Study on genetic diversity of Paris polyphylla population from Vietnam and China

Article *in* Plant Cell Biotechnology and Molecular Biology · January 2016

CITATION
1

READS
136

7 authors, including:

Hoang Tan Quang
Hue University
22 PUBLICATIONS **28** CITATIONS
SEE PROFILE

Tan Trinh Huu
Hue university Vietnam
2 PUBLICATIONS **1** CITATION
SEE PROFILE

Nguyen Duc Huy
Hue University
22 PUBLICATIONS **86** CITATIONS
SEE PROFILE

Plant Cell Biotechnology and Molecular Biology 17(1&2):57-63; 2016 ISSN: 0972-2025

STUDY ON GENETIC DIVERSITY OF *Paris polyphylla* POPULATION FROM VIETNAM AND CHINA

HOANG TAN QUANG*, CAI THI QUYNH THOA, TRINH HUU TAN, NGUYEN THANH GIANG, NGUYEN DUC HUY, TRUONG THI BICH PHUONG AND VAN THI YEN

Department of Molecular Biology, Institute of Biotechnology, Hue University, (Ngoc Anh, Phu Thuong, Phu Vang, Thua Thien Hue 47000) Vietnam [HTQ, THT, NTG, NDH].
College of Sciences, Hue University, Vietnam [CTQT, TTBP].
College of Agriculture and Forestry, Hue University, Vietnam [VTY].
[*For Correspondence: E-mail: htquang@hueuni.edu.vn]

ABSTRACT

We investigated genetic diversity of 55 samples of valuable medicinal plant *Paris polyphylla* from different ecology regions (Thua Thien Hue, Vinh Phuc, Lao Cai (Vietnam) and Jilin (China)) using RAPD technique. We obtained total of 125 DNA polymorphic bands which average 15.63 polymorphic bands each primer used. The amplicon each primer varied 9-23 bands and their sizes ranged 257 to 2,448 bp. Among four regions, Thua Thien Hue showed highest genetic diversity level (H_o = 0.351) while Lao Cai was lowest (H_o = 0.198). The degree of genetic differentiation among populations were high (G_{st} = 0.301). Jaccard's similarity coefficient values were from 0.021 to 0.910 with a mean of 0.302. A phenetic tree classified population into four main groups. Group one was Thua Thien Hue population with coefficients of genetic similarity approximately of 0.42 and group II contained population from Vinh Phuc, group III consisted of population from Thua Thien Hue, Vinh Phuc, Lao Cai, and Jilin, whereas group IV involved population from Thua Thien Hue and Jilin. Our study indicated that *P. polyphylla* genetic relationship and diversity levels were high.

Keywords: Genetic diversity; *Paris polyphylla*; polymorphic bands; RAPD.

INTRODUCTION

Paris, a mostly self-pollination medicinal plant consists of approximately 24 species and is found in the temperate zones and tropical regions of Europe and Asian continent. Several *Paris* species have been reported to be used as haemostatic and anti-inflammatory agents to treat traumatic injuries, snake bites, abscess, parotitis and mastitis (Deb et al., 2015; Shah et al., 2012; Wang et al., 2013). Currently, *Paris polyphylla* Smith is extensively studied in traditional systems of Indian, Chinese and Vietnamese medicines mainly for its anticancerous property. Due to its importance medicinal valuable activity, *P. polyphylla* demand is dramatically increasing. However, *P. polyphylla* is mainly harvested from wild nature. Moreover, illegal and unscientific exploitation coupled with habitat destruction decreased the natural population of the herb, consequence this species is under vulnerable category. Thus, restoration and conservation of the natural population of this potential herb is prerequisites (Negi et al., 2014).

Since the introduction of RAPD markers in 1990 (Williams et al., 1990), application in plant genetic analysis has increased in an exponential manner. Molecular diversity studies in some threatened and rare plants using RAPD polymorphism and Shannon's index have reported (Verma et al., 2007). RAPD has been known with reproducibility challenges but still of relevant use in species with no sequence knowledge. In comparison to other genetically statistic methods, RAPD has advantages such as required simply technique, easy performance and inexpensive, however, RAPD still provides exactly in diversity of genetic level (Liu and Cordes, 2004; Williams et al., 1990). Although the morphology of

P. polyphylla has been extensively studied, information about its molecular phylogeny and population genetics is still limited (Zheng et al., 2012). There have been reported studies on *P. polyphylla* molecular diversity using RAPD (Tang, 2003; Zhang et al., 2004), SSR or ISSR techniques (He et al., 2007a,b; Zheng et al., 2012). However, these studies are limited within *P. polyphylla* populations grown in China. Therefore, we aim to study and compare the genetic variability of *P. polyphylla* populations from Vietnam and China.

MATERIALS AND METHODS

Sample Collection

Rhizomes of *P. polyphylla* were collected from 3 regions in Vietnam and 1 region in China (Table 1). Samples from Thua Thien Hue (Vietnam) were harvested by farmers. Samples from Vinh Phuc, Lao Cai (Vietnam) and Jilin (China) were supported by Tam Dao centre for plant seeds and materials (Tam Quan commune, Tam Dao district, Vinh Phuc province, Vietnam). Selected rhizomes were planted in Styrofoam box for budding at 25°C, light/dark schedule approximate 10/14 hours. The fresh leaves were used directly for DNA extraction.

DNA Isolation

Genomic DNA of *Paris polyphylla* leaf was isolated as described by Cota-Sánchez et al., (2006) with slight modifications. Briefly, leaf pieces were washed with tap water, cut into small piece (5×5 mm, 200 mg), grinded in liquid nitrogen and homogenized with 500 µL CTAB isolation buffer (100 mM Tris. HCl, 20 mM ethylenediaminete-traacetic acid, 3% hexadecyltrimethylammonium bromide, 0,2% β-mecaptoethanol and 1.4 M NaCl, pH 8) in a 1.5 mL eppendorf tube and incubated for 1 h at 65°C. Five microliters of β-mecaptoethanol were added and mixed through for 30 sec, then were incubated at 37°C for 15 min. Genomic DNA was purified by one volume of phenol:chloroform:isoamine alcohol solution (25:24:1), and precipitated with two volumes of cold ethanol 100%. DNA pellets were dried for overnight at room temperature and resuspended in 50 µL double distilled water (DDW). Total DNA concentration and quality were determined using NanoDrop ND-1000 (Thermo, USA) at 260/280 nm. Finally, DNA was diluted to a final concentration of 50 ng/µL for PCR amplification.

PCR-RAPD

Fifty-five of genomic DNA were used as template for RAPD analysis (Table 1). PCR reaction was carried out according to Quang et al., (2011). Each reaction was contained 0.5 unit of Taq DNA polymerase in 1x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM each of dNTP (PCR Master Mix, M7502, Promega), 20 pmol primer and 50 ng genomic DNA in a 20 µL final volume. Amplification was performed in a thermocycle (MJ Mini, Bio-rad, USA) under the following conditions: 2 min at 92^{o}C; followed by 42 cycles for 1 min of denaturing at 92°C; 1 min of annealing at 36°C and a 2 min extension at 72°C; a final extension for 10 min at 72^{o}C. Amplicons were separated on a 1.4% agarose gel for 6 hrs at 40 V. A total of 21 decamer oligonucleotides primers (Operon Technologies, USA) were examined. Then, eight primers were selected for further evaluation based on the quality and number of bands amplified (Table 2). The PCR amplicons were analyzed using Gel documentation system with Quantity One software ver. 4.1 (Bio-rad, USA).

Table 1. The genotype code and locality of collected samples

Locality	Sample codes	Distance (Kilometer from Thua Thien Hue)
Thua Thien Hue	1AL - 30AL	0
Vinh Phuc	31TD - 45TD	~820
Lao Cai	46LC - 48LC	~1020
Jilin	49TQ - 55TQ	> 5000

Data Analysis

PCR-RAPD amplicons were scored as presence (1) or absence (0) of a band in each sample. These data were used to compile a binary matrix for cluster analysis using the NTSYS-pc software (numerical taxonomy system, Exeter software, USA) ver. 2.1. Genetic similarity trees was generated according to Jaccard's similarity coefficient using the similarity for qualitative data (SIMQUAL) routine (Jaccard, 1908). The similarity coefficients were then used to construct a phenetic tree using the unweighted pair-group method with arithmetical averages (UPGMA) routine.

Table 2. Number of selected RAPD markers in diversity analyzing of *P. polyphylla* populations

Primers	Sequence (5'-3')	Number of amplification genotypes	Total of bands	Band size variations (bp)	Unique bands
A1	CAGGCCCTTC	41	12	469-1.444	1
A13	CAGCACCCAC	55	23	360-2.448	0
B10	CTGCTGGGAC	51	20	320-2.554	0
N2	ACCAGGGGCA	43	14	257-1.596	0
A7	GAAACGGGTG	47	14	414-2.046	1
F4	GGTGATCAGG	42	9	282-1.522	0
B4	GGACTGGAGT	38	18	346-2.407	1
N3	GGTACTCCCC	43	15	411-1.946	0

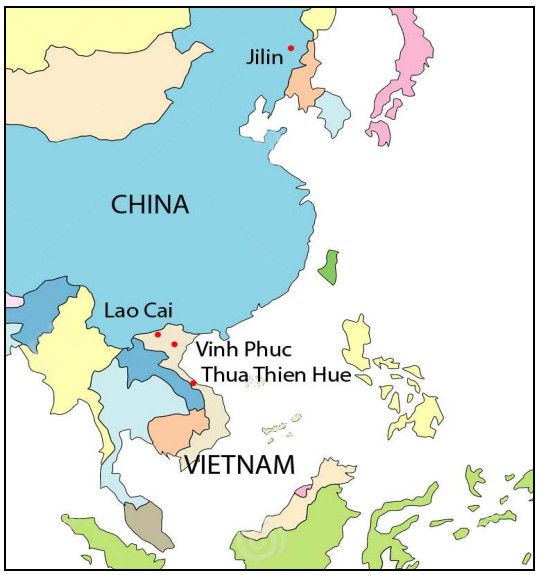

Fig. 1. Sample collection regions

The percentage of polymorphic band (PPB) = (number of polymorphic bands) / (total bands) × 100.

Shannon's index was used to estimate genetic diversity within a population (H_o), average diversity within all populations (H_{pop}), and total diversity among all individuals within the species (H_{sp}) as follow:

H_o = $-\sum p_i \log_2 p_i$, where p_i is the frequency of a given RAPD fragment

H_{pop} = $1/n \sum H_o$ where n is the number of populations

H_{sp} = $\sum p_s \log_2 p_s$ where p_s is frequency of RAPD fragment in the entire sample.

The proportion of diversity within populations was estimated as H_{pop}/H_{sp}, whereas the proportion of diversity among populations (G_{st}) was estimated as $G_{st}=(H_{sp} - H_{pop})/H_{sp}$.

RESULTS AND DISCUSSION

RAPD Analysis

Among of twenty-first tested random primers, we obtained eight primers that showed high numerous of PCR amplicons. These selected primers generated total of 125 bands, ranging in size from approximately 257 (N2) to 2,448 bp (A13) (Table 2), and the number of PCR amplicons of each primers varied from 9 (F4) to 23 (A13). All of bands were polymorphic (with a mean of 15.63 bands per primer) and three bands were unique (A1-1,182, A7-716, and B4-1,948). A13 was specific primer for *P. polyphylla* populations and amplified twenty three PCR-RAPD products (Fig. 2). Our study is in accordance to report by Zhang et al (2004) but higher than that of Tang (2003) on polymorphic bands per primer in *P. polyphylla* of 15.37 and 7.17 polymorphic, respectively. The amplification variation of each tree using these selected primers indicated that 4AL, 6AL and 7AL primers generated highest diversity (64 bands) whereas 27AL primer resulted in lowest diversity (8 bands). The average amplicon was 41.87 bands of each tree.

In this study, the percentage of polymorphic band (PPB) of all samples was 100%, higher than PPB value from other reports. Zhang et al (2004) reported that the PPB of *P. polyphylla* var. *yunnanensis* was 75,14%, PPB of *P. polyphylla* var. *chinensis* was 80,31%. In previous study using ISSR technique the PPB of *P. polyphylla* var. *yunnanensis* was 90,44% (He et al., 2007a) and PPB of *P. polyphylla* from Yunnan and Sichuan province (China) was 93.63% (He et al.,

2007b). Tang (2003) analyzed the genetic diversity and interspecies relationship among 10 species and 1 variant of genus *Paris*, the PPB value was 95.30%. *P. cronquist* showed highest polymorphism (90.2%) while *P. forrestii* was lowest (79.0%). According to Songyun and Soukup (2000), *Paris polyphylla* Sm. consist of 10 sub-species (including *P. polyphylla* var. *polyphylla, yunnanensis, chinensis, nana, alba, stenophylla, minor, latifolia, pseudothibetica*, and *kwantungensis*), samples from Vietnam and China maybe belong to different sub-species so the PPB value was high (100%).

PBB of population level of this study ranged from 87.30% (Lao Cai) to 99.11% (Thua Thien Hue). The PBB of two *P. polyphylla* var. *yunnanensis* populations was 57.4% and 54.67%, while PPB of two *P. polyphylla* var. *chinensis* populations was 56.33% and 57.75% (Zhang et al., 2004) which are considered, lower than that PBB value in our study. These results indicated that *P. polyphylla* populations from Thua Thien Hue, Tam Dao, Lao Cai and Jilin have high level of genetic diversity.

Cluster Analysis

The data obtained from RAPD analysis were combined and phenetically analysed to determine the genetic similarity among the sample. The Jaccard's coefficient of genetic similarity (GS) were calculated using 125 RAPD combinations for 55 genotypes. The GS values changed from 0.021 (27AL and 47LC) to 0.910 (6AL and 7AL) with a mean of 0.302.

Fig. 3 showed a UPGMA phenetic tree produced from the Jaccard's coefficients. The fifty five samples classified into four main groups (I, II, III and IV) with the genetic similarity between group I and II was approximately 0.24, whereas the genetic similarity between group III with groupp I and II was approximate 0.28. Moreover, group IV showed lowest genetic similarity to group I, II, and III with GS value of 0.12. In present study, the genetic relationship of *P. polyphylla* was long distance, indicating the genetic diversity level was high.

Group I contained only samples from Thua Thien Hue (22 samples) with GS approximately of 0.42. Group II involved almost samples from Vinh Phuc (14/16 samples). Group III consisted of 14 samples from all of collected sample regions, including Thua Thien Hue (7 samples), Vinh Phuc (2 samples), Lao Cai (2 samples), and Jilin (3 samples), whereas group IV included 1 sample from Thua Thien Hue, 2 samples from Jilin. Among of four groups, group III and IV had low level of genetic similarity.

Population Diversity

The total amplicon among of four populations varied from 63 (Lao Cai) to 113 (Thua Thien Hue). The population from Lao Cai showed the lowest frequency of polymorphic bands (87.30%), while Thua Thien Hue had the highest polymorphism (99.11%). As expected, Lao Cai displayed the lowest value of Shannon's within population diversity index (H_o = 0.198), whereas highest population diversity was Thua Thien Hue (H_o = 0.351) (Table 3). The average diversity within populations (H_{pop}) was 0.288, which accounted for 69.90% of the total diversity found in the species (H_{sp} = 0.412). The proportion of diversity among populations (G_{st}) value of 0.301 indicated a high degree of genetic differentiation among populations.

In previous reports of genus *Paris*, the G_{st} value ranged from 0.195 to 0.515 (He et al., 2007a,b; Zhang et al., 2004). The G_{st} value of our study was similar to 8 populations in Yannan and Sichuan provinces (China) using ISSR marker (Gst = 0.363) (He et al., 2007b); lower than that of 4 *P. polyphylla* populations (0.515) using RAPD technique (Zhang et al., 2004) but higher than G_{st} value of 7 other *P. polyphylla* var. *yunnanensis* populations (0.195) (He et al., 2007a).

Table 3. The percentage of polymorphic loci and Shannon index (H_o)

	A Luoi	Tam Dao	Lao Cai	Jilin
PBB	99.11	99.01	87.30	97.47
H_o	0.351	0.315	0.198	0.291

Table 4. Shannon's estimates of the genetic diversity of *P. polyphylla* populations

	H_{pop}	H_{sp}	H_{pop}/H_{sp}	G_{st}
Mean	0.288	0.412	0.699	0.301

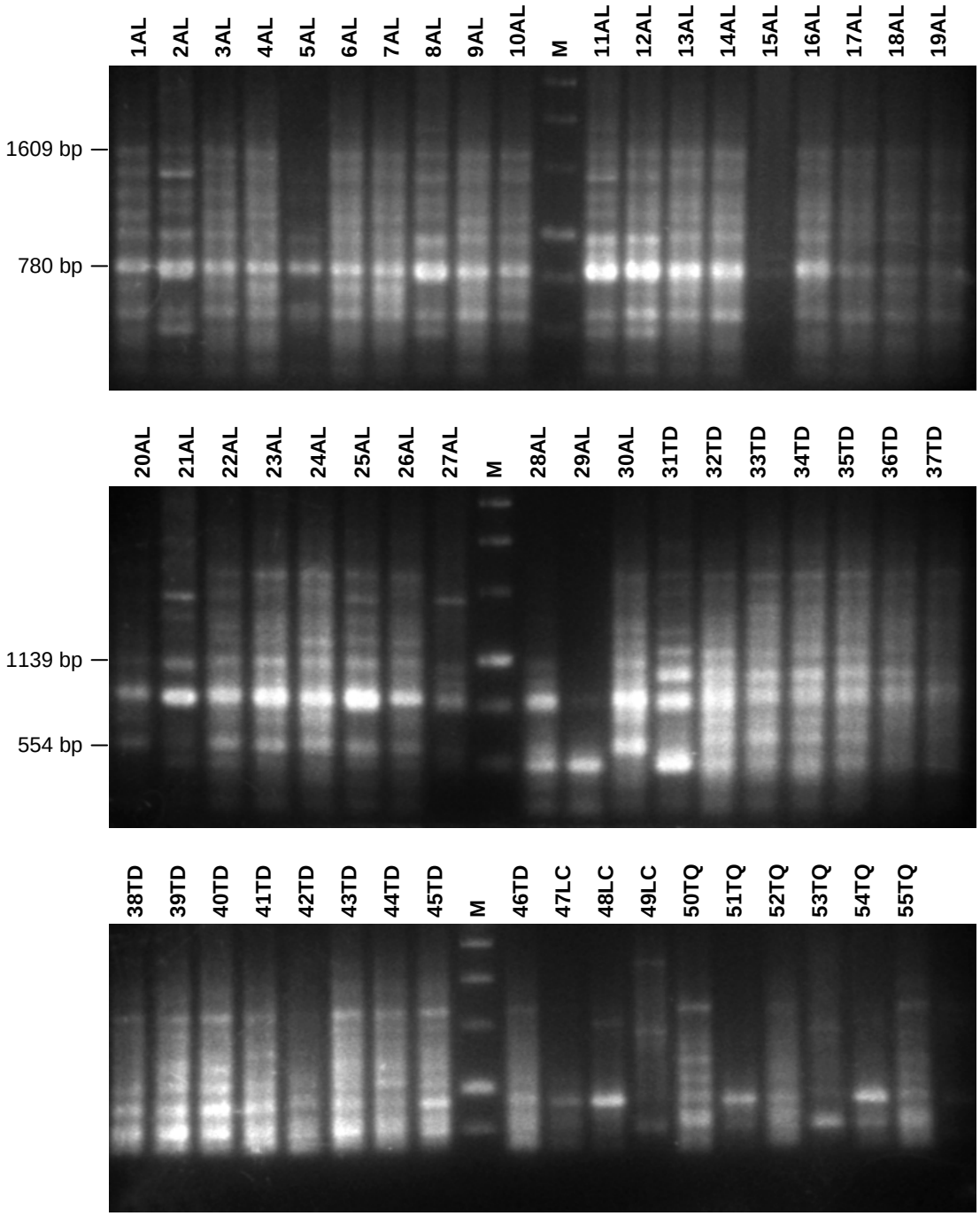

Fig. 2. PCR amplicon of A13 primer from 55 samples. Lane M, DNA1 kb ladder; lane 1-55 represent PCR amplicon of A13 primer of each sample. PCR amplicon was separated on 1.4% agarose gel at 40V for 6h. Gel was stained with 0.05% ethidium bromide and visualized under UV light. The image was taken and analyzed using Gel documentation system (Bio-rad)

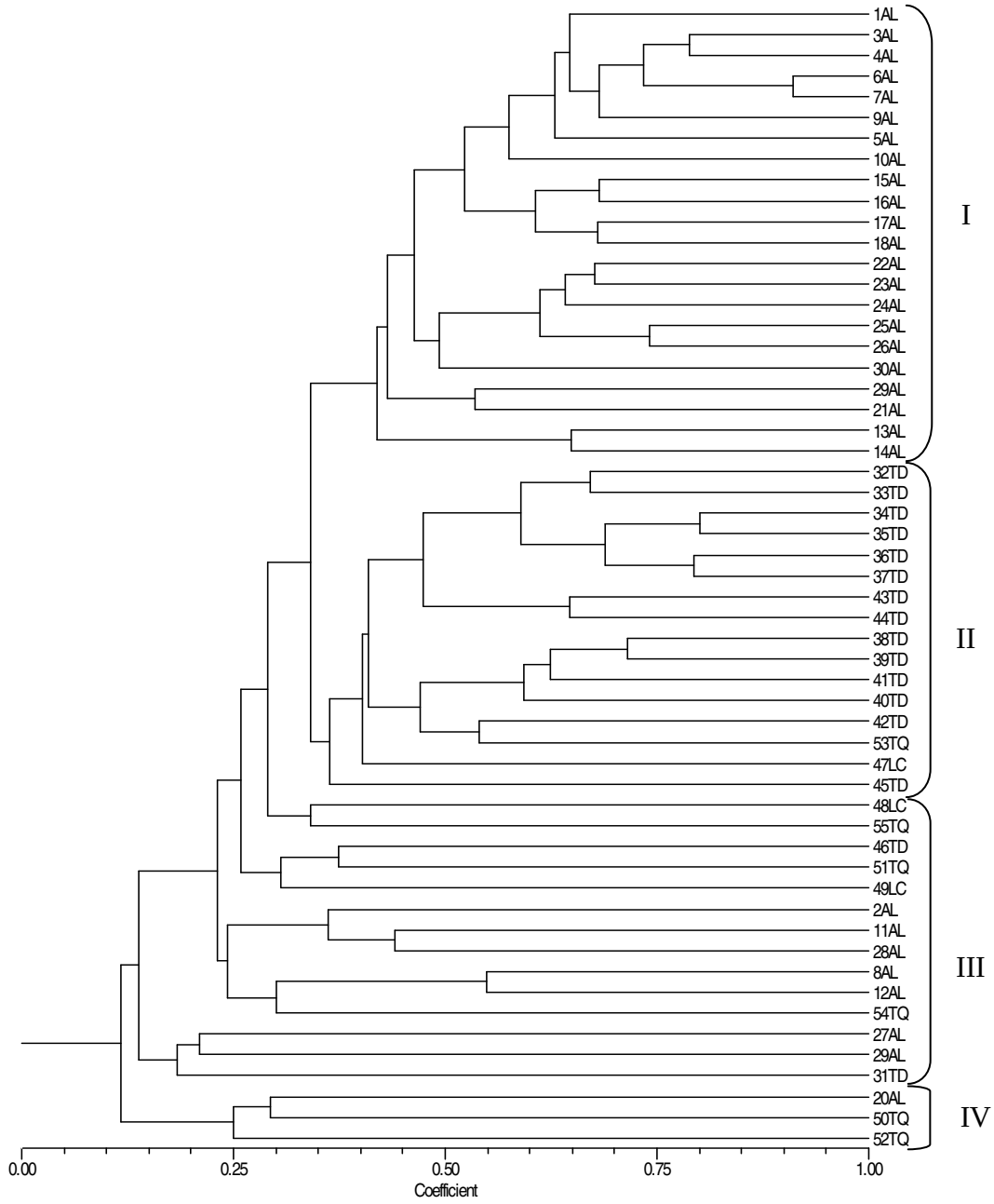

Fig. 3. Phenogram of 55 *Paris polyphylla* trees derived using the UPGMA grouping method based on Jaccard coefficients obtained from RAPD analyses

CONCLUSION

In our study, eight selected random primers (A1, A13, B10, N2, A7, F4, B4, and N3) are suitable for genetic diversity analysis of *P. polyphylla* populations. This is first report on genetic diversity of *P. polyphylla* in Vietnam. RAPD analysis of 55 samples shows that there are 125 DNA fragments were amplified. The genetic diversity is high within studied populations. Thua

Thien Hue has the highest diversity level (H_o = 0.351) while Lao Cai is lowest diversity level (H_o = 0.198). Moreover, the degree of genetic differentiation among populations is also high (G_{st} = 0.301). A phenetic tree classifies population into four main groups which group I contains 22 samples from Thua Thien Hue, group II includes almost samples from Vinh Phuc, group III consisted of 14 samples from Thua Thien Hue (7 samples), Vinh Phuc (2 samples), Lao Cai (2 samples), and Jilin (3 samples), and group IV includes 1 sample from Thua Thien Hue, 2 samples from Jilin.

ACKNOWLEDGEMENTS

This study was supported by the grant from Vietnam Ministry of Education and Training (B2014-DHH-122).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Cota-Sánchez, J.H., Remarchuk, K., Ubayasena, K. 2006. Ready-to-use DNA extracted with a CTAB method adapted for herbarium specimens and mucilaginous plant tissue. Plant Mol. Biol. Rep. **24**(2):161-167.

Deb, C., Jamir, L.S., Jamir, N. 2015. Studies on vegetative and reproductive ecology of *Paris polyphylla* Smith: A vulnerable medicinal plant. American Journal of Plant Sciences. **6**: 2561-2568.

He, J., Wang, H., Li, D.Z., Chen, S.F. 2007a. Genetic diversity of *Paris polyphylla* var. *yunnanensis*, a traditional Chinese medicinal herb, detected by ISSR markers. Planta Med. **73**(12):1316-1321.

He, J., Yang, B.Y., Chen, S.F., Gao, L.M., Wang, H. 2007b. Assessment of genetic diversity of *Paris polyphylla* (Trilliaceae) by ISSR markers. Acta Metallurgica Sinica. **29**(4):388-392.

Jaccard, A. 1908. Nouvelles recherches sur la distribution florale. Bulletin de la Société Vaudoise de Sciences Naturelles. **44**:223-270.

Liu, Z.J., Cordes, J.F. 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture. **238**:1-37.

Negi, J.S., Bisht, V.K., Bhandari, A.K., Bhatt, V.P., Singh, P., Singh, N. 2014. *Paris polyphylla*: Chemical and biological prospectives. Anticancer Agents Med. Chem. **14**(6):833-839.

Quang, H.T., Xuan, L.T., Mai, N.T., Trung, N.V., Lien, N.T.T., Phuong, T.T.B., Loc, N.H. 2011. Genetic variability of "Thanh tra" pummelo (*Citrus grandis* Osbeck) in Thua Thien Hue, Vietnam. Ann. Biol. Res. **2**(4):306-314.

Shah, S.A., Mazumder, P.B., Dutta-Choudhury, M. 2012. Medicinal properties of *Paris polyphylla* Smith: A review. J. Herb. Med. Toxicol. **6**(1):27-33.

Songyun, L., Soukup, V.G. 2000. PARIS Linnaeus, Sp. Pl. 1: 367. 1753. Flora of China. **2**:88-95.

Tang, R. 2003. Tissue culture and molecular systematics studies os *Paris* Master thesis. Sichuan University, China.

Verma, S., Karihaloo, J.L., Tiwari, S.K., Magotra, R., Koul, A.K. 2007. Genetic diversity in *Eremostachys superba* Royle ex Benth. (Lamiaceae), an endangered Himalayan species, as assessed by RAPD. Genet. Resour. Crop. Evol. **54**:221-229.

Wang, Y.F., Li, G., Tang, L., Li, R.Y., Yang, C.Y., Peng, J.M. 2013. Breeding system of *Paris polyphylla* var. *yunnanensis*. Zhongguo Zhong Yao Za Zhi. **38**(17):2773-2778.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**:6531-6535.

Zhang, J., Yu, H., Zhang, S., Ding, C. 2004. RAPD variation within and among four populations of *Paris polyphylla*. Chinese Biodiversity. **12**(5):517-522.

Zheng, J.Y., Wang, H., Chen, X.X., Wang, P., Gao, P., Li, X.N., Zhu, G.P. 2012. Microsatellite markers for assessing genetic diversity of the medicinal plant *Paris polyphylla* var. *chinensis* (Trilliaceae). Genet. Mol. Res. **11**(3):1975-1980.