The fixation index (F_{IS}) of each population ranged from -0.538 to 0.329. Two populations showed signs of significant departure from Hardy-Weinberg equilibrium after Bonferroni corrections (Table 1). When populations were assigned to three genetic groups as STRUCTURE suggested (see below), group A exhibited the highest values in most genetic diversity indices (N_A, N_E, A_P, I, and H_E), while group C had the highest H_O value. However, group B, which was comprised of the largest number of individuals, showed the lowest genetic diversity (Table 1). The genetic differentiation (F_{ST}) between each pair of populations ranged from 0.000 to 0.522 with an average value of 0.201. Most of the F_{ST} statistics were significant at the 5% nominal level (Table 3). The lowest estimate of gene flow (Nm) was detected between population GZWMNS and ZYHCL (0.229), and the highest value was found between BNGD and JDXC (97.406) (Table 3).

Table 3. Genetic differentiation (pairwise FST) (below diagonal) and gene flow (Nm) (above diagonal) values among 12 ***Amorphophallus yunnanensis*** populations in China.

	GZWMNS	BMHC2018	ZYHCL	WMZX	SZFHG	XSSQG	HHLC	FYNG	BNGD	DSQ	JDXC	LYLZW
GZWMNS		0.424	0.229	6.000	0.495	0.479	0.375	0.380	0.309	0.396	0.452	2.126
BMHC2018	**0.371**		1.120	0.760	0.959	0.637	2.340	0.744	0.586	1.023	0.912	0.708
ZYHCL	**0.522**	**0.183**		0.371	0.477	0.368	0.663	0.434	0.325	0.541	0.534	0.387
WMZX	**0.040**	**0.247**	**0.403**		0.906	0.765	0.933	0.641	0.567	0.762	0.919	4.129
SZFHG	**0.336**	**0.207**	**0.344**	**0.216**		-	-	4.341	1.304	2.025	3.815	1.333
XSSQG	**0.343**	**0.282**	**0.405**	**0.246**	0.000		-	37.686	1.204	2.439	5.526	1.312
HHLC	**0.400**	**0.097**	**0.274**	**0.211**	0.000	0.000		-	2.217	-	-	1.069
FYNG	**0.397**	**0.252**	**0.366**	**0.281**	**0.054**	**0.007**	0.000		3.126	7.550	69.975	0.926
BNGD	**0.447**	**0.299**	**0.435**	**0.306**	**0.161**	**0.172**	**0.101**	**0.074**		14.028	97.406	0.732
DSQ	**0.387**	**0.196**	**0.316**	**0.247**	**0.110**	**0.093**	0.000	**0.032**	**0.018**		-	0.944
JDXC	**0.356**	**0.215**	**0.319**	**0.214**	**0.062**	**0.043**	0.000	**0.004**	**0.003**	0.000		1.372
LYLZW	**0.105**	**0.261**	**0.393**	**0.057**	**0.158**	**0.160**	**0.190**	**0.213**	**0.254**	**0.209**	**0.154**	

Bold values indicate significance of the F_{ST} values at the 5% nominal level.

Table 4. Results of the analysis of molecular variance (AMOVA). All fixation indexes are significant ($P < 0.001$).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*F*-statistics
Among groups	2	46.404	0.380	18.332	F_{CT}=0.183
Among populations within groups	9	36.450	0.167	8.041	F_{SC}=0.098
Within populations	184	264.497	1.528	73.627	F_{ST}=0.264

d.f., degrees of freedom.

Genetic structure among populations

The ΔK value suggested $K = 3$ was the optimal genetic cluster number for the STRUCTURE analysis (Fig. 2A). Placing these genetic groups in a geographic context, group A was comprised of two populations in southern part of Yunnan province (ZYHCL and BMHC2018), group B included populations that are distributed in the relatively northern part of Yunnan except the population BNGD, and group C consisted of three populations (GZWMNS, WMZX, and LYLZW) in Guizhou and Guangxi provinces (Fig. 2B). One population (BMHC2018) showed signs of genetic admixture (Fig. 2B). Results of the unrooted NJ tree and the PCA plot (Fig. 3; Fig. 4) were generally congruent with the genetic clustering of STRUCTURE.

The hierarchical AMOVA, which was conducted with predefined clusters of the STRUCTURE results, indicated that most of the genetic variation (73.63%) was distributed within populations, followed by the variation among genetic groups (18.33%). Only 8.04% of the variation was among populations within groups ($P < 0.001$) (Table 4). A significant IBD pattern of *A. yunnanensis* populations was revealed by the Mantel test (r^2=0.219, $P < 0.01$) (Fig. 5A). However, no significant correlation between the genetic and environmental distances was detected ($P = 0.441$) (Fig. 5B).

DISCUSSION

We developed 12 SSR markers with RAD-seq and used them to assess the genetic diversity of *A. yunnanensis* in southwestern China (Table 1). At the population level, the genetic diversity of *A. yunnanensis* (H_O and H_E) was lower than that reported in a previous study of *Amorphophallus konjac* in southwestern China (t-test, $P < 0.05$) (Gao *et al.*, 2018b). *Amorphophallus konjac* is the most widely distributed *Amorphophallus* species in China. Although *A. yunnanensis* occurs in many countries across Southeast Asia, the distribution ranges of *A. yunnanensis* populations in China are restricted to the Yunnan-Guizhou plateau (Li *et al.*, 2010; Pan *et al.*, 2015). The restricted geographic range may be an explanation for the low genetic diversity in *A. yunnanensis*. The loss of genetic diversity can also be attributed to the habitat fragmentation caused by human activities (Long *et al.*, 2003; Ren and Pan, 2013).

Our genetic structure tests assigned the 12 populations of *A. yunnanensis* into three genetic groups. The genetic structure was further confirmed by the higher percentage of variation among groups than among populations in AMOVA. Group A containing two populations (ZYHCL and BMHC2018) in the southern region of Yunnan province, exhibited the highest genetic diversity. The gene flow between populations in group A and all other populations was relatively low (Table 3). The private allele number was the largest in group A among the three genetic groups (Table 1). If gene flow is low, there are more private alleles, and if gene flow is high, private alleles are rarer (Curry *et al.*, 2019; Mullen, 2007). Besides, it is noteworthy that group A deviated from Hardy-Weinberg equilibrium (HWE), implying inbreeding or population stratification (Wigginton *et al.*, 2005). The inbreeding events in group A might also contribute to the excessive of private alleles. In general, the most abundant rare alleles observed in Group A provided further evidence

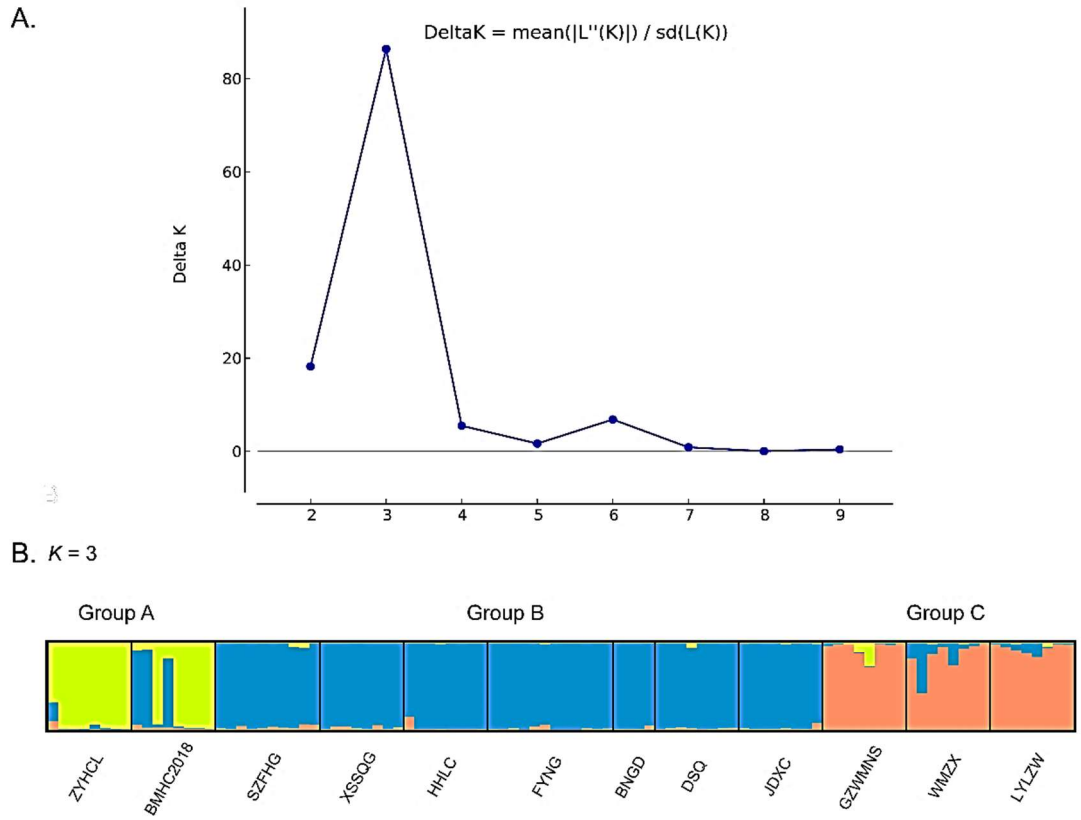

Fig. 2. The STRUCTURE analysis of ***Amorphophallus yunnanensis*** populations in China. **A**. ΔK estimates of the posterior probability distribution. **B**. Estimated population structure of Amorphophallus yunnanensis populations with K =3.

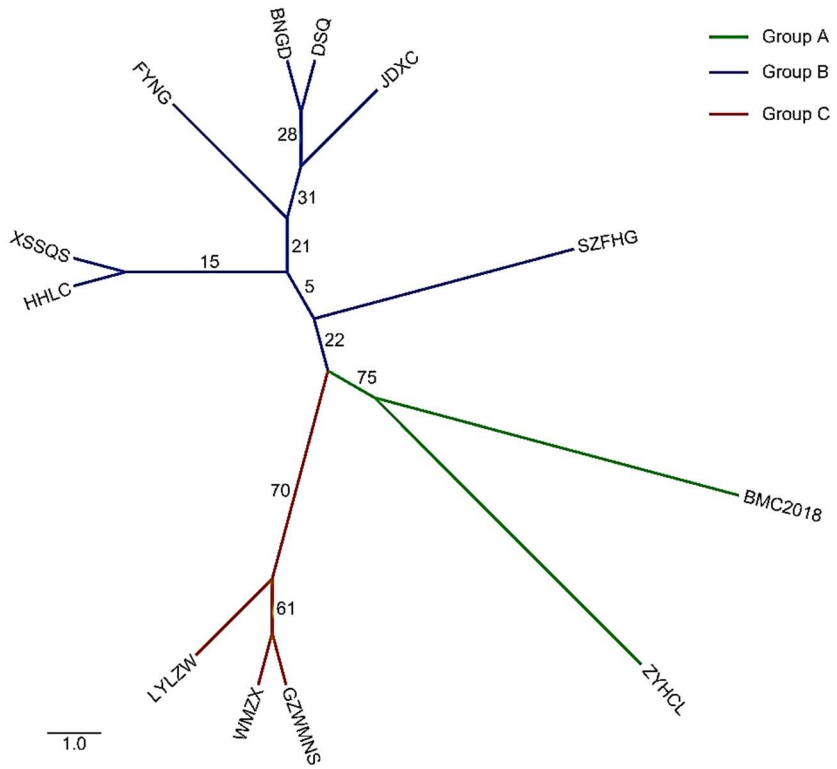

Fig. 3. Neighbour-joining tree of the 12 populations of ***Amorphophallus yunnanensis*** in China. Bootstrap values are shown at each branch. The three genetic groups are shown in different colours.

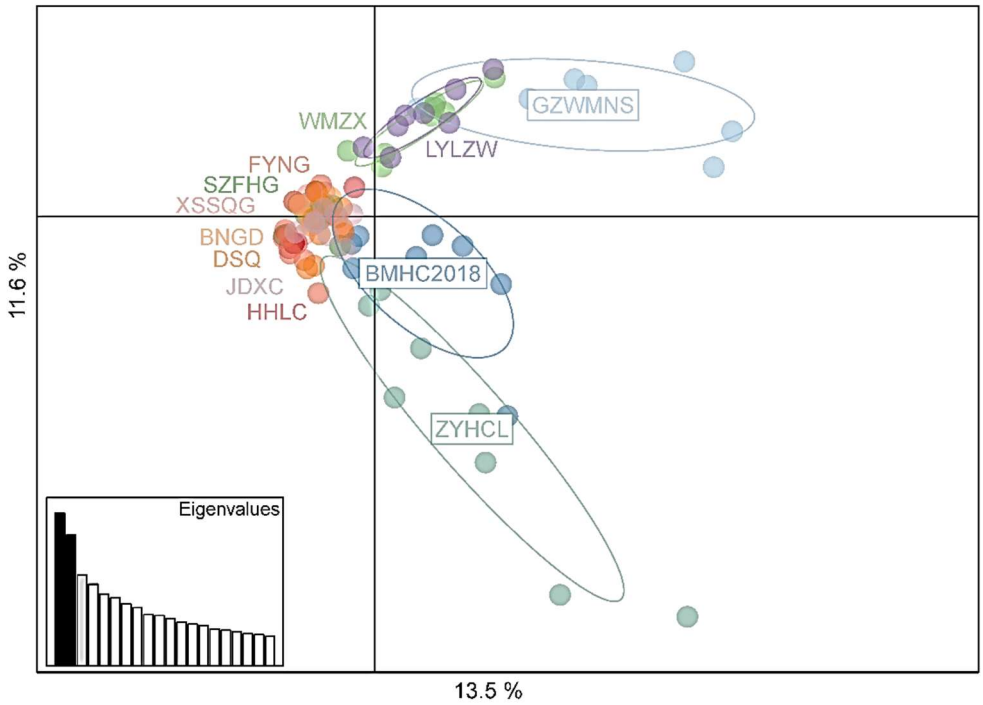

Fig. 4. Scatter plot of ***Amorphophallus yunnanensis*** populations in China according to their coordinates on the first two principal components of the principal component analysis. The first two axes represent 13.5 % and 11.6 % of the total variation, respectively.

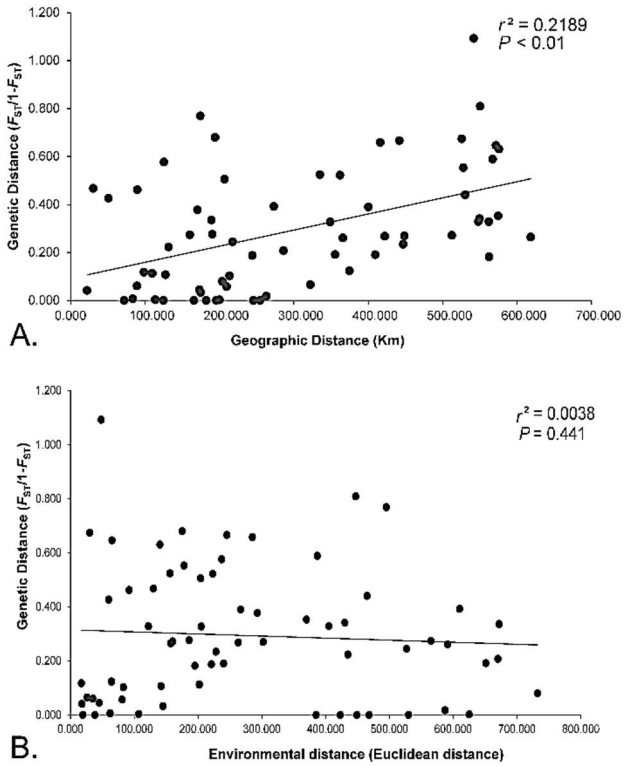

Fig. 5. Mantel tests of the pairwise geographic, environmental and genetic correlations of 12 ***Amorphophallus yunnanensis*** populations in Southwestern China. (A) Correlation of pairwise geographic distance versus pairwise genetic distance (FST/(1-FST)); (B) Correlation of pairwise environmental distance versus pairwise genetic distance (FST/(1-FST)).

for genetic uniqueness in this region. Group B included the rest of the Yunnan populations, while group C comprised populations located at the edge of Yunnan-Guizhou plateau.

Mantel test suggested that the genetic differentiation pattern of *A. yunnanensis* fits isolation-by-distance model. The interpretation for the IBD is the migration drift equilibrium or the steppingstone colonization pattern (Orsini *et al.*, 2013). These two processes can potentially be distinguished by the fact that stepping-stone colonization could lead to lower genetic diversity in populations colonized later (Prunier and Holsinger, 2010). Our findings on the genetic diversity of *A. yunnanensis* are consistent with a stepping-stone colonization scenario extending outwards from the southern regions to the northern area of China (Kurniawan *et al.*, 2011; Liu, 2003). There are no signs of less human disturbance or comparatively well-conserved habitat in the region of group A. We concluded that this area might be a diversification centre of *A. yunnanensis*. *Amorphophallus* species is self-incompatible, and it attracts certain rove beetles as pollinators (Tang *et al.*, 2020). The relatively weak dispersal ability of these insects could hinder the pollination between different populations. Furthermore, the seeds of this plant are mainly dispersed by birds with poor fly ability. The gene flow analysis also indicated that the gene exchange mostly occurred among neighbouring populations (Table 3). Limitations related to reproductive strategies may have imposed constraints

on the long-distance dispersal of *A. yunnanensis* (Yuan *et al.*, 2012).

Knowledge of the genetic diversity in this species is increasingly important for implementing effective conservation strategies (Francisco-Ortega *et al.*, 2000; Li *et al.*, 2018). *Amorphophallus yunnanensis* has lower genetic diversity than other *Amorphophallus* species (Gao *et al.*, 2018b). Significant genetic differentiation (F_{ST}) (average 0.201) was observed in most populations examined (Table 3). With declining habitats, conservation actions are necessary for wild resources of *Amorphophallus* (Long *et al.*, 2003). Here we discovered the geographic differentiation pattern of *A. yunnanensis* populations in China, revealing three distinct genetic groups. Conservation biology aims to preserve the adaptive genetic variation within species (Fraser and Bernatchez, 2010). According to the evolutionarily significant unit (ESU) criteria that ESUs are populations or groups of populations demonstrating significant divergence (Dizon *et al.*, 1992), these groups should be treated as distinct ESUs. Specifically, group A should be considered a high conservation priority because it contains the most abundant rare alleles and has the highest genetic diversity.

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LITERATURE CITED

Baird, N.A., P.D. Etter, T.S. Atwood, M.C. Currey, A.L. Shiver, Z.A. Lewis, E.U. Selker, W.A. Cresko, E.A. Johnson. 2008. Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. Plos One **3(10)**: e3376.

Chae, W.B., S.J. Hong, J.M. Gifford, A.L. Rayburn, E.J. Sacks and J.A. Juvik. 2014. Plant morphology, genome size, and SSR markers differentiate five distinct taxonomic groups among accessions in the genus *Miscanthus*. GCB Bioenergy **6(6)**: 646–660.

Chua, M., T.C. Baldwin, T.J. Hocking and K. Chan. 2010. Traditional uses and potential health benefits of *Amorphophallus konjac* K. Koch ex N.E.Br. J. Ethnopharmacol. **128(2)**: 268–278.

Curry, C.J., P.A. White and J.N. Derr. 2019. Genetic analysis of African lions (*Panthera leo*) in Zambia support movement across anthropogenic and geographical barriers. Plos One **14(5)**: e0217179.

Dizon, A.E., C. Lockyer, W.F. Perrin and D.J. Sisson. 1992. Rethinking the stock concept: a phylogeographic approach. Conserv. Biol. **6(1)**: 24–36.

Earl, D.A. and B.M. Vonholdt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conser. Genet. Res. **4(2)**: 359–361.

Ekué, M.R.M., O. Gailing and R. Finkeldey. 2009. Transferability of simple sequence repeat (SSR) markers developed in *Litchi chinensis* to *Blighia sapida* (Sapindaceae). Plant Mol. Biol. Rep. **27(4)**: 570–574.

Evanno, G., S. Regnaut and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. **14(8)**: 2611–2620.

Excoffier, L. and H.E.L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. **10(3)**: 564–567.

Faith, D.P. 1994. Genetic diversity and taxonomic priorities for conservation. Biol. Conserv. **68(1)**: 69–74.

Francisco-Ortega, J., A. Santos-Guerra, S.C. Kim and D.J. Crawford. 2000. Plant genetic diversity in the Canary Islands: a conservation perspective. Am. J. Bot. **87(7)**: 909–919.

Fraser, D.J. and L. Bernatchez. 2010. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol. Ecol. **10(12)**: 2741–2752.

Gao, Y., S. Yin, H. Yang, L. Wu and Y. Yan. 2017. Genetic diversity and phylogenetic relationships of seven *Amorphophallus* species in southwestern China revealed by chloroplast DNA sequences. Mitochondrial DNA A **29(5)**: 679–686.

Gao, Y., S. Yin, C. Liu, H. Wang, H. Chu and L. Tang. 2018a. A rapid approach for SSR development in *Amorphophallus paeoniifolius* using RAD-seq. Taiwania **63(3)**: 281–285.

Gao, Y., G. Yuan, C. Niu and L. Tang. 2018b. Genetic diversity and population structure of the devel's tongue arum (*Amorphophallus konjac*) in southwestern China. Pak. J. Bot. **50(3)**: 999–1004.

Goslee, S.C. and D.L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. J. Stat. Softw. **22(7)**: 1–19.

Goudet, J. 1995. FSTAT Vers. 2.9.3.: a computer program to calculate F-satistics. J. Hered. **86(6)**: 485–486.

Gupta, P.K., S. Rustgi, S. Sharma, R. Singh, N. Kumar and H.S. Balyan. 2003. Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol. Genet. Genomics. **270(4)**: 315–323.

Hedrick, P.W. 1983. Genetics of populations. Science Books International, Boston.

Jiang, X., Y. Chen, X. Chen, S. Yan, M. Xia, J. Shen and J. Zhang. 2019. Geographical distribution and characteristics of Konjac resources in Xishuangbanna, Yunnan Province. Agricultural Science. **1(4)**: 1–4.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. **24(11)**: 1403–1405.

Kurniawan, A., I.P.A.H. Wibawa and B. Adjie. 2011. Species diversity of *Amorphophallus* (Araceae) in Bali and Lombok with attention to genetic study in A. paeoniifolius (Dennst.) Nicolson. Biodiversitas **12(1)**: 7–11.

Li, C., J.L. Chen, Y.Y. Li and D.K. Tian. 2018. Genetic diversity of *Begonia versicolor* (Begoniaceae), a narrow endemic species in southeast Yunnan of China. Taiwania **63(1)**: 49–53.

Li, H., G. Zhu, P.C. Boyce, M. Jin, W.L.A. Hetterscheid, J. Bogner and N. Jacobsen. 2010. Araceae, In: Flora of China, vol. 23: 23–33. Missouri Botanical Garden Press, St. Louis.

Liu, P. 2003. Konjac, China Agriculture Press, Beijing.

Long, C., H. Li, Z. Ouyang, X. Yang, Q. Li and B. Trangmar. 2003. Strategies for agrobiodiversity conservation and promotion: A case from Yunnan, China. Biodivers. Conserv. **12(6)**: 1145–1156.

Mullen, S.P. 2007. Conservation and the genetics of populations. Copeia. **2007(3)**: 774–775.

Nei, M., F. Tajima and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. **19(2)**: 153–170.

Orsini, L., J. Vanoverbeke, I. Swillen, J. Mergeay and L. De Meester. 2013. Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. Mol. Ecol. **22(24)**: 5983–5999.

Pan, C., A.W. Gichira and J.M. Chen. 2015. Genetic variation in wild populations of the tuber crop *Amorphophallus konjac* (Araceae) in central China as revealed by AFLP markers. Genet. Mol. Res. **14(4)**: 18753–18763.

Peakall, R. and P.E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. Bioinformatics **28(19)**: 2537–2539.

Pritchard, J.K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155(2)**: 945–959.

Prunier, R. and K. Holsinger. 2010. Was it an explosion? Using population genetics to explore the dynamics of a recent radiation within *Protea* (*Proteaceae* L.). Mol. Ecol. **19(18)**: 3968–3980.

Rao, V.R. and T. Hodgkin. 2002. Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell Tiss. Org. **68(1)**: 1–19.

Ren, P. and M. Pan. 2013. Population genetic structure of five *Amorphophallus* species from the south of Yunnan province by inter-simple sequences(ISSR) markers. J. Wuhan Univ. Nat. Sci. **28(2)**: 85–86.

Rozen, S. and H. Skaletsky. 2000. Primer 3 on the WWW for general users and for biologist programmers, In: Methods in Molecular Biology, vol. 132:365–386. Humana Press, USA.

Santosa, E., C.L. Lian, Y. Pisooksantivatana and N. Sugiyama. 2007. Isolation and characterization of polymorphic microsatellite markers in *Amorphophallus paeoniifolius* (Dennst.) Nicolson, Araceae. Mol. Ecol. Notes. **7(5)**: 814–817.

Setsuko, S., K. Sugai, I. Tamaki, K. Takayama, H. Kato and H. Yoshimaru. 2020. Genetic diversity, structure, and demography of *Pandanus boninensis* (Pandanaceae) with sea drifted seeds, endemic to the Ogasawara Islands of Japan: Comparison between young and old islands. Mol. Ecol. **29(6)**: 1050–1068.

Sinnott, R.W. 1984. Virtues of the Haversine. Sky and Telescope. **68(2)**: 159.

Smouse, P.E. and J.C. Long. 1992. Matrix correlation analysis in anthropology and genetics. Am. J. Phys. Anthropol. **35(S15)**: 187–213.

Srzednicki, G. and C. Borompichaichartkul. 2020. Konjac glucomannan-production, processing, and functional applications, CRC Press, Boca Raton.

Takezaki, N., M. Nei and K. Tamura. 2010. Software for constructing population trees from allele frequency data and computing other population statistics with windows interface. Mol. Biol. Evol. **27(4)**: 747–752.

Tang, R., B. Huang, W. Sun and G. Chen. 2020. Pollination biology of *Amorphophallus albus* (Araceae), an endemic plant in the dry-hot valley of Jinsha River. Plant Science Journal. **38(4)**: 458–466.

Thiel, T., W. Michalek, R.K. Varshney and A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. **106(3)**: 411–422.

Tian, Z., F. Zhang, H. Liu, Q. Gao and S. Chen. 2016. Development of SSR markers for a Tibetan medicinal plant, *Lancea tibetica* (Phrymaceae), based on RAD sequencing. Appl. Plant. Sci. **4(11)**: 1600076.

Tomlinson, P.B., S.J. Mayo, J. Bogner and P.C. Boyce. 1998. The genera of Araceae. Kew Bull. **53(2)**: 505.

Van Oosterhout, C., W. F. Hutchinson, D.P.M. Wills, P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes **4(3)**: 535–538.

Wigginton, J.E., D.J. Cutler and G.R. Abecasis. 2005. A note on exact tests of Hardy-Weinberg equilibrium. Am. J. Hum. Genet. **76(5)**: 887–893.

Wu, X., X. Xu, D. Ma, R. Chen and Y. Cao. 2019. Virulence structure and its genetic diversity analyses of *Blumeria graminis* f. sp. tritici isolates in China. BMC Evol. Biol. **19(1)**: 183.

Yin, S., Y. Yan, L. You, Q. Chen, Y. Zhou, K. Chen, R. Li, Z. Yang, L. Man and Y. Gao. 2019. Newly developed genomic SSRs reveal genetic diversity in wild and cultivated *Amorphophallus albus* germplasms. Plant Mol. Biol. Rep. **37(4)**: 365–375.

Yuan, J.H., F.Y. Cheng and S.L. Zhou. 2012. Genetic structure of the tree peony (*Paeonia rockii*) and the Qinling Mountains as a geographic barrier driving the fragmentation of a large population. Plos One **7(4)**: e34955.

Zalapa, J.E., H. Cuevas, H. Zhu, S. Steffan, D. Senalik, E. Zeldin, B. McCown, R. Harbut and P. Simon. 2012. Using next-generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences. Am. J. Bot. **99(2)**: 193–208.