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# GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE DVEL'S TONGUE ARUM (*AMORPHOPHALLUS KONJAC*) IN SOUTH-WESTERN CHINA

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**Abstract**

*Amorphophallus konjac*, also known as the devil's tongue arum, is the most widely utilised *Amorphophallus* species in China. To evaluate its genetic diversity and population structure, genetic variation in 18 wild *A.konjac* populations (182 individuals) from south-western China was assessed with 13 expressed sequence tag-derived simple sequence repeats (EST-SSRs). In total, 107 alleles were obtained from 13 loci in 18 populations. A moderate to high genetic diversity was detected ($N_A$ varied from 1.308 to 3.846, $H_O$ ranged between 0.256 and 0.615, $H_E$ ranged between 0.145 and 0.516, $I$ ranged between 0.205 and 0.947). The pair wise genetic distance was significantly correlated with geographic distance in the mantel test, suggesting that isolation by distance was one of the reasons for the genetic variation in *A. konjac* populations. Genetic structure analysis divided the 18 populations into three groups, which were significantly related to their geographic origins. When developing conservation strategies, these genetic groups should be treated as distinct evolutionarily significant units. The results of our study suggest that the devil's tongue arum in south-western China contains a high level of genetic variation. There is much potential in these materials for the genetic improvement.

**Key words:** *Amorphophallus konjac*; Genetic diversity; Population structure; EST-SSR; Conservation strategy.

## Introduction

*Amorphophallus* (Araceae) is a genus of perennial herbs. About 200 *Amorphophallus* species are found in the tropic and sub-tropic areas of continental south-east Asia, Australia, Pacific islands and Africa (Li *et al.*, 2010). Indochina Peninsula as well as south-western China is recognised as one centre of origin of *Amorphophallus* (Liu, 2004). Sixteen species (seven endemic) are distributed in China (Li *et al.*, 2010). *Amorphophallus* species have been historically used as a food source and traditional medicine in China. Of these, *A. konjac*, which is known as the devil's tongue arum, is the most widely utilised species and is a diet food because of its high fibre content (Li *et al.*, 2010). However, the *A. konjac* industry faces some serious problems, such as genetic decline and bacterial soft rot disease (Wu *et al.*, 2012).

One of the foundations for breeding improvement is the genetic diversity of crops (Vellve, 1993). Wild populations are precious gene pools for crop improvement (Oyama *et al.*, 2006). But human activities have led to a decline in wild populations of *A. konjac* (Wang & Xie, 2009). Molecular markers are powerful and reliable tools for genetic diversity studies (Chae *et al.*, 2014). Genetic diversity in *Amorphophallus* has been assessed using different markers, such as the DNA random amplified polymorphisms (Zhang *et al.*, 2001), amplified fragment length polymorphisms (AFLPs) (Pan *et al.*, 2015) and inter-simple sequence repeats (Ren & Pan, 2013).

Simple sequence repeats (SSRs) are short sequences with tandemly repeated motifs (1-6 bp), which exist extensively in the genome (Ekué *et al.*, 2009). A handful of studies have identified/characterised microsatellites and have developed markers for *Amorphophallus* species. Santosa *et al*., (2007) isolated 19 polymorphic SSR markers from *A. paeoniifolius* through probe hybridisation and sequencing, and Pan *et al*., (2012) obtained 13 SSR markers from *A. konjac* using the same method. Mandal *et al*., (2016) conducted genetic diversity analyses of *A. paeoniifolius* populations using SSR primers developed from *A. konjac* and found 11 cross-genus transferable markers. Zheng *et al*., (2013) sequenced transcriptomes of *A. konjac* as well as *A. bulbifer*, and identified expressed sequence tag-derived simple sequence repeats (EST-SSRs) based on these transcriptome sequences.

The genetic information of *Amorphophallus* species in south-western China has not been comprehensively illustrated. Toevaluate the molecular resources, EST-SSR markers were used to unveil the genetic variations in the wild *A. konjac* populations across south-western China in this study.

## Materials and Methods

In total, 18 wild populations (3–20 individuals per population, 182 individuals total) of *A. konjac* were sampled throughout south-western China (Fig. 1, Table 1). Leaves of each sample were collected, and dried in silica gels. DNA was extracted with genomic DNA extraction kits of the plant (Tiangen, Beijing, China), and its quality was visualised with 0.8% (w/v) agarose gels.

In all, 13 polymorphic EST-SSRs reported by Zheng *et al*., (2013) were employed in the molecular analysis. The forward primers were fluorescently labelled with 5'-HEX, 5'-6-FAM and 5'-ROX, respectively (Table 2). Polymerase chain reactions (PCRs) with a volume of 15μL were conducted with 1 × Buffer ($Mg^{2+}$ plus) (Takara Biotechnology (Dalian) Co. Ltd, China), 0.1 mMdNTP (Takara), 0.1 μM forward/reverse primer, 0.8 U Taq polymerase (rTaq, Takara) and 1μL genomic DNA. Initial denaturation took place at 94°C for 6 min, 30 cycles of 94°C for 35 s, 55°C for 40 s, and 72°C for 45 s, with the final extension (72°C) for 7 min. Capillary electrophoresis of amplified products was conducted using ABI 3730 and genotyped by software GENEMAPPER version 2.3 (Applied Biosystems).

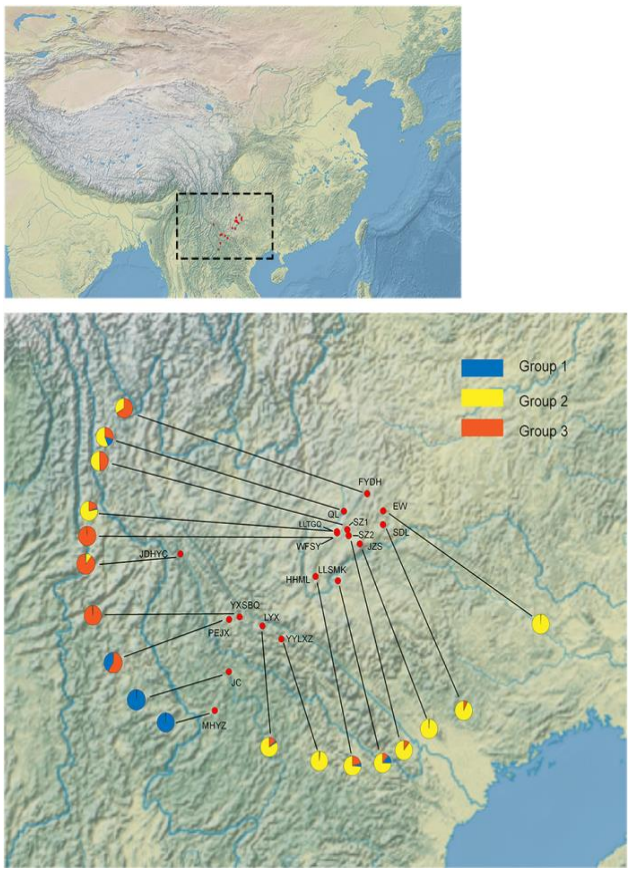


Fig. 1. Sampling locations and results of model-based structure analysis of the *Amorphophallus konjac* populations. The pie charts indicate the membership proportions of each population of $K = 3$.

Genetic diversity indices (total allele number, $N_A$; allelic richness, $A_R$; expected heterozygosity, $H_E$; observed heterozygosity, $H_O$; Shannon's information index, $I$; fixation index, $F_{IS}$) were computed using GENALEX 6.5 (Peakall & Smouse, 2012). To infer genetic differentiation among populations, population differentiation ($F_{ST}$) was calculated with 1000 permutations by Arlequin 3.5.1 (Excoffier & Lischer, 2010). Geographic distances were calculated and isolation by distance (IBD) was tested with a mantel test in GENALEX 6.5 (Peakall & Smouse, 2012).

Population structure was detected by STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) with an admixture model. Simulations were run with the cluster number ($K$) ranging from 1 to 15, and 10 repeats for each cluster. Each run included 100,000 repetitions as burn-in and 500,000 Markov Chain Monte Carlo chains. The most probably genetic clusters were determined by $\Delta K$ (Evanno *et al.*, 2005). Assignment coefficients (q) were treated with CLUMPP (Jakobsson and Rosenberg, 2007), and the result was visualised by DISTRUCT 2.1 (Rosenberg, 2004). Discriminant analysis of the principal component (DAPC) was accessed with 'ADEGENET' package in R (Jombart, 2008). A neighbour-joining (NJ) tree produced with $D_A$ distance was drawn using the POPTREE 2 software (Nei *et al.*, 1983; Takezaki *et al.*, 2010), and 1000 bootstraps were computed.

Genetic variation among different hierarchical levels of populations was quantified using the analysis of molecular variance (AMOVA) within ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010) with 1023 permutations.

**Table 2. Characteristics of 13 microsatellites.**

| Locus | Primer sequence | Repeat motif | Annealing temperature (°C) | Allele range (bp) | $N_A$ | $H_T$ | $H_O$ | $H_E$ | Fluorescent dye |
|---|---|---|---|---|---|---|---|---|---|
| AK-EST-SSR50 | F: CCGCTTCCTCAAAACCTGTA<br>R: AGAGGAAAGGAGAGCTTGGG | (GA)6 | 54 | 123-129 | 5 | 0.119 | 0.062 | 0.110 | 5'-6-FAM |
| AK-EST-SSR61 | F: TCGATCTATGCAATCCACCA<br>R: TTCCCGCTCTCTGTTCTTGT | (TC)8 | 54 | 192-204 | 5 | 0.106 | 0.064 | 0.077 | 5'-6-FAM |
| AK-EST-SSR108 | F: CGCCAATATATAACGGCCAA<br>R: CTCCGCTCTTCCAGACATTC | (AGA)6 | 54 | 268-289 | 8 | 0.677 | 0.824 | 0.503 | 5'-6-FAM |
| AK-EST-SSR8 | F: GGTCGATTCTTGAGCGAACT<br>R: ATTGGGCGTGGCATTAGTAG | (ACA)5 | 54 | 109-124 | 10 | 0.788 | 0.859 | 0.555 | 5'-HEX |
| AK-EST-SSR98 | F: CTTCAGTGTCGGAGGGAGAG<br>R: GAGGAGATGCCCAGAGTCAG | (GAG)5 | 54 | 234-259 | 8 | 0.521 | 0.292 | 0.287 | 5'-HEX |
| AK-EST-SSR273 | F: CTCCATGGTCTTGTCCAGGT<br>R: CTCGACAAACTCTTCTCCCC | (CGC)5 | 54 | 264-267 | 2 | 0.479 | 0.507 | 0.397 | 5'-HEX |
| AK-EST-SSR26 | F: CCATCTCTAGCTGAGGCGAG<br>R: AATTGGCAGAGGATGAGTCG | (AC)6 | 52 | 184-190 | 9 | 0.406 | 0.219 | 0.252 | 5'-ROX |
| AK-EST-SSR68 | F: AAAACCCCATCAAAACCCAT<br>R: GAGAAGTCGAGCGGAAGATG | (ACCGCT)4 | 54 | 116-122 | 9 | 0.680 | 0.759 | 0.537 | 5'-6-FAM |
| AK-EST-SSR253 | F: CACACGGAATTATAAGCGCA<br>R: GATCCACACACGAAAACACG | (CT)8 | 54 | 205-207 | 12 | 0.654 | 0.902 | 0.517 | 5'-6-FAM |
| AK-EST-SSR27 | F: AAATAGGGCACCGAATTGTG<br>R: CCATTGTCAAGCAGAGACGA | (TTCA)5 | 54 | 262-289 | 8 | 0.689 | 0.748 | 0.480 | 5'-6-FAM |
| AK-EST-SSR114 | F: TCACCAAATCTATCCCCAGC<br>R: TATTATGAAGTCTACCTGATCTGGAC | (AT)6 | 54 | 150-158 | 15 | 0.514 | 0.395 | 0.408 | 5'-HEX |
| AK-EST-SSR95 | F: CTGAGGAGGGTTGGAGGAG<br>R: CTCCCGCCTCTCGGATTAC | (TGC)5 | 54 | 198-216 | 7 | 0.488 | 0.151 | 0.186 | 5'-HEX |
| AK-EST-SSR190 | F: GGGAAAGAGAAGGGAGCAAG<br>R: CGGCATGGAAAGAAATAGGA | (GAA)5 | 52 | 273-276 | 9 | 0.630 | 0.554 | 0.418 | 5'-ROX |

$N_A$, Number of alleles; $H_T$: Total Expected Heterozygosity; $H_O$: Observed heterozygosity; $H_E$: Expected heterozygosity

**Table 1. Summary of population locations and genetic diversity for 18 populations of *Amorphophallus konjac*.**

| Pop | Latitude | Longitude | Altitude/m | *N* | $N_A$ | $A_R$ | $A_P$ | *I* | $H_O$ | $H_E$ | $F_{IS}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MHYZ | 21.905 | 101.427 | 769 | 3 | 1.308 | 1.277 | 0 | 0.205 | 0.256 | 0.145 | -0.75 |
| EW | 25.266 | 104.6 | 1964 | 4 | 1.615 | 1.615 | 0 | 0.427 | 0.615 | 0.308 | -1 |
| FYDH | 25.554 | 104.299 | 1749 | 9 | 2.692 | 1.92 | 0.077 | 0.674 | 0.477 | 0.389 | -0.072 |
| HHML | 24.161 | 103.328 | 1454 | 20 | 3.538 | 2.055 | 0.385 | 0.759 | 0.415 | 0.41 | -0.008 |
| JC | 22.558 | 101.692 | 883 | 19 | 2.769 | 1.682 | 0.769 | 0.52 | 0.273 | 0.284 | 0.134 |
| JDHYC | 24.542 | 100.779 | 1207 | 14 | 3.846 | 2.373 | 0.231 | 0.947 | 0.495 | 0.516 | 0.162 |
| JZS | 24.709 | 104.159 | 2001 | 6 | 1.615 | 1.615 | 0 | 0.427 | 0.615 | 0.308 | -1 |
| LLSMK | 24.088 | 103.746 | 1844 | 7 | 2 | 1.697 | 0.077 | 0.515 | 0.537 | 0.337 | -0.411 |
| LLTGQ | 24.916 | 103.729 | 1919 | 9 | 2.462 | 1.854 | 0 | 0.648 | 0.537 | 0.402 | -0.128 |
| LYX | 23.332 | 102.324 | 1467 | 10 | 3.154 | 2.171 | 0.538 | 0.811 | 0.486 | 0.462 | 0.04 |
| PEJX | 23.438 | 101.696 | 1346 | 5 | 2.923 | 2.602 | 0.308 | 0.876 | 0.4 | 0.495 | 0.25 |
| QL | 25.259 | 103.862 | 1884 | 4 | 2.385 | 1.952 | 0 | 0.665 | 0.423 | 0.405 | 0.039 |
| SDL | 25.034 | 104.597 | 1528 | 15 | 2.231 | 1.707 | 0 | 0.54 | 0.598 | 0.355 | -0.331 |
| SZ1 | 24.951 | 103.923 | 1894 | 14 | 2.462 | 1.959 | 0 | 0.66 | 0.507 | 0.396 | -0.246 |
| SZ2 | 24.848 | 103.95 | 2059 | 20 | 2.615 | 1.696 | 0.077 | 0.543 | 0.596 | 0.348 | -0.504 |
| WFSY | 24.894 | 103.731 | 2053 | 9 | 2.077 | 1.796 | 0.154 | 0.552 | 0.615 | 0.356 | -0.628 |
| YXSBQ | 23.479 | 101.895 | 1100 | 4 | 2.077 | 1.772 | 0.308 | 0.521 | 0.346 | 0.311 | -0.134 |
| YYLXZ | 23.109 | 102.682 | 1461 | 10 | 1.846 | 1.64 | 0 | 0.467 | 0.577 | 0.319 | -0.742 |

**Table 3. Genetic differentiation (pair wise $F_{ST}$) (below diagonal) and significance levels (above diagonal) values among 18 *Amorphophallus konjac* populations.**

| | MHYZ | EW | FYDH | HHML | JC | JDHYC | JZS | LLSMK | LLTGQ | LYX | PEJX | QL | SDL | SZ1 | SZ2 | WFSY | YXSBQ | YYLXZ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MHYZ | | * | * | * | NS | * | * | * | * | * | NS | * | * | * | * | * | * | * |
| EW | 0.452 | | * | NS | * | * | NS | NS | NS | NS | * | * | NS | NS | NS | * | * | NS |
| FYDH | 0.382 | 0.131 | | NS | * | * | * | NS | * | * | * | * | * | NS | * | * | * | * |
| HHML | 0.337 | 0.029 | 0.093 | | * | * | NS | NS | NS | NS | * | NS | NS | NS | NS | * | * | NS |
| JC | 0.073 | 0.351 | 0.349 | 0.293 | | * | * | * | * | * | * | * | * | * | * | * | * | * |
| JDHYC | 0.3 | 0.143 | 0.134 | 0.102 | 0.269 | | * | * | * | * | * | * | * | * | * | * | * | * |
| JZS | 0.452 | 0 | 0.131 | 0.029 | 0.351 | 0.143 | | NS | NS | NS | * | * | NS | NS | NS | * | * | NS |
| LLSMK | 0.345 | 0.059 | 0.116 | 0.032 | 0.31 | 0.118 | 0.059 | | NS | NS | * | NS | NS | NS | NS | * | * | NS |
| LLTGQ | 0.33 | 0.034 | 0.103 | 0.033 | 0.257 | 0.107 | 0.034 | 0.05 | | NS | * | NS | NS | NS | NS | * | * | NS |
| LYX | 0.288 | 0.035 | 0.117 | 0.03 | 0.236 | 0.096 | 0.035 | 0.049 | 0.023 | | * | NS | NS | * | * | * | * | * |
| PEJX | 0.23 | 0.233 | 0.18 | 0.177 | 0.163 | 0.129 | 0.233 | 0.206 | 0.184 | 0.155 | | NS | * | * | * | * | NS | * |
| QL | 0.291 | 0.114 | 0.116 | 0.085 | 0.26 | 0.126 | 0.114 | 0.106 | 0.079 | 0.076 | 0.156 | | * | NS | * | * | NS | * |
| SDL | 0.36 | 0.012 | 0.103 | 0.026 | 0.305 | 0.127 | 0.012 | 0.047 | 0.019 | 0.027 | 0.19 | 0.091 | | * | NS | * | * | NS |
| SZ1 | 0.384 | 0.067 | 0.043 | 0.046 | 0.313 | 0.106 | 0.067 | 0.074 | 0.054 | 0.056 | 0.181 | 0.099 | 0.053 | | * | * | * | * |
| SZ2 | 0.361 | 0.008 | 0.102 | 0.018 | 0.285 | 0.125 | 0.008 | 0.042 | 0.02 | 0.026 | 0.193 | 0.081 | 0.01 | 0.045 | | * | * | NS |
| WFSY | 0.428 | 0.25 | 0.127 | 0.196 | 0.371 | 0.195 | 0.25 | 0.227 | 0.207 | 0.21 | 0.221 | 0.19 | 0.223 | 0.132 | 0.217 | | * | * |
| YXSBQ | 0.414 | 0.327 | 0.194 | 0.269 | 0.355 | 0.198 | 0.327 | 0.283 | 0.253 | 0.245 | 0.201 | 0.21 | 0.282 | 0.214 | 0.275 | 0.25 | | * |
| YYLXZ | 0.395 | 0.008 | 0.123 | 0.02 | 0.342 | 0.134 | 0.008 | 0.042 | 0.03 | 0.032 | 0.212 | 0.1 | 0.012 | 0.06 | 0.011 | 0.237 | 0.31 | |

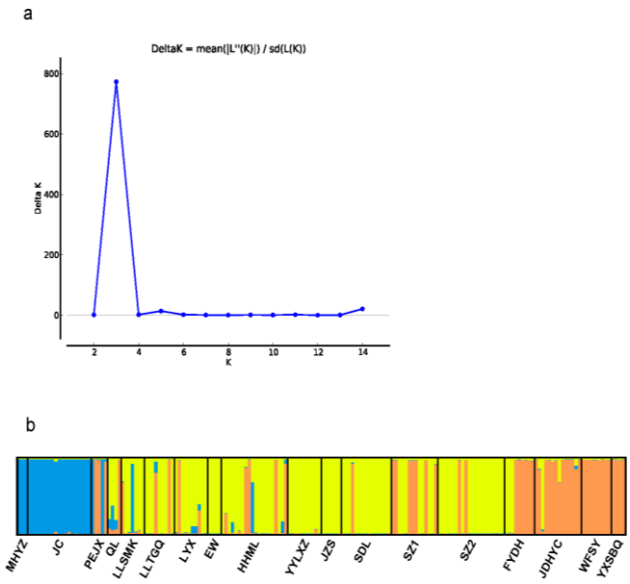


Fig. 2. The structure analysis of 18 *Amorphophallus konjac* populations. (a) Δ*K* estimates of the posterior probability distribution. (b) Estimated population structure of *Amorphophallus konjac* populations with *K* =3.

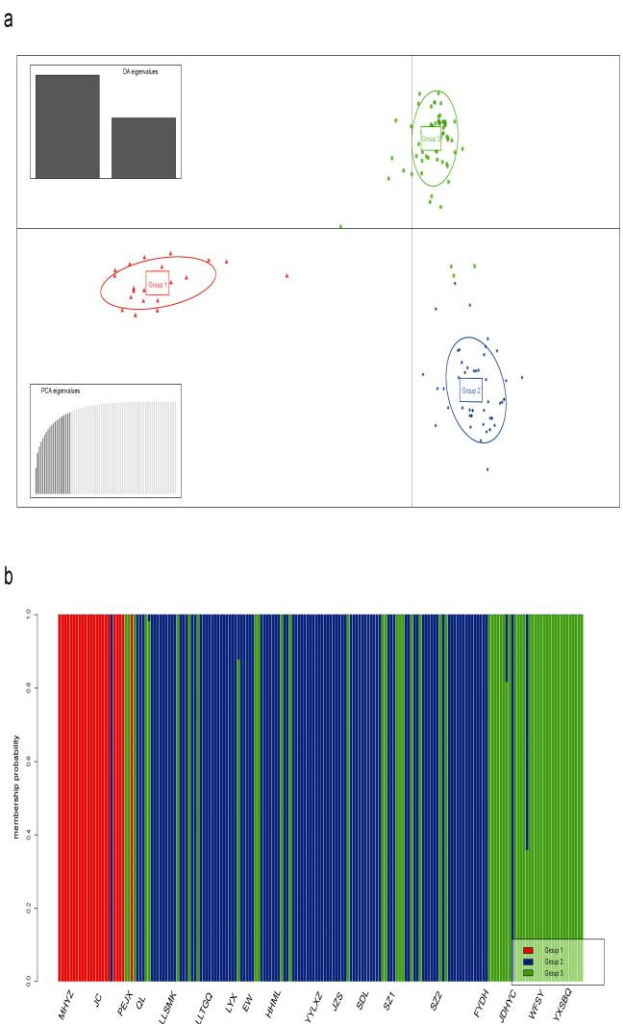


Fig. 3. Discriminant analysis of principal component (DAPC) of 18 *Amorphophallus konjac* populations. (a) Plot of the first two dimensions of DAPC. (b) Bar plot of the three clusters identified with R adegenet package.

## Results

**Genetic diversity:** The mean allele numbers ($N_A$) for each population varied from 1.308 (MHYZ) to 3.846 (JDHYC), and the allelic richness ($A_R$) ranged between 1.277 (MHYZ) and 2.602 (PEJX). Besides, the private alleles were found in 10 populations. The highest frequency of private alleles was detected in population JC ($A_P$ = 0.769) and the lowest was found in SZ2, LLSMK, and FYDH ($A_P$=0.077). The mean value of $H_O$ of all populations was 0.487, ranging from 0.256 (MHYZ) to 0.615 (EW, JZS, and WFSY). The average $H_E$ was 0.363 and the highest value was 0.516 (JDHYC), while the lowest was 0.145 (MHYZ). Thefixation index ($F_{IS}$) per population ranged from -1.000 (EW and JZS) to 0.250 (PEJX), and the average value was -0.296 (Table 1).

**Genetic structure:** Based on STRUCUTRE analysis, three genetic clusters was detected (Fig. 2). The first group mainly consisted of two populations (JC and MHYZ) from the southern regions (Xishuangbanna Dai Autonomous Prefecture). The second group contained LLTGQ, SZ1, SZ2, QL, EW, SDL, JZS, LLSMK, HHML, YYLXZ, and LYX, which were from east of the Wumeng Mountains. Group 3 included populations PEJX, YXSBQ, JDHYC, WFSY, and FYDH from west of the Wumeng Mountains (Fig. 1). In addition, DAPC analysis (Fig. 3) and the unrooted NJ tree constructed using $D_A$ distance (Fig. 4) divided populations into three genetic clusters. The population structure was confirmed with AMOVA. When assigning the populations into three clusters, 28.045% of the variation was found among clusters, with 5.82% of the variation being among populations and 66.135% being within populations ($p$<0.001) (Table 3).

**Genetic differentiations:** Pair wise $F_{ST}$ values of each population pair ranged from 0 (between population GZS and EW) to 0.452 with most population pairs (306 out of 360 pairs) detected with significant values ($p$<0.05) (Table 4). The mantel test showed that there was a significant correlation between the pair wise genetic distance and geographic distance ($r^2$=0.218, $P$ = 0.001) (Fig. 5).

## Discussion

**Genetic diversity of *A. konjac* populations:** In this study, moderate to high genetic diversity was detected ($H_O$ ranged between 0.256 and 0.615, $H_E$ ranged between 0.145 and 0.516, $I$ ranged between 0.205 and 0.947), higher than that reported in a previous study on *A. konjac* populations in central China ($H$ varied from 0.066 to 0.202, $I$ varied from 0.113 to 0.313) (Pan *et al.*, 2015). The differences in genetic diversity were caused by the using of different DNA markers (SSR vs. AFLP). The co-dominant nature of microsatellites led to the relatively higher genetic diversity (Ridout & Donini, 1999; Varshney *et al.*, 2007). Besides, *A. konjac* in China was believed to have spread from the south-western region into the northern parts of its range. The lower genetic diversity in central China was consistent with the 'abundant centre' model in which populations of the edge had lower densities and less genetic diversity compared with core populations (Inbar *et al.*, 2010). The genetic diversity in our study was also relatively higher than several genetic researches of *Amorphophallus* species from other countries, such as Indonesia and India (Kurniawan *et al.*, 2011; Anil *et al.*, 2014).

**Table 4. Results of the analyses 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 | 183.727 | 1.108 | 28.045 | $F_{CT}$=0.280 |
| Among populations within groups | 15 | 95.526 | 0.23 | 5.82 | $F_{SC}$=0.081 |
| Within populations | 297 | 774.922 | 2.614 | 66.135 | $F_{ST}$=0.339 |

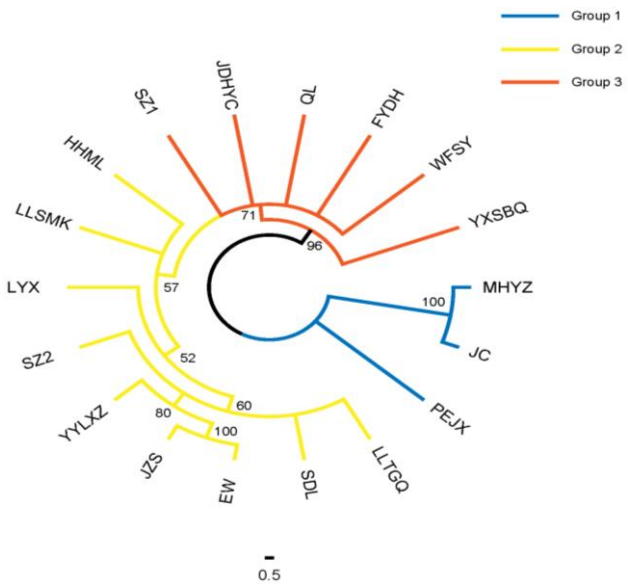


Fig. 4. Neighbour-joining tree of the 18 populations of *Amorphophallus konjac*. Bootstraps that were higher than 50% are shown.

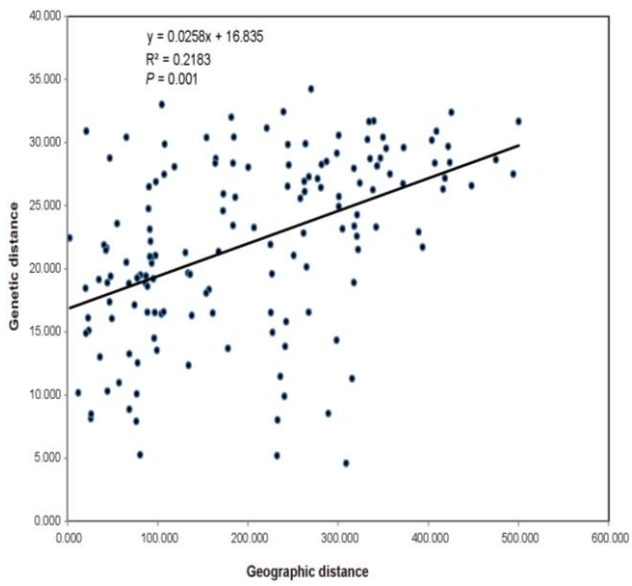


Fig. 5. The mantel test between pair wise genetic and geographic distances (km) of 18 *Amorphophallus konjac* populations.

**Population structure:** The 18 populations were divided into three groups by all the three tests. The structure patterns were strongly related to their geographical regions. Group 1 contained two populations (MHYZ and JC) from Xishuangbanna. This region had a subtropical climate that differed greatly from other areas in this study. Populations MHYZ and JC had a long period of local adaption and were differentiated from other north populations long ago according to the molecular evidence that the highest proportion of private alleles was found in population JC (Table 1). In addition, the genetic barrier between groups 2 and 3 was correlated with the natural geographic barrier of the Wumeng Mountains, which ran from northeast to southwest, hindering genetic exchange between these geographic regions. Similar geographic barriers had been observed in other plants, such as *Paeonia rockii* (Yuan *et al.*, 2012) and *Castanea mollissima* (Liu *et al.*, 2013). The genetic structure was further confirmed by AMOVA ($p$<0.001) (Table 3). Many introgressions were observed between populations in proximity to each other, indicating that hybridisation occurred among these populations.

**Genetic differentiation:** Low to moderate levels of genetic differentiation ($F_{ST}$) (average 0.162) was reported in this research. Mantel test also suggested that the pattern of genetic variation between populations for *A. konjac* was driven by isolation due to distance. In addition, *A. konjac* could reproduce clonally via the vegetative organ, and sexually via seeds pollinated by insects (Li *et al.*, 2010). This reproducing system might have imposed constraints on long-distance dispersal.

**Conservation strategies:** There was a moderate to high genetic diversity in *A. konjac*. Population structure analyses showed that three significant genetic groups were observed and geographically adjacent populations tended to group together. These genetic groups should be treated as distinct evolutionarily significant units when making conservation strategies. Specifically, two populations from the Xishuangbanna region were isolated from the other populations and harboured most of the rare alleles. Relatively low genetic diversity was also observed in these populations. The highest conservation priority was needed for these two populations. Despite widely distributed groups maintaining comparatively high genetic variability, most of the populations in these regions were suffering from habitat fragmentation due to human activity (Li *et al.*, 2010). Thus, *in situ* conservation should be applied to preserve genetic diversity.

## Conclusions

In this study, 182 individuals from 18 wild populations were collected from south-western China, and the molecular variation and genetic structure were identified with 13 EST-SSRs. The results showed that the *A. konjac* materials in southern-western China contained a high level of genetic diversity. Population structure analyses revealed three major genetic groups, which were separated mainly by geographic barriers and different climates. Finally, evolutionarily significant units were identified and conservation strategies were suggested for these wild populations. These genetic materials could be used to improve the *Amorphophallus* crop, particularly in the control of bacterial soft rot disease.

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**References**

Anil, S.R., E.A. Siril and S.S. Beevy, 2014. Diversity analysis in *Amorphophallus* using isozyme markers. *Int. J. Veg. Sci.*, 20: 305-321.

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: 646-660.

Ekué, M.R., 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: 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: 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: 564-567.

Inbar, M., G. Eli, R. Uri, F. Adam, D. Alon, T. Yaron and G. Sarig, 2010. The change in genetic diversity down the core-edge gradient in the eastern spadefoot toad (*Pelobates syriacus*). *Mol. Ecol*., 19: 2675-2689.

Jakobsson, M. and N.A. Rosenberg, 2007. Clumpp: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23: 1801-1806.

Jombart, T., 2008. Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24: 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: 7-11.

Li, H., G. Zhu, P.C. Boyce, M. Jin, W.L.A. Hetterscheid, J. Bogner and N. Jacobsen, 2010. Araceae. Flora of china, Science Press, Beijing.

Liu, P., 2004. Konjac. China Agriculture Press, Beijing.

Liu, W., M. Kang, H. Tian and H. Huang. 2013. A range wide geographic pattern of genetic diversity and population structure of *Castanea mollissima* populations inferred from nuclear and chloroplast microsatellites. *Tree Genet.& Genomes*, 9: 975-987.

Mandal, R., S. Nag, J. Tarafdar, S. Mitra, R. Mandal, S. Nag, J. Tarafdar and S. Mitra. 2016. A comparison of efficiency parameters of SSR markers and genetic diversity analysis in *Amorphophallus paeoniifolius* (dennst.) nicolson. *Braz. Arch. Biol. Techn.*, 59: 1-7.

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

Oyama, K., S. Hernández-Verdugo, C. Sánchez, A. González-Rodríguez, P. Sánchez-Peña, J.A. Garzón-Tiznado and A. Casas. 2006. Genetic structure of wild and domesticated populations of *Capsicum annuum* (Solanaceae) from northwestern mexico analyzed by RAPDs. *Genet Resour. Crop Ev.*, 53: 553-562.

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: 18753-18763.

Pan, C., Y.N. You, Y. Diao, Z.L. Hu and J.M. Chen. 2012. Isolation and characterization of microsatellite loci for the herbaceous tuber crop, *Amorphophallus konjac* (Araceae). *Genet. Mol. Res.*, 11: 4617-4621.

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: 2537-2539.

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

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 WuhanUniv Nat Sci.*, 28: 85-86.

Ridout, C.J. and P. Donini. 1999. Use of AFLP in cereals research. *Trends Plant Sci.*, 4: 76-79.

Rosenberg, N.A. 2004. Distruct: A program for the graphical display of population structure. *Mol. Ecol. Resour.*, 4: 137-138.

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: 814-817.

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: 747-752.

Varshney, R.K., K. Chabane, P.S. Hendre, R.K. Aggarwal and A. Graner. 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Sci.*, 173: 638-649.

Vellve, R. 1993. The decline of diversity in european agriculture. *Ecologist*, 23: 64-69.

Wang, S. and Y. Xie. 2009. China species red list, Higher Education Press, Beijing.

Wu, J., X. Liu, D. Ying, Z. Ding and Z. Hu. 2012. Authentication and characterization of a candidate antagonistic bacterium against soft rot of *Amorphophallus konjac*. *Crop Prot.*, 34: 83-87.

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.

Zhang, Y.J., X.G. Zhang, P.Y. Liu and C.J. Feng. 2001. RAPD analysis of *Amorphophallus* germplasms. *J. Southwest Agri. Uni.*, 23: 418-421.

Zheng, X., C. Pan, Y. Diao, Y. You, C. Yang and Z. Hu. 2013. Development of microsatellite markers by transcriptome sequencing in two species of *Amorphophallus* (Araceae). *BMC Genomics*, 14: 490.

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