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Full-length article

Biodiversity in cultivated *Panax notoginseng* populations[1]

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Key words

Panax notoginseng; AFLP; genetic diversity; phenotypes; saponins; breeding

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Abstract

Aim: *Panax notoginseng* is a cultivated ginseng species highly valued for its various pharmacological activities mostly associated with triterpenoid saponin glycosides. It would be of great interest to understand biodiversity in this ginseng species after its long history of domestication. **Methods:** We collected 92 random sampled 3-year-old *P notoginseng* plants from 4 counties of Wenshan prefecture in Yunnan province, China and documented their morphological features of plant height, stem color, number of leaves/leaflets and dry weight of tap root. Their genetic diversity was evaluated by fluorescent amplified fragment length polymorphism (fAFLP) analysis. **Results:** Among the samples collected, variable morphological features were observed. For these 4 populations (Zhulijie, Shangliuhe, Bazai and Jinbuhuan) analyzed by fAFLP, percentage of polymorphic bands among the total number of 582 discrete bands were 74.05%, 45.36%, 38.83% and 51.89% respectively. Mean genetic heterozygosity were 0.166, 0.093, 0.094 and 0.125. On the other hand, Nei genetic distances among populations were all <0.03. Further analysis of molecular variance (AMOVA) attributed most (93.5%) genetic diversity to within population variation. Principal coordinates analysis (PCA) did not group any population distinctively. **Conclusion:** This domesticated ginseng species still maintains a fair level of biodiversity and this conclusion is consistent with the local practice of non-selective collection of seeds for next season planting. There was no genetic drift in populations. Biodiversity of *P notoginseng* can be exploited to improve this important herb through breeding. Two possible strategies include inbreeding for pure lines and hybrid breeding with genetic divergent parents for hybrid vigor.

Introduction

Panax notoginseng (Burk) F H Chen belongs to Araliaceae. Its root is one of the most highly valued traditional Chinese medical herbs for its hemostatic and restorative properties. In the last 20 years, a great deal of chemical and pharmacological studies on *P notoginseng* has been carried out. Compounds isolated from notoginseng include saponins, flavonoids, non-protein amino acids, polysaccharide, fatty acids, aliphatic alkenes and peptides. Dammarene-type triterpenoid saponin glycosides are considered to be the primary pharmacologically effective components that exert various effects on the blood, cardiocerebral vascular system, central nervous system and endocrine system[1–6].

According to historical records, *P notoginseng* has been cultivated for more than 400 years in Wenshan prefecture (Yunnan province, China), the most important notoginseng producing area in China[7]. This ginseng species is unique in the way that it can only be found in farms, not in the wild. There is the question on extent of biodiversity in such cultured ginseng populations. Our previous study on *P notoginseng* plants from one single farm found variations in both genetic makeup and contents of ginsenosides (Rg_1, Rb_1, Re, Rd and notoginsenoside R_1)[8], suggesting the

presence of biodiversity in one cultured population. This research was designed to evaluate biodiversity of *P notoginseng* species that are represented by random samples collected from farms in 4 major production counties in Wenshan prefecture. The 3-year-old plants were documented for various morphological features and their genetic diversity evaluated by fluorescent amplified fragment length polymorphism (fAFLP) analysis.

Materials and methods

Plant material ninty-two randomly selected 3-year-old *P notoginseng* plants were harvested in December of 2005 from 4 farms located in 4 counties in Wenshan prefecture (for details see Table 1). Whole plants were photographed and morphological features of plant height (without peduncle), stem color, number of leaves and number of leaflets for each leaf were recorded. One leaf with 8–10 leaflets for each plant was collected and dried with silica gel in sealed plastic bags immediately. Dried leaves were frozen in liquid nitrogen and ground into powder, then stored at –80 ^{o}C. Tap roots were harvested, cleaned, dried in an oven and weighed. For quantitative morphological features, general calculations and Student's *t*-test were conducted with Microsoft Excel 2003.

fAFLP analysis DNA was extracted from powdered leaves using DNeasy Plant Mini kit (Qiagen, CA, USA). The quality of DNA was checked by electrophoresis on 0.8% agarose gel. fAFLP analysis was conducted as previously reported[8]. Selective amplification was carried out with different combinations of *Eco*RI and *Mse*I selective primers (Applied Biosystems, Foster City, CA, USA). 4 selective AFLP primer combinations were used in this study: *Eco*RI-ACT/*Mse*I-CAC (1B), *Eco*RI-ACA/*Mse*I-CAC (2B); *Eco*RI-ACA/*Mse*I-CTC (2F) and *Eco*RI-AGG/*Mse*I-CAC (7B). After selective amplification, 0.6 μL PCR product was mixed with ROX size standard (0.6 μL) and Hi-Di formamide (8.8 μL), then subjected to capillary electrophoresis using 3730xl DNA analyzer (Applied Biosystems).

Statistical analysis An AFLP Excel Macro[9] was used to convert allele size data from GeneMapper 3.7 (Applied BiosystemsA) into binary form, to indicate the presence (1) or absence (0) of alleles. GenAlEx 6[10] was used to compute allele frequency in populations, genetic heterozygosity within populations, and the average Nei genetic distance among populations. It was also used for analysis of molecular variance (AMOVA) and principal coordinates analysis (PCA).

Results and Discussion

Diversity in morphological features In order to have a good representation of *P notoginseng* at the species level, samples were collected from the main production area of Wenshan prefecture, Yunnan province of China. Of 4 independent farms located in 4 counties, each had a plantation area of more than 1 hectare and experience of more than 10 years of plantation of *P notoginseng* (Table 1). Randomly selected 3-year-old plants were harvested from each farm. Morphological features of these plants were recorded (Table 2).

Differences among samples in morphological features were observed even within the same farms. Plants had different stem colors, were of a wide range of heights, had variable numbers of leaves, had different numbers of leaf-

Table 1. *Panax notoginseng* samples from Wenshan prefecture, Yunnan province.

Population	Sample number	Location	Altitude (m)
Zhulijie, Wenshan County (ZLJ)	23	N23°19.036′/E104°21.684′	1398
Shangliuhe, Pingba County (SLH)	25	N23°15.056′/E104°05.731′	1750
Bazai, Maguan County (BZ)	21	N23°00.596′/E104°04.456′	1724
Jinbuhuan, Yansan County (JBH)	23	N23°32.009′/E104°23.508′	1541

Table 2. Morphological features of *Panax notoginseng* samples. [‡]Mean±SD. [b]$P<0.05$ *vs* total mean.

Population	ZLJ	SLH	BZ	JBH	Total
Sample number	23	25	21	23	92
Stem color (green/green-purple/purple)	18/2/3	12/0/13	14/2/5	14/5/4	58/9/25
Plant height (without peduncle, cm)[‡]	32.0±6.7	37.7±6.8	40.1±8.0[b]	31.1±6.9[b]	35.2±7.9
Number of leaves[‡]	4.5±0.7	4.4±0.6	4.7±0.5	4.6±0.9	4.5±0.7
Leaflets per leaf[‡]	6.9±0.7[b]	6.6±0.8	6.5±0.4	5.6±0.8[b]	6.4±0.8
Dry weight of tap root (g)[‡]	3.89±1.99	4.29±2.69	3.66±2.59	2.1±1.23[b]	3.49±2.33

lets per leaf and, importantly, yielded tap roots of very variable dry weight. It is noted that JBH population had lower plant heights, lower number of leaflets and lower yield of tap root than total population, most likely due to general poor growth of plants on the farm.

Genetic diversity in *P notoginseng* fAFLP analysis of 92 samples generated a total number of 582 discrete peaks, among which 509 polymorphic peaks were detected (see Table 3 for a summary).

In these 4 populations: Zhulijie, Wenshan County (ZLJ), Shangliuhe, Pingba County (SLH), Bazai, Maguan County (BZ), Jinbuhuan, Yansan County (JBH), analyzed by fAFLP, the percentages of polymorphic bands were 74.05%, 45.36%, 38.83% and 51.89%, respectively. Mean genetic heterozygosity were 0.166, 0.093, 0.094 and 0.125. For the total population of 92 samples, the percentage of polymorphic bands was 87.5% and the mean heterozygosity was 0.155. These results were consistent with the genetic diversity reported in wild populations of *Panax quinquefolius* revealed by random amplified polymorphic DNA (RAPD)[11] and allozyme analysis[12], although results from different techniques are not strictly comparable. Compared with the previous report of genetic diversity in *P notoginseng*[13], we report similar levels of genetic diversity present in larger numbers of samples from 4 more representing populations. In reference to the highly variable morphological features documented for the same samples, we conclude that a fair level of genetic diversity is present even in cultured *P notoginseng* species. Further AMOVA attributed most (28.0%+22.2%+18.0%+25.3%=93.5%) genetic diversity to within population variations.

No obvious genetic drift among populations Another objective of this study was to evaluate possible genetic drift or isolation of any population. According to farm owners, these 4 farms had been self sufficient in seeds for many years. Nei genetic distance was used to estimate genetic distance among populations (Table 4).

It was found that Nei genetic distances between populations were all <0.030, suggesting a lack of obvious genetic diversity between populations. This agrees with the AMOVA result of a small contribution by among population variation to total variation (6.5%). The fact that PCA did not group any population distinctively (data not shown) provides further support to the conclusion that *P notoginseng* populations are not significantly different from each other genetically. In other words, this species lacks genetic drift or isolation in its populations. This finding might be explained by the lack of geographical isolation and possible exchange of plantation materials during the history of cultivation in Wenshan prefecture. On the other hand, 400 years might not be long for accumulation of significant differences between populations even under full isolation.

Table 3. Summary of genetic diversity in *Panax notoginseng* by fAFLP.

Population	ZLJ	SLH	BZ	JBH	Total
Samples number	23	25	21	23	92
Number of bands	471	416	372	422	582
Percentage of polymorphic bands	74.1%	45.4%	38.8%	51.9%	87.5%
Mean heterozygosity	0.166	0.093	0.094	0.125	0.155
Contribution to genetic variance by AMOVA analysis	28.0%	22.2%	18.0%	25.3%	6.5%*

*Variation among populations.

Table 4. Nei genetic distance between populations.

Population	ZLJ	SLH	BZ	JBH
ZLJ	0.000			
SLH	0.007	0.000		
BZ	0.009	0.007	0.000	
JBH	0.016	0.014	0.021	0.000

The presence of morphology diversity together with genetic diversity in 4 populations of *P notoginseng* leads to our conclusion that *P notoginseng* still maintains a fair level of biodiversity at the species level despite a few hundred years of cultivation and the lack of gene exchange with wild plants. We reason that this maintenance of biodiversity in cultivation is associated with the local practice of non-discriminative harvest of seeds in winter time when roots remain underground (the most valuable part, to be harvested in next spring). Another possible contribution factor is the poor germination of seed after storage. Seeds harvested can only be used for next season planting. Poor viability of seeds made it difficult to maintain any unusual plants. The presence of biodiversity in a cultivated population is good for preservation and the future survival of *P notoginseng* as a species. On the other hand, such diversity is associated with non-uniform performance in farms. There is a lot of room for improvement and this biodiversity will form the basis of future improvement. Individual plants should be evaluated for their morphological traits and pharmacologically active components. One possible approach is to continuously self-pollinate an elite individual to create good performing and more homogeneous populations (in-breeding). An alternative approach is to use 2

genetic divergent parent plants for cross pollination to create hybrids, followed by selection for better performance in hybrid populations (hybrid vigor).

Author contribution

Yan HONG, Hwee-ling KOH, Chong-ren YANG and Ying-jun Zhang designed research; Dong WANG and Deborah HONG performed research; Yan HONG and Hwee-ling KOH contributed new analytical tools and reagents, Dong WANG and Yan HONG analyzed data and wrote the paper.

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