Genetic Diversity and Genetic Relationships of Amomum tsao-ko Based on Random Amplified Polymorphic DNA Markers

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Abstract

Amomum tsao-ko is a perennial herb of the ginger family. Fruit is used as a medicine and is often added to foods as a spice, but little information on its genetic features is currently available. In this study, 96 A. tsao-ko samples from eight populations were analyzed using RAPD molecular markers. Twelve RAPD primers produced 492 loci, 98.98% (487) of which were polymorphic. Marker index (MI) values and mean polymorphism information content (PIC) represented 8.095 and 0.197, respectively. At the population level, the percentage of polymorphic bands (PPB) was 57.18%. Nei's gene diversity (H) was 0.121, and Shannon's information index (I) was 0.275. The genetic differentiation coefficient among populations was Gst=0.084, which was similar to the 6% genetic variation among populations analyzed by AMOVA, indicating that there was no genetic differentiation among the populations of A. tsao-ko. A Mantel test (r = 0.153, P >0.05) further indicated that the geographic distance was not related to estimates of genetic distance. UMPGA cluster and principal coordinate analysis (PCoA) all divided 96 A. tsao-ko samples into three major groups. © 2018 Friends Science Publishers

Keywords: Genetic diversity; A. tsao-ko; RAPD marker; Polymorphic

Introduction

Amomum tsao-ko Crevostet Lemaire is a perennial herb of the genus Zingiberaceae, stem tufted, plant height up to 3 meters, leaf blade narrowly elliptic or oblong, yellow flower, and the fruit is red when ripe, the dry fruit is a traditional Chinese herbal medicine (Fig. 1). It is mainly distributed in southwest China and Vietnam at altitudes ranging from 1300 to 1800 m (Wu and Larsen, 2000; Xia et al., 2004). Previous research showed the major active compounds in A. tsao-ko were essential oils (EOs), which can be separated and extracted from the dried fruit using steam distillation, microwave assisted extraction, and ultrasonic extraction (Feng et al., 2010; Li et al., 2011). The EOs mainly consisted of monoterpane hydrocarbons and oxygenated monoterpenes such as 1, 8-cineole, α-pinene, β-pinene, terpineol, geraniol and geranial (Liu et al., 2011). The fruit of A. tsao-ko has the effects of eliminating phlegm and dampness as well as warming stomach and cold-disspelling, and its also used as a spice in cooking (Lim, 2013; Shi et al., 2014). Since the edible fruits of A. tsao-ko have been overharvested for trade, it is listed as “Near Threatened” by IUCN (Leong-Skornickova et al., 2012). Yunnan province (SW China) is the main production area of A. tsao-ko, and the planting area accounts for approximately 90% of China.

Fig. 1: Plant, flower and fruit morphology of Amomum tsao-ko. A, plant morphology; B, flower; C, fresh fruit; D, dry fruit

Traditionally, the fruit shape is the main basis for A. tsao-ko classification can be classified as round, oval, long and shuttle type. Due to multivariate environmental factors and ecological plasticity, it is hard to figure out the genetic
relationships of A. tsao-ko populations according to morphological traits. At present, the molecular genetics research of A. tsao-ko germplasm resources is still not perfect. Insensitive to environmental factors relative to morphological data, molecular markers are remarkably efficient in providing abundant information. Numerous molecular detection types have been applied to evaluate the genetic diversity of Amomum L. species including random amplified polymorphic DNA (RAPD) in A. villosum (Wang et al., 2000; Xu and Ding, 2005), amplified fragment length polymorphisms (AFLPs) in Thai Amomum species (Kaewsri et al., 2007), plus inter-simple sequence repeats (ISSRs) in Amomi Fructus (Zhang et al., 2011).

The survival and development of species relies largely on genetic diversity (Booy et al., 2000). Quantification of the genetic diversity of populations contributes to the analysis of its evolutionary potential and the prediction of its future fate and is of great importance to preservation and utilization of germplasm resources. RAPD is an efficient tool for exploring genetic variation and assessing diversity, and it has been widely employed for germplasm recognition in many plants (Nagl et al., 2011; Shafi et al., 2016; Bakhsh et al., 2017; Baruah et al., 2017; Kumari et al., 2017).

As a traditional Chinese medicine and spice, research on A. tsao-ko has mainly concentrated on its chemical composition (Yang et al., 2008; Liu et al., 2011; Zhang et al., 2012). Whereas, the genetic diversity of A. tsao-ko has been the subject of little research so far, this severely limits the preservation and use of the spice. Microsatellite (SSR) markers have recently been employed in genetic analysis of 60 A. tsao-ko individuals from three populations (Baoshan population, Maguan population and Fadou population) and low genetic diversity was detected using few markers (nine SSR markers) (Yang et al., 2014). Lately, Ma et al. (2017a, b) used five pairs of SSRs to analyze 44 A. tsao-ko. The results showed that genetic diversity of A. tsao-ko in Jinping County was higher than that of Yang et al. (2014).

As an effective and instrument approach to identify genetic variation, RAPD can produce markers which covering the genome without prior knowledge of their sequence (Williams et al., 1990). ARAPD marker system was suitable for the determination of genetic differentiation and diversity in A. tsao-ko germplasm in this study, serving three purposes, that was, to estimate genetic diversity of different geographically cultivated populations in Yunnan Province, reveal the distribution pattern of genetic variability within/among populations, and provide suggestions for the preservation and utilization of such important herbal medicine.

Materials and Methods

The experiments were carried out from October 2016 to May 2017. A. tsao-ko resources survey and leaf collection were conducted in October-November 2016, which have a wide range of representative, and cover the traditional A. tsao-ko production area in Yunnan Province (N22°53'24"-N 24°49'48", E 98°18'36"-E 103°31'48").

The genomic DNA extraction, RAPD reaction system optimization and primer screening were carried out between December 2016 and February 2017. Then PAPD-PCR amplification of A. tsao-ko samples and data analysis were conducted from March 2017 to May 2017.

Plant Materials

Plant materials, totaled 96 individuals of A. tsao-ko, were collected from Yunnan Province, China (Fig. 2 and Table 1). There were eight geographic populations, each comprising 12 individuals that were distributed at a distance of over 50 m. Young leaf materials were collected and later reserved in the lab at the temperature of 80°C before DNA extraction.

DNA Extraction

A modified cetethyltrimethyl ammonium bromide (CTAB) was employed to extract genomic DNA (Doyle, 1987). After extraction, the purity of DNA was estimated by NanoDrop-2000 version 1.0 spectrophotometer, the quality of DNA was determined by running on one percent agarose gel electrophoresis. Finally, the DNA was adjusted to 20 ng/μL for PCR amplification.

RAPD Amplification

Individual RAPD-PCRs were conducted in a 25 μL reaction volume, which contained 40 ng DNA, 4.0 mmol/L MgCl2, 2.0U Taq DNA polymerase, 0.4 μmol/L per primer, 0.2 mmol/L dNTPs and 2.5 μL of 10× PCR reaction buffer (Mg2+-free). ETC-811PCR Thermocycler was used in all amplifications (Eastwin, Beijing, China). PCR program was carried at initial denaturing at 95°C for five minutes (one cycle); followed by 95°C for one minute, 36°C for one minute, 72°C for 1.5 minutes (forty cycle); a final extension at 72°C for eight minutes. Fragments were parted on 5% denaturing polyacrylamide gels in 10×TBE buffer, followed by visualization of silver nitrate staining.

Statistical Analyses

Data procured were input into a binary matrix for analysis. A missing or ambiguous band was scored as 0 (absent) while distinct and visible bands were marked as 1 (present). The population was assumed to be in Hardy-Weinberg equilibrium, genetic polymorphism parameters for each population with the use of POPGENE 1.32 (Yeh et al., 1999). PIC values was calculated by formula PIC=2f(1-f) (Roldán-Ruiz et al., 2000). MI (Marker index) was calculated using the formula given by Prevost and Wilkinson (1999).
Table 1: Population information and number of Amomumtsao-ko samples used in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Population</th>
<th>No. of Voucher samples</th>
<th>Latitude (N)</th>
<th>Longitude (E)</th>
<th>Altitude (m)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>Pop1</td>
<td>12</td>
<td>22°54'36&quot;</td>
<td>103°13'12&quot;</td>
<td>1665</td>
<td>Jinhping, Yunnan</td>
</tr>
<tr>
<td>Y</td>
<td>Pop2</td>
<td>12</td>
<td>23°30&quot;</td>
<td>102°55'12&quot;</td>
<td>2108</td>
<td>Yuyang, Yunnan</td>
</tr>
<tr>
<td>L</td>
<td>Pop3</td>
<td>12</td>
<td>22°53'24&quot;</td>
<td>102°24'43&quot;</td>
<td>1880</td>
<td>Lvxian, Yunnan</td>
</tr>
<tr>
<td>P</td>
<td>Pop4</td>
<td>12</td>
<td>23°24'</td>
<td>103°31'48&quot;</td>
<td>1721</td>
<td>Pingbian, Yunnan</td>
</tr>
<tr>
<td>LC</td>
<td>Pop5</td>
<td>12</td>
<td>22°53'46&quot;</td>
<td>99°49'12&quot;</td>
<td>1924</td>
<td>Lancang, Yunnan</td>
</tr>
<tr>
<td>YX</td>
<td>Pop6</td>
<td>12</td>
<td>24°17'24&quot;</td>
<td>100°6'36&quot;</td>
<td>1811</td>
<td>Yuxian, Yunnan</td>
</tr>
<tr>
<td>B</td>
<td>Pop7</td>
<td>12</td>
<td>24°49'48&quot;</td>
<td>98°46'48&quot;</td>
<td>1873</td>
<td>Baoshan, Yunnan</td>
</tr>
<tr>
<td>D</td>
<td>Pop8</td>
<td>12</td>
<td>24°45'0&quot;</td>
<td>98°18'36&quot;</td>
<td>1822</td>
<td>Lianghe, Yunnan</td>
</tr>
</tbody>
</table>

Table 2: Primer sequences, number of amplified and polymorphic bands, percentage of polymorphic bands, and PIC and MI values of RAPD primers used for genotyping A. tsao-ko

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer Sequence</th>
<th>TNB</th>
<th>NPB</th>
<th>PPB</th>
<th>PIC</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA4</td>
<td>ATTCGCGGCTT</td>
<td>37k</td>
<td>60k</td>
<td>97.297</td>
<td>0.187</td>
<td>7.088</td>
</tr>
<tr>
<td>OPA7</td>
<td>GAAACGGGTG</td>
<td>26k</td>
<td>60k</td>
<td>100.000</td>
<td>0.184</td>
<td>4.776</td>
</tr>
<tr>
<td>OPA8</td>
<td>GTACGTCAGG</td>
<td>42k</td>
<td>60k</td>
<td>97.619</td>
<td>0.191</td>
<td>7.842</td>
</tr>
<tr>
<td>OPA9</td>
<td>GGCGTCAGGC</td>
<td>52k</td>
<td>60k</td>
<td>100.000</td>
<td>0.173</td>
<td>9.006</td>
</tr>
<tr>
<td>OPA19</td>
<td>CAAACATTGAG</td>
<td>46k</td>
<td>60k</td>
<td>100.000</td>
<td>0.175</td>
<td>8.070</td>
</tr>
<tr>
<td>OPA20</td>
<td>GTTCGCGATCC</td>
<td>41k</td>
<td>60k</td>
<td>100.000</td>
<td>0.179</td>
<td>7.330</td>
</tr>
<tr>
<td>OPB1</td>
<td>GTTCGCTCC</td>
<td>50k</td>
<td>60k</td>
<td>100.000</td>
<td>0.212</td>
<td>10.622</td>
</tr>
<tr>
<td>OPB5</td>
<td>TGGCCCTTCTC</td>
<td>32k</td>
<td>60k</td>
<td>96.875</td>
<td>0.157</td>
<td>5.018</td>
</tr>
<tr>
<td>OPB7</td>
<td>GTGCACGAGG</td>
<td>33k</td>
<td>60k</td>
<td>96.970</td>
<td>0.231</td>
<td>7.960</td>
</tr>
<tr>
<td>OPB18</td>
<td>CCACGACGGT</td>
<td>43k</td>
<td>60k</td>
<td>100.000</td>
<td>0.239</td>
<td>10.275</td>
</tr>
<tr>
<td>OPB19</td>
<td>AGCAGACGGG</td>
<td>45k</td>
<td>60k</td>
<td>97.778</td>
<td>0.212</td>
<td>9.315</td>
</tr>
<tr>
<td>OPC4</td>
<td>CGCGATCTC</td>
<td>45k</td>
<td>60k</td>
<td>100.000</td>
<td>0.221</td>
<td>9.935</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>41k</td>
<td>60k</td>
<td>98.878</td>
<td>0.197</td>
<td>8.095</td>
</tr>
</tbody>
</table>

*TNB: total number of bands; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; PIC: polymorphism information content; MI: marker Index

Results

Characteristics of RAPD Markers

Of total 120 RAPD primers were used for screening, 12 were able to produce consistent and distinguishable bands (Fig. 3). Table 2 showed characteristics of the 12 RAPD primers. 492 bands were amplified for 96 A. tsao-ko individuals. 487 (98.98%) of them were polymorphic. The highest number of amplification bands was 52 (OPA9), and the lowest with 26 (OPA7) averaging 41 fragments per primer. PIC values ranged from 0.157 (OPB5) to 0.239 (OPB18) averaging 0.197. The highest MI was observed for the primer OPB1 (10.622) and the lowest for the primer OPB7 (4.776), with an average of 8.095.

Analysis of Genetic Diversity

Within populations, the percentage of polymorphic bands was 51.21 ~ 64.92% with an average of 57.18%, and H, 0.114 to 0.140 with an average of 0.121, and I, 0.189 to 0.232 with an average of 0.202. Average Na per locus was 1.572, and average Ne per locus was 1.180. Ne and polymorphic content were found highest in Pop1 and Pop3, and lowest in Pop4 (Table 3). These results revealed that A. tsao-ko had relatively low genetic diversity both within and among populations.

Genetic Differentiation in Populations

A. tsao-ko genetic differentiation analysis among eight populations exposed that Ht was 0.133, Hs was 0.121, Gst was 0.084, and Nm was 5.425 (Table 3). Most of the genetic variation (91.6%) appeared within the population.
Table 3: Mean values genetic diversity parameters for eight *A. tsao-ko* populations

<table>
<thead>
<tr>
<th>Population ID</th>
<th>PPB (%)</th>
<th>Na (%)</th>
<th>Ne (%)</th>
<th>H (Na)</th>
<th>F</th>
<th>Ht</th>
<th>Hs (%)</th>
<th>Gst (%)</th>
<th>Nm (%)</th>
<th>Hs/Ht</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pop1</td>
<td>69.00</td>
<td>1.619</td>
<td>1.681</td>
<td>0.129</td>
<td>0.216</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pop2</td>
<td>57.66</td>
<td>1.577</td>
<td>1.712</td>
<td>0.119</td>
<td>0.199</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pop3</td>
<td>64.92</td>
<td>1.649</td>
<td>1.206</td>
<td>0.140</td>
<td>0.232</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pop4</td>
<td>56.25</td>
<td>1.563</td>
<td>1.168</td>
<td>0.115</td>
<td>0.193</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pop5</td>
<td>51.21</td>
<td>1.512</td>
<td>1.185</td>
<td>0.118</td>
<td>0.190</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pop6</td>
<td>53.23</td>
<td>1.532</td>
<td>1.169</td>
<td>0.114</td>
<td>0.189</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pop7</td>
<td>54.23</td>
<td>1.542</td>
<td>1.171</td>
<td>0.116</td>
<td>0.193</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pop8</td>
<td>58.06</td>
<td>1.581</td>
<td>1.178</td>
<td>0.121</td>
<td>0.202</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>57.18</td>
<td>1.572</td>
<td>1.180</td>
<td>0.121</td>
<td>0.202</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.96</td>
<td>1.990</td>
<td>1.187</td>
<td>0.133</td>
<td>0.235</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PPB: percentage of polymorphic bands; Na: observed number of alleles; Ne: effective number of alleles; H: Nei’s gene diversity; F: Shannon’s Information index; Ht: total genetic diversity; Hs: genetic diversity within populations; Gst: genetic differentiation among populations; Nm: gene flow estimated from Gst

Table 4: Analysis of molecular variance (AMOVA) based on RAPD markers in *A. tsao-ko*

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom (df)</th>
<th>Sum of squares (SS)</th>
<th>Estimate of variance components</th>
<th>Percentage of total variance (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Populations</td>
<td>7</td>
<td>863.573</td>
<td>4.506</td>
<td>6%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within Populations</td>
<td>88</td>
<td>6098.167</td>
<td>69.297</td>
<td>94%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>6961.740</td>
<td>73.803</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

This can be further backed up by another molecular variance analysis (AMOVA), which revealed that variation in populations accounted for 94% of the entire variation (p<0.001) (Table 4). The genetic distance among populations of *A. tsao-ko* varied from 0.0094 to 0.0239 (Table 5). Pop4 and Pop7 had the shortest genetic distance, demonstrating a closer relationship between them. In contrast, Pop2 and Pop5 had the longest genetic distance, signifying a more distant relationship between them. Furthermore, the genetic distances and geographic distances did not show significant correlations by a Mantel test (r = 0.153, P > 0.05).

Cluster Analysis and Principal Coordinates Analysis (PCoA)

Revealed by the data from RAPD marker, the simple matching coefficient varied from 0.78 to 0.96 with an average of 0.8175. Fig. 4 depicts a dendrogram based on the similarity matrix. In this dendrogram, 96 individuals of *A. tsao-ko* were clustered into three major groups. Cluster I included 17 samples of *A. tsao-ko* from seven different populations (all except Pop7); Cluster II consisted of 48 samples; and cluster III comprised the remaining 31 samples. On the strength of the genetic similarity matrix, PCoA was conducted to further comprehend the genetic relationships among *A. tsao-ko* samples (Fig. 5). The first two principal components represented 10.52% and 5.82% of the entire molecular variation correspondingly. The PCoA plot revealed a similar grouping of samples to the dendrogram analysis.

Discussion

*A. tsao-ko* is an important crop with drug and diet attributes. It has been cultivated in China historically, especially in Yunnan Province (Yang et al., 2014). To date, few studies have applied this technology to study the genetic diversity of *A. tsao-ko*. In the present study, the genetic diversity of eight *A. tsao-ko* populations was found to be significantly different, but the level of diversity was lower than the average (0.22 or 0.23) genetic diversity of various other plant populations based on RAPD, AFLP, and ISSR dominant markers (Nybom, 2004). The results revealed that *A. tsao-ko* had relatively low genetic diversity, which is in accordance with that reported by Yang et al. (2014). In addition, low genetic diversity has also been detected in *A. Fructus* (Zhang et al., 2011).
A. tsao-ko thrives in moist, well-drained, organic matter-rich soil in shade or partial shade. Owing to its strict environmental requirements, this plant is mainly distributed in Yunnan province, southwestern China (Wu and Larsen, 2000), and this narrow distribution may be the main reason for the low level of genetic diversity (Hamrick and Godt, 1996).

The current results reveal a low level of genetic diversity at the species level (H=0.134). Nei’s gene diversity (H) was relatively low in Pop6 (Yuxian population) at 0.114, whereas maximum H (0.140) was observed in Pop3 (Lvchun population). Amomum tsao-ko cultivation (Pop1 and Pop3) has existed for more than 300 years in Yunnan, of which Honghe state has the longest cultivation history. Relatively strong artificial selection occurred during the process of introduction of the A. tsao-ko into other states, with individuals exhibiting high yield, high germination rate, and good disease resistance being more likely to be introduced, leading to reduced genetic diversity in other areas.

It is genetic variation available within a population that bestows a plant the evolutionary potential and ability to survive in tough environmental conditions. Both POPGENE

<table>
<thead>
<tr>
<th>Population</th>
<th>Pop1</th>
<th>Pop2</th>
<th>Pop3</th>
<th>Pop4</th>
<th>Pop5</th>
<th>Pop6</th>
<th>Pop7</th>
<th>Pop8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pop1</td>
<td>****</td>
<td>0.9838</td>
<td>0.9899</td>
<td>0.9894</td>
<td>0.9821</td>
<td>0.9853</td>
<td>0.9856</td>
<td>0.9863</td>
</tr>
<tr>
<td>Pop2</td>
<td>0.0163</td>
<td>****</td>
<td>0.9834</td>
<td>0.9841</td>
<td>0.9764</td>
<td>0.9793</td>
<td>0.9856</td>
<td>0.9838</td>
</tr>
<tr>
<td>Pop3</td>
<td>0.0101</td>
<td>0.0167</td>
<td>****</td>
<td>0.9901</td>
<td>0.9849</td>
<td>0.9837</td>
<td>0.9879</td>
<td>0.9861</td>
</tr>
<tr>
<td>Pop4</td>
<td>0.0109</td>
<td>0.0158</td>
<td>0.0099</td>
<td>****</td>
<td>0.9858</td>
<td>0.9868</td>
<td>0.9906</td>
<td>0.988</td>
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<td>Pop5</td>
<td>0.0181</td>
<td>0.0239</td>
<td>0.0152</td>
<td>0.0143</td>
<td>****</td>
<td>0.9846</td>
<td>0.9856</td>
<td>0.9826</td>
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<td>Pop6</td>
<td>0.0148</td>
<td>0.021</td>
<td>0.0164</td>
<td>0.0133</td>
<td>0.0155</td>
<td>****</td>
<td>0.9866</td>
<td>0.986</td>
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<td>Pop7</td>
<td>0.0145</td>
<td>0.0145</td>
<td>0.0121</td>
<td>0.0094</td>
<td>0.0145</td>
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<td>0.989</td>
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<td>0.0138</td>
<td>0.0163</td>
<td>0.014</td>
<td>0.0121</td>
<td>0.0176</td>
<td>0.0141</td>
<td>0.0111</td>
<td>****</td>
</tr>
</tbody>
</table>

Fig. 4: UPGMA dendrogram of 96 Amomum tsao-ko samples based on RAPD molecular markers

Fig. 5: Two-dimensional principal coordinates analysis created from RAPD-based genetic similarity estimates for 96 samples of Amomum tsao-ko. PC 1 and PC 2 represent the first and second principal coordinates, respectively (91.6%) and AMOVA (94%) analyses revealed that the majority of A. tsao-ko genetic differentiation existed within populations, which also indicated that this species is heterologous (Hamrick and Godt, 1996; Bussell, 1999). Hamrick (1987) reported 16 species of cross-pollinated plants with high gene flow (Nm). The Nm of A. tsao-ko among populations was nearly 4-fold greater than that of the 16 species, and this level of migration would prevent divisions among populations. Hartl and Clark (1997) noted that the influence of genetic drift exerted on genetic differentiation could be hindered by high gene flow. The two significant factors in genetic differentiation among populations are genetic drift and selection. The genetic drift can be prevented from causing genetic differentiation when Nm is above 1. The relatively low genetic differentiation and high gene flow in A. tsao-ko indicated both random mating among populations and that the species does not face genetic drift or population decline. Habitat heterogeneity is the major cause for the genetic differentiation of A. tsao-ko.
among populations. Wang (1994) noted the genetic identity from the same species but indifferent populations it was approximately 0.90 in spermatophytes. In this study, the genetic identity of A. tsao-ko between populations (0.9855) was higher than 0.90, which indicated that the genetic differentiation between populations was due to different growth environments. This result coincides with the hypothesis of high gene flow among A. tsao-ko populations.

Mantel test results showed no significant correlation between geographical distance and genetic distance. This may be attributed to A. tsao-ko originating in Jingping (Honghe State) and Maguan (Wenshan State) counties gradually being introduced to other parts of Yunnan Province, which may have resulted in high levels of gene flow among populations over long geographic distances. Thus, the geographic distance was not a major factor in contributing to the degree of genetic differentiation among A. tsao-ko populations. Results of the Mantel test were also supported by genetic identity and cluster analyses. As indicated in the cluster analysis, eight populations of A. tsao-ko were divided into three major groups (Fig. 4). We noticed that it was phylogeographic structure where populations near each other, such as Pop1 and Pop2, did not cluster together. There are two possible reasons for this phenomenon, one is due to the small sample size of each population and the other is a high level of gene flow and gene exchange (Wang et al., 2016).

In this study, the cluster analysis unequivocally divided 96 A. tsao-ko samples into three main groups. According to dendrograms, little or no location specificity existed among the A. tsao-ko genotypes, as reported for Galega officinalis (Wang et al., 2012) and Ricinus communis (Bhaveshb et al., 2010; Pecina-Quintero et al., 2013). Opportunities to migrate A. tsao-ko seedlings from growers in one area to the neighboring region or from growers in different agro-climatic areas for the procurement of high-yielding plant material for A. tsao-ko farming are possible explanations for the above results.

The longstanding survival of a species largely counts on a high level of genetic diversity. As it stands, economic activities and artificial selection (seedling breeding process) are posing considerable threats to A. tsao-ko populations, which may face imminent extinction in lack of effective conservation measures. According to the results herein, populations with higher levels of genetic diversity, such as Pop1 and Pop3, should be protected first via in situ conservation. Further, it is necessary to establish a germplasm nursery in a native area such as Jingping County.

Conclusion

This study, first of its kind, has demonstrated that the RAPD is a reliable and powerful tool to evaluate genetic diversity and relationships among A. tsao-ko genotypes. At the population level, the percentage of polymorphic bands (51.21~64.92%) and short genetic distance (0.0094 to 0.0239) revealed the low genetic diversity in A. tsao-ko, which suggested that there is a need to bolster the conservation of A. tsao-ko resources.

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References

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