

GENETIC DIVERSITY OF *Panax vietnamensis* var. *langbianensis* POPULATIONS IN LAM VIEN PLATEAU – VIETNAM DETECTED BY INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

Le Ngoc Trieu¹, Nong Van Duy², Tran Van Tien^{1,✉}

¹Dalat University, 01 Phu Dong Thien Vuong, Da Lat City, Lam Dong Province, Vietnam

²Tay Nguyen Institute of Scientific Research, Vietnam Academy of Science and Technology, 116 Xo Viet Nghe Tinh, Dalat City, Vietnam

✉To whom correspondence should be addressed. E-mail: tientv@dlu.edu.vn

Received: 21.12.2019

Accepted: 27.12.2019

SUMMARY

Panax vietnamensis var. *langbianensis* is a new variety from Lam Vien plateau of Vietnam. In this study, inter simple sequence repeat (ISSR) markers were employed to investigate the genetic diversity and variability of 115 individuals belonging to two naturally distributed populations of this variety, which classified by habitat. Genetic diversity at the taxon level was high ($H_{eT} = 0.284$ and $PPB_T = 97.2\%$). The result showed lightly higher genetic diversity in population in Lac Duong region ($H_{eLD} = 0.228$ and $PPB_{LD} = 81.5\%$) as compared to those located in Dam Rong region ($H_{eDR} = 0.213$ and $PPB_{DR} = 79.4\%$). The interpopulation gene differentiation was high ($G_{ST\ Total} = 0.221$) with the genetic distance among populations was $D_{LD-DR} = 0.191$. Gene flow within populations was $Nm = 0.8793$. In Lac Duong population, the genetic diversity of older group ($H_{eLD\ O} = 0.233$; $PPB_{LD\ O} = 77.1\%$) was higher than of younger group ($H_{eLD\ Y} = 0.214$; $PPB_{LD\ Y} = 72.4\%$) and the intergroup gene differentiation was $G_{STD\ L} = 0.0205$ with the genetic distance between these two group was $D_{LD\ O-Y} = 0.0061$ showed the risk of reduction in genetic diversity. In Dam Rong population, the genetic diversity of older group ($H_{eDR\ O} = 0.204$; $PPB_{DR\ O} = 75.2\%$) was equal to younger group ($H_{eDR\ Y} = 0.209$; $PPB_{DR\ Y} = 72.7\%$) and the intergroup gene differentiation was $G_{STD\ R} = 0.0304$ with the genetic distance between them was $D_{DR\ O-Y} = 0.01393$ showed the stability in genetic diversity. Data for genetic diversity and variation from this study can be used to further investigate and protect this variety for conservation and development purposes and for sustainable exploiting and use of these valuable natural resources.

Keywords: Genetic diversity, ISSR, *Panax vietnamensis* var. *langbianensis*, Vietnam

INTRODUCTION

The name “Ginseng” is used to refer any of the species of slow-growing perennial plants with fleshy roots, belonging to the genus *Panax* of the family Araliaceae. They are the most famous and valuable medicinal plants in the world. In 2016, a new variety of *Panax vietnamensis* var. *vietnamensis* was described in Lam Vien plateau of Vietnam with two populations and was registered as *Panax vietnamensis* var. *langbianensis* based on the distinguishable DNA barcodes and morphological characteristics (Duy *et al.*, 2016).

However, currently the variety in Lam Vien plateau is considered to be endangered and its distribution is limited. There are only two indigenous

populations that have been recorded within Lam Vien plateau. Field investigation research has identified approximately 200 - 250 individual plants remaining within the two studied regions. Both habitats of the rediscovered variety were narrow and one of them has been influenced by human interventional activities. However, the genetic diversity of this variety has not been investigated in Vietnam. This is true even given that it is well known the reduction in genetic diversity is an actual risk to *P. vietnamensis* var. *langbianensis*. Genetic variation is currently understood as a critical variable to the long-term survival of a population or species (Beardmore, 1983; Anatonovis, 1984). Understanding the genetic variation and diversity within and among populations of rare and

endangered taxa is essential when developing management strategies for both *in situ* and *ex situ* conservation activities (Hogbin, Peakall, 1999). Thus, estimating inter- and intra-population genetic diversity is critical to the protection and long-term availability of *P. vietnamensis* var. *langbianensis* in both terms of ecological biodiversity and medically-related uses. Current research methods support the use of molecular markers as a suitable and accurate tool for population genetic diversity detection.

The advantages of inter simple sequence repeat (ISSR) lies within in its low-cost, convenience of use, and high-level of reliability in reproducing results (Zietkiewicz *et al.*, 1994; Nagoaka, Ogihara, 1997; Lu *et al.*, 2009; Roy, Chakraborty, 2009). As such, ISSR methods have established widespread and accepted use for applications in population genetic studies of both wild and cultivated plants (Roy, Chakrabort, 2009).

In the current study, the ISSR marker system was employed to induce DNA fingerprints for the estimation of genetic diversity and the identification of genetic differentiation in wild found *P. vietnamensis* var. *langbianensis* populations which distributed in their natural habitats. The objectives of this study were as follows: (1) to estimate genetic diversity identified and analyzed in sample sets according to total investigated taxon, population and

age group levels; (2) to analyze genetic relationships and differentiation among two populations and two age groups belonging to each populations and (3) to contribute and catalogue the data of this study for use in the conservation and sustainable utilization of the researched medicinal plants within Vietnam.

MATERIALS AND METHODS

Plant materials

From March 2012 to May 2014, a total of 115 individuals presenting natural populations of the *Panax* taxon from Lac Duong District and Dam Rong District (Lam Dong Province, Vietnam), which corresponded to 2 naturally distributive populations of the new variety, were sampled across their original habitats (Table 1). Sixty four individuals from the Lac Duong population and 51 individuals from the Dam Rong population were randomly collected for DNA extraction. Chosen individuals for sampling were separated from each other at least 50 m.

Fresh leaves were collected, kept fresh if DNA extraction within 36 hours or dried in sealed bags with silica gel if DNA extraction executed over 36 hours later and brought to the laboratory where each sample was extracted and preserved at a constant -20°C for DNA analysis.

Table 1. Geographic localities of *P. vietnamensis* var. *langbianensis* populations in this study.

Sample quantity	Age group	Population	Geographic localities	Longitude/Latitude range
32 samples (Lxo)	>8	LD	Lac Duong District	108°25'38" E
32 samples (Lxy)	≤ 6			12°05'01"N, 1900 m asl.
26 samples (Dxo)	>8	DR	Dam Rong District	108°11'35" E
25 samples (Dxy)	≤ 6			12°05'35"N, 1850 m asl.

L: belong to LD population; D: belong to LD population; x Number for sample identification; o: old group and y: young group.

DNA extraction purification and quantification

Total genomic DNA was extracted and purified using CTAB protocol I (Weising *et al.*, 2007) with a modification of adding 10% SDS to the extraction buffer which was then dissolved in water for the subsequent use. The DNA concentration (C) was calculated as follows: $C (\mu\text{g} / \mu\text{L}) = \text{OD}_{260} \times 50$. The $\text{OD}_{260}/\text{OD}_{280}$ ratio was also calculated to determine DNA purity (Weising *et al.*, 2007).

ISSR-PCR amplification

ISSR primers used in this study were synthesized by Bioneer Corporation (Republic of Korea), according to the primer set published by the University of British Columbia and Zagazig University (Admed, 2005). 60 ISSR primers were initially screened, and 20 of them, which yielded bright, clear bands and at least possessed one polymorphic band in both populations, were used for

the analysis of all 115 samples (Table 2). Six individuals, including 3 from LD population and 3 individuals from DR population were used for the primer screening. PCR amplification was repeated for those working primers to check the stability and reproducibility of ISSR DNA fingerprints.

PCR was performed in 20 μ L reactions containing 2 mM $MgCl_2$, 0.25 mM each of dNTPs, 1U Taq DNA polymerase (ThermoScientific), 0.2 μ M primer and approximately 30 ng DNA templates. The amplifications were performed in a Peqstar 96X Universal Gradient thermocycler (PEQLAB Biotechnologie GmbH, Germany) with the following program: initial denaturation at 94°C for 5 min; 10 cycles of 94°C for 45 s, annealing temperature +5 (Ta +5)°C (Table 2) for 45 s, decreased 0.5°C/cycle, 72°C for 1 min 30 s; 36 cycles of 94°C for 45 s, annealing temperature for 45 s, 72°C for 1 min 30 s; Final extension at 72°C for 15 min; the amplification products were separated in 2% agarose gel, using TBE buffer at 60 V for 3 hours, stained with ethidium bromide (0.5 μ g/ml), and photographed under 254/312 nm wavelength lights using Micro Doc Gel Documentation System (Clever Scientific, USA).

Data analysis

Since ISSR markers are dominantly inherited, each band was assumed to represent the phenotype at a single biallelic locus (Williams *et al.*, 1990). ISSR bands were scored as presence (1) or absence (0) characters, to construct the binary data matrix. POPGENE software was used to calculate genetic diversity parameters: the percentage of polymorphic bands (*PPB*), the average expected heterozygosity (H_e), the gene differentiation (G_{ST}), the genetic distance among investigated sample sets (D) and gene flow (Nm) (Yeh *et al.*, 1997). The Nei's genetic distance between pair of sample sets was calculated as: $D_{XY} = -\ln(I_{XY})$ is based on the concept of genetic identity (I_{XY}): $I_{XY} = J_{y0} / \sqrt{(J_X \times I_Y)}$, where: J_X = average homozygosity in the first sample set, J_Y = average homozygosity in the second sample set, J is calculated by formula $J = 1 - H_{eT}$, J_{XY} = average inter-sample set homozygosity = j_{XY}/L with j_{XY} is homozygosity among two sample sets and L is the number of investigated loci. $J_{XY} = \sum_{XYjk} (p_{Xjk} \times p_{Yjk})$, where p_{Xjk} and p_{Yjk} are frequencies of K^{th} allele at locus j^{th} in the first and the second sample sets, respectively (Vicente *et al.*, 2003).

Similarity coefficients between pairs of samples and UPGMA dendrogram for genetic relationship

among all studied samples were calculated and established by using NTSYSpc 2.1 (Numerical Taxonomy and Multivariate Analysis System) software (Rohlf, 2004).

RESULTS

Genetic diversity

The twenty selected primers yielded 428 reproducible bands for total investigated samples. For species level, the number of bands per primer varied between 12 (HB15) and 32 (17899B), with an average of 21.4. For whole taxon, the *PPB* per primer varied from 87.0 % (UBC 826) to 100 % (12 from 20 used primers) with an average of $PPB_{Total} = 97.2\%$. In LD population, *PPB* per primer varied between 41.7% (HB15) and 100% (HB9), with an average of $PPB_{LD} = 81.5\%$, the older group possessed average value of $PPB_{LD O} = 77.1\%$ and the counterpart in younger group was $PPB_{LD Y} = 72.4\%$. In DR population, *PPB* per primer varied between 56.5% (UBC826) and 100% (HB12), with an average of $PPB_{DR} = 79.4\%$, the older group possessed average value of $PPB_{DR O} = 75.2\%$ and the counterpart in younger group was $PPB_{DR Y} = 72.7\%$. There was the reduction of *PPB* from whole taxon to population to age group levels (Table 2).

Among the two investigated populations, LD population possessed the higher level of genetic diversity ($H_{eLD} = 0.228$ and $PPB_{LD} = 81.5\%$), while DR population harbored lower level ($H_{eDR} = 0.213$ and $PPB_{DR} = 79.4\%$).

In LD population, the genetic diversity of older group ($H_{eLD O} = 0.233$; $PPB_{LD O} = 77.1\%$) was higher than of younger group ($H_{eLD Y} = 0.214$; $PPB_{LD Y} = 72.4\%$). In DR population, the genetic diversity of older group ($H_{eDR O} = 0.204$; $PPB_{DR O} = 75.2\%$) was equal to younger group ($H_{eDR Y} = 0.209$; $PPB_{DR Y} = 72.7\%$).

Genetic relationship

In LD population, the gene similarity coefficients ranged from 65.65% to 89.49% with a mean of 77.80% (Fig. 3.), these coefficients ranged from 68.46% to 86.92% with a mean of 78.49% for older group and from 69.16% to 89.49% with a mean of 79.10% for younger group. The intergroup gene differentiation among two age groups was $G_{STDL} = 0.0205$ with the genetic distance between them was $D_{LD O-Y} = 0.0061$.

In DR population, the gene similarity coefficients ranged from 60.51% to 87.62% with a mean of 77.65% (Fig. 4.), these coefficients ranged from 63.08% to 86.21% with a mean of 78.49 % for older group and from 67.52% to 87.62% with a mean of 77.74 % for younger group. The intergroup gene differentiation was $G_{STDR} = 0.0304$ with the genetic distance between these was $D_{DR.O.Y} = 0.01393$

The gene similarity coefficients among the

individuals of taxon were varied, ranging from 0.6565 to 0.8949 with a mean of 0.778 (Fig.5). Among the two investigated populations, the interpopulation gene differentiation was relatively high ($G_{ST\ Total} = 0.221$) with the genetic distance among them was $D_{LD-DR} = 0.191$ this means 19.1 % differentiation among populations existed. The gene flow within populations was $Nm = 0.8793$ showed that the migration among the two populations was medium.

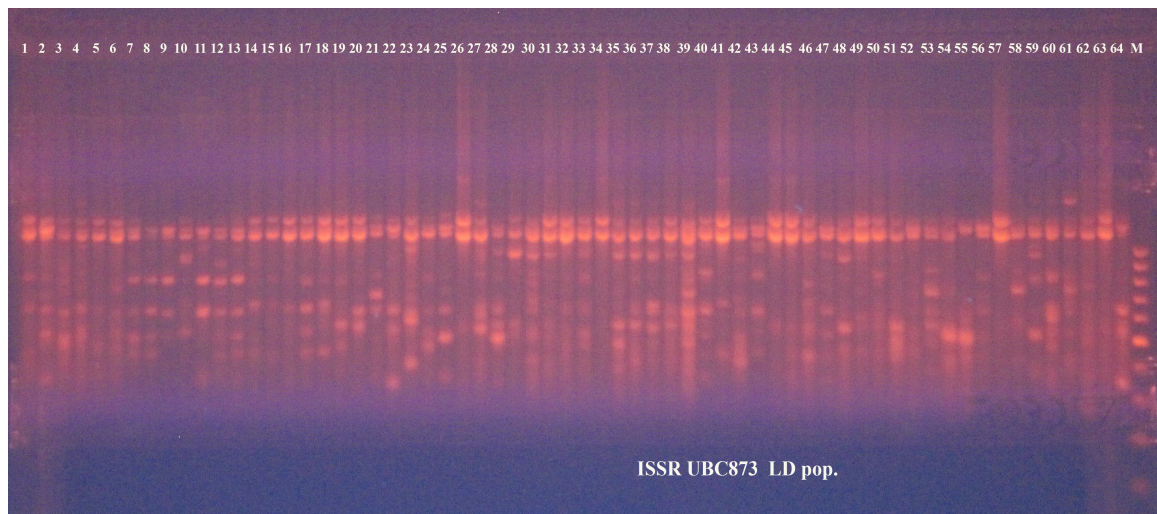


Figure 1. Gel electrophoresis image for DNA fingerprint of LD population using UBC873 primers.

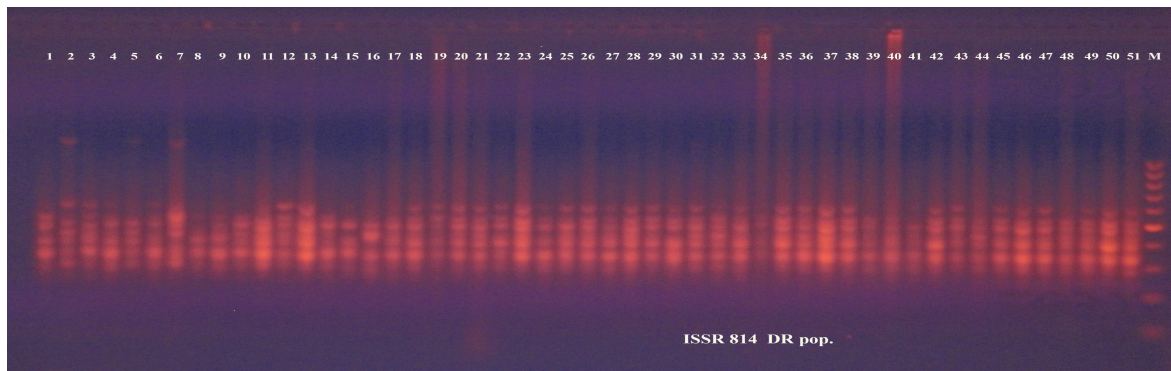


Figure 2. Gel electrophoresis image for DNA fingerprint of DR population using 814 primers.

DISCUSSION

The ISSR markers in this study yielded reproducible polymorphic bands in 115 individuals which corresponded to investigate samples belonging to two populations of *P. vietnamensis* var.

langbianensis in the LamVien plateau - Vietnam, which was classified by habitat. This method provides a highly effective and reliable molecular-level tool for analyzing genetic diversity and genetic relationships within the variety.

Table 2. ISSR primers used in this study and their amplification characteristic.

Primer code	Sequence (5' → 3')	T _a (°C)	Total recorded bands for whole taxon	PPB (%)						
				Whole taxon	LD population			DR population		
					Pop.	Older group	Younger group	Pop.	Older group	Younger group
808	(AG) ₈ C	52	14	92.9	92.9	92.9	57.1	85.7	85.7	78.6
814	(CT) ₈ TG	51,5	15	100.0	86.7	86.7	66.7	80.0	66.7	73.3
844A	(CT) ₈ AC	52	19	100.0	89.5	88.5	78.9	94.7	94.7	84.2
17898A	(CA) ₆ AC	54,5	19	100.0	84.2	84.2	68.4	63.2	63.2	47.4
17898B	(CA) ₆ GT	54,5	27	100.0	77.8	77.8	74.1	96.3	81.5	92.6
17899A	(CA) ₆ AG	54	19	100.0	94.7	89.5	84.2	94.7	84.2	89.5
17899B	(CA) ₆ GG	54	32	100.0	87.5	84.4	87.5	96.9	93.8	90.6
HB8	(GA) ₆ GG	52	18	94.4	72.2	66.7	61.1	72.2	66.7	55.6
HB9	(GT) ₆ GG	52	19	100.0	100.0	84.2	100.0	84.2	84.2	73.7
HB10	(GA) ₆ CC	52	28	89.3	75.0	71.4	67.9	82.1	78.6	82.1
HB11	(GT) ₆ CC	52	17	94.1	70.6	70.6	58.8	76.5	76.5	76.5
HB12	(CAC) ₃ GC	52	23	100.0	47.8	39.1	47.8	100.0	100.0	91.3
HB15	(GTG) ₃ GC	52	12	100.0	41.7	41.7	41.7	91.7	91.7	75.0
UBC 807	(AG) ₈ T	54	23	87.0	69.6	52.2	65.2	60.9	56.5	52.2
UBC 826	(AC) ₈ C	54	23	95.7	87.0	87.0	69.6	56.5	56.5	43.5
UBC 842C	(GA) ₈ CG	51,5	26	96.2	92.3	92.3	61.5	65.4	65.4	57.7
UBC 842T	(GA) ₈ TG	51,5	26	100.0	76.9	69.2	73.1	61.5	61.5	65.4
UBC 856C	(AC) ₈ CA	52	25	100.0	92.0	84.0	84.0	92.0	92.0	92.0
UBC 856T	(AC) ₈ TA	52	19	94.7	94.7	89.5	89.5	63.2	52.6	63.2
UBC 873	(GACA) ₄	52	24	100.0	87.5	87.5	87.5	62.5	54.2	58.3
Total			428							
Average			21.4	97.2	81.5	77.1	72.4	79.4	75.2	72.7

This study reports the genetic diversity at taxon and population levels and also at individual groups which was classified by age. The extent of genetic variation within two natural populations and within two different age groups of each population, the gene differentiation and the genetic distance among them were showed.

Surveyed on the wide range of species which share the same life history traits with currently investigated variety (dicotyledon, long-lived perennial life form, endemic, outcrossing breeding system and ingested seed dispersal mechanism), Hamrick and Godt (1996) reported that the genetic diversity based on allozyme marker were $PPB = 42-46\%$; $H_e = 0.10-0.14$; $G_{ST} = 0.14-0.24$, and Nymbom (2000) reported that the genetic diversity were $H_e = 0.19-0.24$; $G_{ST} = 0.17-0.23$ based on RAPD markers. Thus, results from this study showed

that *P. vietnamensis* var. *langbianensis* in the Lam Vien plateau - Vietnam possessed a high level of genetic diversity and interpopulation gene differentiation.

Achieved results showed the higher population genetic diversity related to *PPB* and heterozygosity than other *Panax* populations which were reported in previous studies based on RAPD (Artyukova *et al.*, 2004), on Allozyme (Jennifer *et al.*, 2004), on AFLP (Zhou *et al.*, 2005; Zhuravlev *et al.*, 2010). However, the similarity coefficients among the pair of samples in the current study were higher (Bai *et al.*, 1997), which showed the limitations of ISSR markers in individual discrimination. The results from this study showed that *PPB* depends on size of sample sets and because of the *PPBs* of different sample sets at the same investigated level were also not equal; there were the genetic differentiation among them.

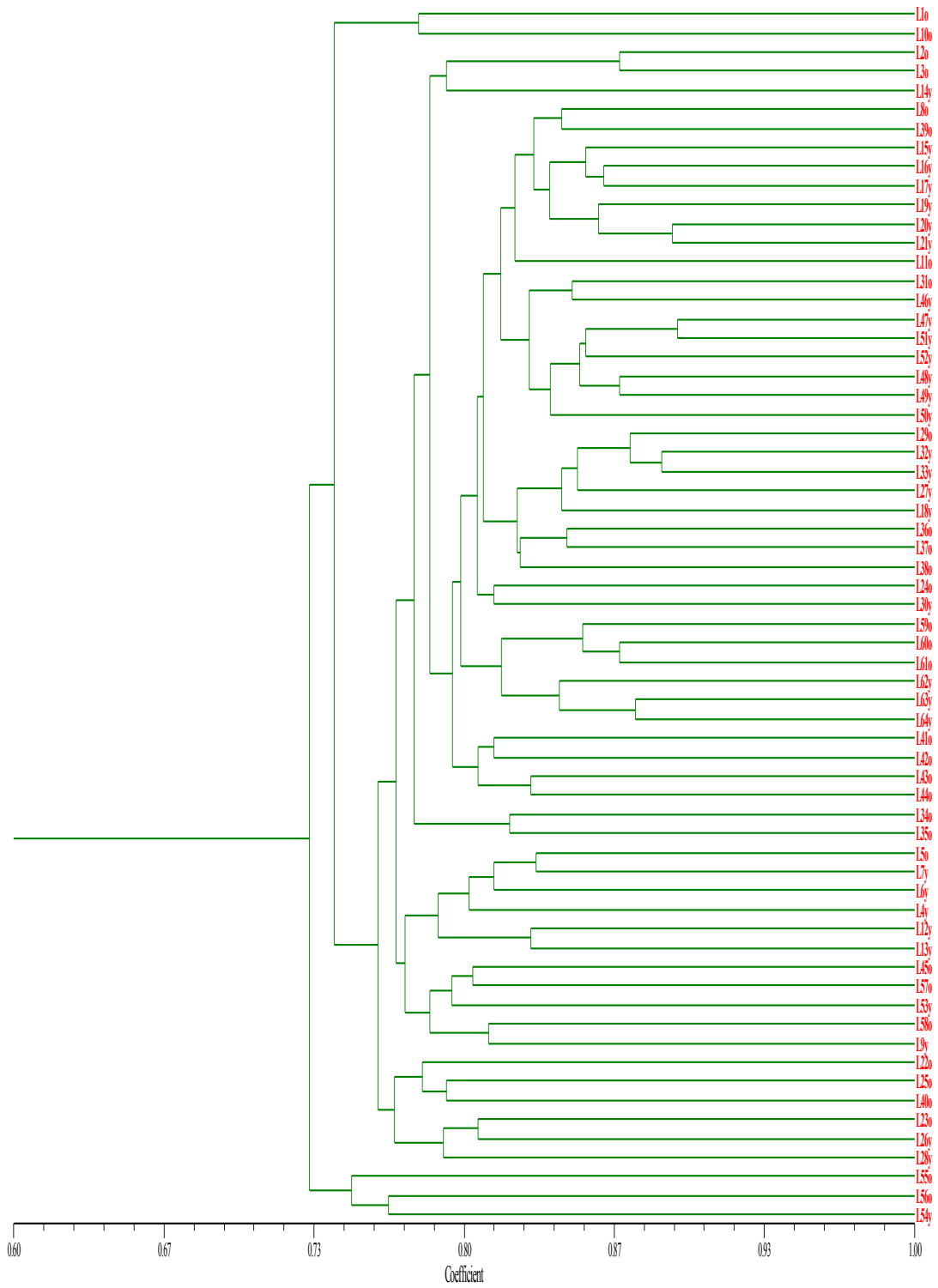


Figure 3. UPGMA dendrogram for genetic relationship among individuals in LD population.

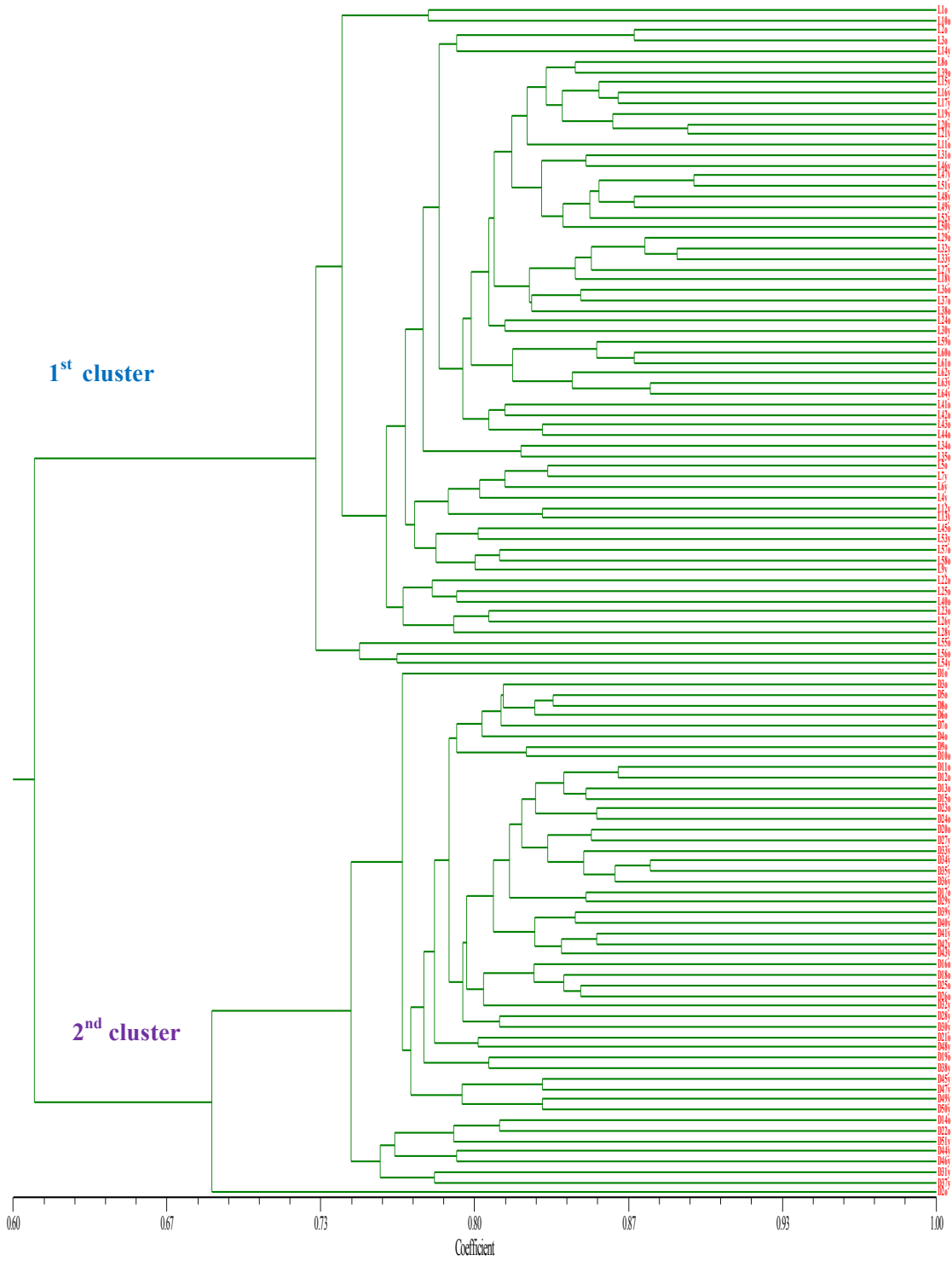


Figure 5. UPGMA dendrogram for genetic relationship among individuals in whole taxon.

Also using ISSR technique to induce DNA fingerprints in *P. ginseng* cultivated in North-East China, Li *et al.* (2011) reported that the genetic diversity was high at the species level ($H_e = 0.2886$; $PPB = 98.96\%$) but lower in type of forest ginseng ($H_e = 0.1702$; $PPB = 57.29\%$). These parameters in separated populations of forest ginseng were ranged as $H_e = 0.1065$ – 0.1520 ; $PPB = 34.38$ – 40.62%). Genetic differentiation was also detected among populations forest ginseng was $G_{ST} = 0.2328$ with $Nm = 1.648$. Comparing to these results, the current study revealed that genetic diversity of the *P. vietnamensis* var. *langbianensis* in Lam Vien plateau of Vietnam was relatively lower compared to *P. ginseng* in taxon level but higher in population level. The genetic differentiation among populations of currently investigated variety was lower than among populations belonging to forest type of *P. ginseng* in North-east China in the study executed by Li *et al.* (2011). The gene flows among the *P. vietnamensis* var. *langbianensis* populations were also lower than those among the forest ginseng populations investigated by Li *et al.* (2011). Due to the long lifespan and overlapping generations of the populations within the prior study, considerable genetic variability has been accumulated and conserved under various selection traits during the evolutionary process (Li *et al.*, 2011) and this may happen to *P. vietnamensis* var. *langbianensis*. The interpopulation gene differentiation was high ($G_{ST-Total} = 0.221$) with the genetic distance among populations was $D_{LD-DR} = 0.191$ and gene flow within populations was $Nm = 0.8793$ may due to the substitution of natural primary jungle by cultivated *Pinus kesiya*, which doesn't re-establish the suitable condition for investigated variety; and establishment new road and the extension of villages and agricultural areas between two habitats of this variety.

Using 20 selected ISSR primers allowed separation the individuals from two investigated populations to be two distinguishable clusters in the UPGMA dendrogram of whole taxon (Fig. 5.). The first and the second clusters corresponded and confirmatively identical to UPGMA dendrogram of LD population and DR population, respectively.

For genetic structure, there were the differences between the two investigated populations. In LD population, the older group possessed the higher genetic diversity than younger group and the genetic differentiation among them was low as $G_{STDL} =$

0.0205 with the genetic distance was $D_{LD-O-Y} = 0.0061$. While in DR population, older and younger groups harbored the same level of genetic diversity but the genetic differentiation among them was high as $G_{STDR} = 0.0304$ with the genetic distance was $D_{DR-O-Y} = 0.01393$. These suggested that even possessed the higher genetic diversity, LD population has coped with the risk of reduction in genetic diversity, and DR population showed the stability in genetic diversity through generations. Studied on *P. quinquefolius* occurred from Georgia to West Virginia, Jennifer and Hamrick (2004) reported that the harvested populations possessed the higher genetic differentiation but lower expected heterozygosity compared to the protected populations due to harvest pressure. In case of current study, there was no harvest pressure to both investigated populations but the same situation with *P. quinquefolius* ranged from Georgia to West Virginia occurred. This can be explained by the actual distribution of the two populations: while DR population's habitat located in natural primary jungle which far from residential areas and almost lacking of human activities, LD population's habitat located near to residential and agricultural areas and was interfered by human unfavorable activities such as coal making, and using the insecticides, and animal hunting. These activities lead to the reduction of pollinators (insects) and dispersive animals (rodents and birds), changes of habitat, especially surface runoff.

CONCLUSION

The understanding on population genetic variability is essential to effective conservation and sustainable management. The relatively high genetic diversity at population and taxon levels are the advantages for conservation and development of the new variety ginseng species in Lam Vien plateau - Vietnam. However, there are some disadvantages for this variety including the number of discovered individuals was small, narrow habitats, endemic, one of two discovered populations showed the reduction in genetic diversity due to the human affects in the habitat. Otherwise, because the re-discovered variety is ginseng, it may cope with the harvested pressure, thus lacking of an actionable conservation strategy may lead to the increased reduction of genetic diversity and reserve of this taxon. Thus, it is critical importance to further investigate and protect this variety for conservation purposes and for sustainable

harvesting and use of these valuable natural resources.

Acknowledgements: *The authors would like to express their sincere thanks to the National Foundation for Science & Technology Development (NAFOSTED) under grant No. 106.11-2012.79 for supporting the research project. We would like to thank Mr. Pham Van Nhi for help with the field investigation and materials collection.*

REFERENCES

- Admed MA (2005) *PCR techniques*. Department of Genetics. Zagazig University, Zagazig, Egypt.
- Anatonovis J (1984) Genetic variation within population. In: Dirzor, R., Sarukan, J. (eds) *Perspectives on plant population biology*. Sinauer, Sunderland pp. 229-241.
- Artyukova EV, Kozyrenko MM, Koren OG, Muzarok TI, Reunova GD and Zhuravlev YN (2004) RAPD and allozyme analysis of genetic diversity in *Panax ginseng* C.A. Meyer and *P. quinquefolius* L. *Russ J Gen* 40(2): 178-185.
- Bai D, Brandle J, Reeleder R (1997) Genetic diversity in North American ginseng (*Panax quinquefolius* L.) grown in Ontario detected by RAPD analysis. *Genome* 40: 111-115.
- Beardmore JA (1983) Extinction, survival and genetic variation. In: Schoenwald-Cox, C.M., Chamber, S.M., Macbryde, B., Thomas L. (eds). *Genetics and Conservation*. Benjamin-Cummings Menlo Park pp. 125-151.
- Duy NV, Trieu LN, Chinh ND, Tran VT (2016) A new variety of *Panax* (Araliaceae) from Lam Vien Plateau, Vietnam and its molecular evidence. *Phytotaxa* 277(1): 047-058.
- Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. *Philos Trans R Soc Lond Ser B Biol Sci* 351:1291-1298.
- Hogbin PM, Peakall R (1999) Evaluation of the contribution of the genetic research to the management of the endangered plant *Zieria prostrata*. *Conservation Biology* 13: 14-522.
- Jennifer MC, Hamrick JL (2004) Genetic diversity in harvested and protected populations of wild American ginseng, *Panax quinquefolius* L. (Araliaceae). *Am J Bot* 91(4): 540-548.
- Li S, Li J, Yang XL, Cheng Z, Zhang WJ (2011) Genetic diversity and differentiation of cultivated ginseng (*Panax ginseng* C. A. Meyer) populations in North-east China revealed by inter-simple sequence repeat (ISSR) markers. *Genet Resour Crop Evol* 58: 815-824.
- Lu X, Liu L, Gong Y, Zhao L, Song X, Zhu X (2009) Cultivar identification and genetic diversity analysis of broccoli and its related species with RAPD and ISSR markers. *Sci Hort* 122: 645-648.
- Nagoaka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphism in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor Appl Genet* 93: 133-139.
- Nybom H, Bartish IV (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspect Plant Ecol Evol Syst* 3: 93-114.
- Rohlf FJ (2004) NTSYSpc - *Numerical Taxonomy and Multivariate Analysis System* Version 2.1 - User Guide. Applied Biostatistics Inc.
- Roy SC, Chakraborty BN (2009) Genetic diversity and relationships among tea (*Camellia sinensis*) cultivars as revealed by RAPD and ISSR based fingerprinting. *Ind J Biot* 8: 370-376.
- Vicente MCD, Lopez C, Fulton T (2003) *Genetic diversity analysis with molecular marker data: Learning module*. International Plant Genetic Resources Institute (IPGRI) and Cornell University.
- Weising K, Nybom H, Wolff K, Kahl G (2005) *DNA fingerprinting in plants principles, methods, and applications* (second Edition). Cpc Press Taylor & Fancies group.
- Williams JK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl Acids Res* 18: 6531-6535.
- Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belg J Bot* 129-157.
- Zhou SL, Xiong GM, Li ZY, Wen J (2005) Loss of genetic diversity of domesticated *Panax notoginseng* Chen as evidenced by ITS sequence and AFLP polymorphism: A comparative study with *P. stipuleanatus* Tsai et Feng. *J Integr Plant Biol* 47(1): 107-115.
- Zhuravlev YN, Reunova GD, Kats IL, Muzarok TI, Bondar AA (2010) Genetic variability and population structure of endangered *Panax ginseng* in the Russian Primorye. *Chin Med* 5: 21.
- Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.

ĐA DẠNG DI TRUYỀN QUẦN THỂ *Panax vietnamensis* var. *langbianensis* Ở CAO NGUYÊN LÂM VIÊN BẰNG CHỈ THỊ PHÂN TỬ ISSR

Lê Ngọc Triệu¹, Nông Văn Duy², Trần Văn Tiến¹

¹Trường Đại học Đà Lạt, 01 Phù Đổng Thiên Vương, thành phố Đà Lạt, tỉnh Lâm Đồng, Việt Nam

²Viện Nghiên cứu Khoa học Tây Nguyên, Viện Hàn Lâm Khoa học và Công nghệ Việt Nam, 116 Xô Viết Nghệ Tĩnh, thành phố Đà Lạt, tỉnh Lâm Đồng, Việt Nam

TÓM TẮT

Panax vietnamensis var. *langbianensis* là một thứ mới ở cao nguyên Việt Nam. Trong nghiên cứu này, chỉ thị phân tử ISSR được sử dụng để khảo sát đa dạng di truyền và biến dị của 115 cá thể thuộc 2 quần thể tự nhiên. Đa dạng di truyền ở mức độ tổng thể nghiên cứu là cao ($H_{eT} = 0.284$ và $PPB_T = 97.2\%$). Kết quả đa dạng di truyền của quần thể ở Lạc Dương ($H_{eLD} = 0.228$ và $PPB_{LD} = 81.5\%$) cao hơn ở quần thể Đam Rong ($H_{eDR} = 0.213$ và $PPB_{DR} = 79.4\%$). Biệt hoá di truyền giữa các quần thể cao h ($G_{ST\ Total} = 0.221$) với khoảng cách giữa các quần thể $D_{LD-DR} = 0.191$. Dòng chảy gen giữa các quần thể as $Nm = 0.8793$. Ở quần thể tại Lạc Dương, độ đa dạng di truyền của nhóm tuổi lớn ($H_{eLD\ O} = 0.233$; $PPB_{LD\ O} = 77.1\%$) là cao hơn so với nhóm tuổi nhỏ ($H_{eLD\ Y} = 0.214$; $PPB_{LD\ Y} = 72.4\%$) và chỉ số biệt hóa gene giữa hai nhóm tuổi này là $G_{STDL} = 0.0205$ với khoảng cách di truyền giữa chúng là $D_{LD\ O-Y} = 0.0061$, điều đó thể hiện sự suy giảm đa dạng di truyền của quần thể. Ở quần thể tại Đam Rông, độ đa dạng di truyền của nhóm tuổi lớn ($H_{eDR\ O} = 0.204$; $PPB_{DR\ O} = 75.2\%$) là tương đương với nhóm tuổi nhỏ ($H_{eDR\ Y} = 0.209$; $PPB_{DR\ Y} = 72.7\%$) và chỉ số biệt hóa gene giữa hai nhóm tuổi này là $G_{STDR} = 0.0304$ với khoảng cách di truyền giữa chúng là $D_{DR\ O-Y} = 0.01393$, qua đó cho thấy tính ổn định di truyền của quần thể này. Dữ liệu về đa dạng và biến động di truyền từ nghiên cứu có thể được sử dụng để khảo sát sâu hơn và làm cơ sở để bảo vệ thứ mới này với mục đích bảo tồn, phát triển và khai thác, sử dụng một cách bền vững nguồn tài nguyên thiên nhiên có giá trị này.

Từ khóa: Genetic diversity, ISSR, *Panax vietnamensis* var. *langbianensis*, Vietnam