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DALP Analysis on Genetic Diversity of *Panax notoginseng*

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Abstract

***Panax notoginseng* (Burk) F.H. Chen is one of the most famous Chinese traditional medicinal plants, which belongs to *Panax genus* under *Araliaceae* family. At the present, *Panax notoginseng* cultivated is a mix colony. Using DALP makers, this article studied on genetic diversity of cultivated populations of *Panax notoginseng* in Wenshan County of Yunnan province and Napo country of Guangxi province. And the results showed that there were 260 polymorphic loci detected from the total of 292 in 13 populations, and there was great part of genetic diversity found between populations and the genetic differentiations were lower within populations. So there is broad prospect in good species breeding. And it can provide basic information for resource protection and sustainable use of *Panax notoginseng*.**

Keywords

***Panax notoginseng*, Genetic Diversity, DALP**

1. Introduction

Panax notoginseng (Burk) F. H. Chen is one of the most famous Chinese traditional medicinal plants, which belongs to *Panax notoginseng* under *Araliaceae* family. Wenshan prefecture is the major producing area, and it is about 400 years that *notoginseng* was cultivated [1]. In the process of cultivation and selection, some excellent genetic resource was lost. To protect that genetic resource and provide basic information for sustainable use of

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Panax notoginseng, we must strengthen the basic genetic research of *Panax notoginseng*. And it has to deeply understand the genetic differentiation of *Panax notoginseng*. Molecular maker is the most direct and accurate method for genetic diversity analysis, so we used DALP molecular maker in genetic diversity.

2. Materials and Methods

2.1. Materials

The three-year *panax notoginseng*' s leaves were collected from Yanshan base in Wenshan county of Yunnan province and Jingxi country of Guangxi province, except yellow *panax notoginseng* and purplish panax *notoginseng*' s leaves that were collected from Zhela base of Wenshan County, Yunnan province. The geographical location and the habitat overview of the population is showed in **Table 1**.

2.2. Methods

Using CTAB to extract Genomic DNA from leaves of *Panax notoginseng*. Detecting DNA concentration is diluted and preserving in −20˚C. Getting 8 primers from 40 random primers that can get clear bands and react stably; they are PS01-PR02, PS02-PR01, PS04-PR01, PS04-PR02, PS05-PR02, PS08-PR01, PS09-PR01and PS10-PR01 (the primers' sequence is in **Table 2**).

Table 1. The habitats and localities of materials.

Code	Sampling position	Population	Altitude (m)	Longitude	Latitude
WM	Matang	PA	1470	104˚5'	23˚49'
WZ	Zhuilijie	PB	1430	204˚24'	23˚37'
WL	Laohuilong	PL	1450	104˚36'	23˚46'
WD	Dongshan	PI	1760	104˚58'	23˚54'
YC	Chongka	PD	1498	104˚25'	23˚32'
YZ	Zhela	PC	1580	104˚35'	23˚62'
YA	Aolongke	PE	1600	105˚6'	23˚6'
YP	Panlong	PJ	1510	104˚31'	23˚54'
MR	Renhe	PG	1600	106˚7'	23˚4'
MB	Bazhai	PH	1480	104˚7'	23˚0'
QB	Niejiao	PF	1992	103˚87'	23˚54'
GNP	Napo	PK	1100	105˚57'	23˚18'

"Code" is the abbreviation of "sampling position", for example, WM = Wenshan matang, YC = Yanshan chongka; PA—PL is the randomizing ID of the population.

Table 2. The number and sequence of primers.

Primer	No.	Sequence (5'-3')
Selective primers	PS01	5'-GTTTTCCCAGTCACGACAGC-3'
Selective primers	PS02	5'-GTTTTCCCAGTCACGACGAC-3'
Selective primers	PS03	5'-GTTTTCCCAGTCACGACACG-3'
Selective primers	PS04	5'-GTTTTCCCAGTCACGACCAG-3'
Selective primers	PS05	5'-GTTTTCCCAGTCACGACCAC-3'
Selective primers	PS06	5'-GTTTTCCCAGTCACGACTCAG-3'
Selective primers	PS07	5'-GTTTTCCCAGTCACGACCCAG-3'
Selective primers	PS08	5'-GTTTTCCCAGTCACGACGC-3' 5'-GTTTTCCCAGTCACGACCTAG-3'
Selective primers	PS09	5'-GTTTTCCCAGTCACGACCGC-3'
Selective primers	PS10	5'-AACAGCTATGACCATGA-3'
Reverse primers	PR01	5'-TTTCACACAGGAAACAGCTATGAC-3'
Reverse primers	PR02	

The PCR system for DALP analysis was as follows: dd H_2O 6.5 uL, 2.5 mmol/L dNTP 1 uL, 25 mmol/L Mg^{2+} 2 uL, 10 × PCR buffer 2.5 uL, 50 - 100 ng template DNA 2 uL, 5 pmol/L selective primer 1 uL, 5 pmol/L reverse primer 3 uL, 1U Taq polymerase in 20 uL reaction system. And the PCR program was as follow: predenaturation 5 min at 94˚C, denatured 30 s at 94˚C, annealed 30 s at 50˚C, 1 min at 72˚C, 12 cycles, and denatured 30 s at 94˚C, annealed 30 s at 50˚C, 30 s at 72˚C 28 cycles, then extend at 72˚C, 4˚C hold.

Using 0/1 matrix and POPGENE1.32 to calculate the Nei's genetic diversity index (H), Shannon information index (I), percentage of polymorphic bases (PPB), genetic differentiation (Gst), diversity index within population (Hs), total genetic diversity (Hs & Ht), the Nei's genetic distance (D) and genetic identity (I). Then the populations were calculated by the MEGA software.

3. Results

1% Agar gel electrophoresis showed that the bands of 192 samples' DNA extracted by CTAB method were the highest purity, best quality, clear and less fragments (As shown in **Figure 1**). The test results of DALP analysis as shown in **Figure 2** to **Figure 5**.

A total of 292 clear bands were amplified from 8 selected DALP primers, 260 (89.05%) of which were polymorphic. The PPB (79.08), Shannon index (0.2817) and the Nei's genetic diversity index (0.1172) of popula-

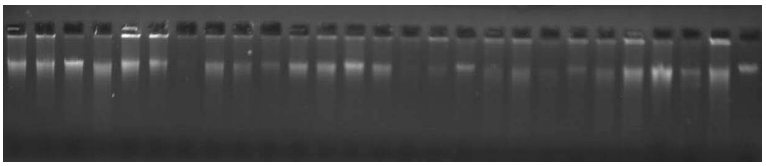

Figure 1. Total DNA extracted by CTAB method.

Figure 2. The PCR amplified results of *Panax notoginseng* cultivated in Wenshan prefecture by primer ps05-pr1.

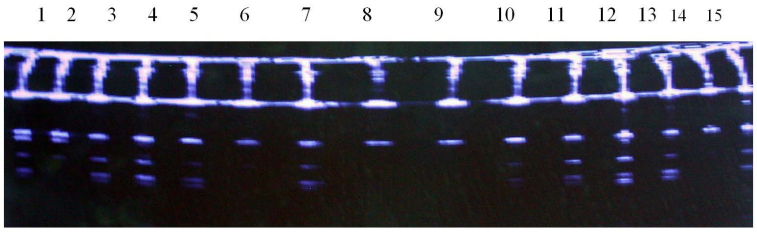

Figure 3. The PCR amplified results of *Panax notoginseng* cultivated in Guangxi province by primer ps05-pr1.

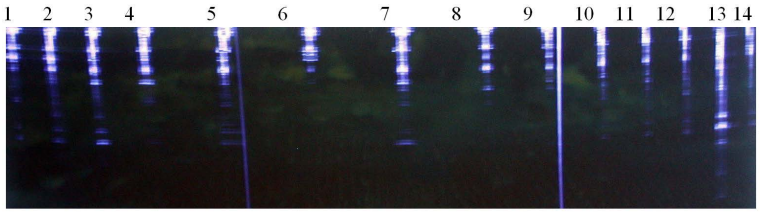

Figure 4. The PCR amplified results of yellow *Panax notoginseng* by primer ps05-pr1.

tions cultivated in Zhela (PC) were highest in Wenshan's populations. And The PPB (40.92), Shannon index (0.0868) and the Nei's genetic diversity index (0.0606) of populations cultivated in Bazhai (PH) were lowest in Wenshan's populations. And there were big difference between the highest one and the lowest one. Overall, the PPB, Shannon index and the Nei's genetic diversity index of Wenshan's populations were lower than Guangxi' s populations' which had more genetic diversity. And the PPB, Shannon index and the Nei's genetic diversity index of *Panax stipuleanatus* which was contrast were next to Guangxi's populations (The results shown in **Table 3**).

Based on the proportion of genetic diversity level in Ht, Ht-Hs and Gst, we know that genetic differentiation between 10 populations cultivated in Wenshan was 0.7732. And it showed that there was 23.38% molecular variation only within populations and there was 77.32% molecular variation between populations, so there was more variation between populations. Genetic differentiation idex (0.1696) of the PK population cultivated in Napo Guangxi province was lowest. There was 83.04% molecular variation within population and only 16.96% molecular variation between populations. The degree of genetic diversity within population was lower than the total degree in 11 populations, and it showed that genetic diversity mainly existed between populations. Based on Wright (1931) [2], the migration number of every populations (Nm*) was over 1, which suggested that there existed sufficient genetic exchanges to prevent the genetic differentiation made by genetic drift among population. The sufficient genetic exchange number was inversely proportional to genetic differentiation level among populations in PD population of Longka and PK population of Napo, which's Nm* was over 1 and the genetic differentiation was low (The results shown in **Table 4**).

The analysis results of genetic distance showed that the genetic distance of every populations ranged from 0.0714 to 0.2408 (**Table 5**). The distance ranged from PB population to PC population was only 0.01. And genetic distance of Wenshan populations was close expecting PG. More distance excited between PK and other populations, especially PA which was over 0.1694. The cluster analysis showed that PB and PC classified one group, PD, PE and PF classified one group, PH classified with PI and PJ, and PG and PK were separate ones.

A total of 150 clear bands were amplified from 8 screened DALP primers, 120 (80%) of which were polymorphic in 96 samples from Wenshan, Guangxi, yellow *Panax notoginsseng*, purple *Panax notoginseng* and *Panax stipuleanatus*. From the **Table 6**, the genetic diversity analysis showed that PPB (81.82), Shannon index

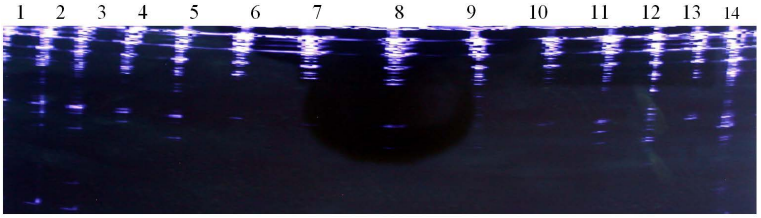

Figure 5. The PCR amplified results of purple *Panax notoginseng* by primer ps05-pr1.

Table 3. Genetic diversity of 11populations in *Panax notoginseng*.

Population	Total number of loci	No. of polymorphic loci	PPB	na*	ne*	The Nei's genetic diversity H*	Shannon index I*
PA	110	84	76.36%	1.3818	1.3055	0.1697	0.243
PB	148	105	70.92%	1.2364	1.1891	0.1051	0.1504
PC	120	94	79.08%	1.2636	1.2109	0.1172	0.2817
PD	135	73	54.54%	1.1818	1.1455	0.0808	0.2466
PE	128	80	62.5%	1.2091	1.1674	0.0928	0.264
PF	80	37	46.35%	1.1545	1.1255	0.0692	0.0989
PG	117	47	73.65%	1.2455	1.1964	0.1091	0.1562
PH	113	45	40.92%	1.1364	1.1091	0.0606	0.0868
PI	127	55	43.65%	1.1455	1.1164	0.0646	0.0926
PJ	138	82	60%	1.2	1.16	0.0889	0.1273
PK	110	90	81.82%	1.8182	1.396	0.2442	0.3782
stipuleanatus	140	112	80%	1.8	1.4168	0.2544	0.3896

Table 4. Analysis on genetic differentiation among populations.

Population	Ht	Hs	Dst	Gst	Nm*
PA	0.1534	0.0750	0.0784	0.5111	0.4783
SD	0.0395	0.0132	0.0263		
PB	0.0955	0.0455	0.05	0.5238	0.4545
SD	0.0304	0.0094	0.021		
PC	0.1091	0.0455	0.0636	0.5833	0.3571
SD	0.0344	0.0094	0.0248		
PD	0.0693	0.0477	0.0216	0.3115	1.1053
SD	0.0220	0.0109	0.0111		
PL	0.0773	0.0273	0.05	0.6471	0.2727
SD	0.0278	0.0061	0.0217		
PF	0.0659	0.0318	0.0341	0.5172	0.4667
SD	0.0229	0.007	0.0159		
PG	0.1102	0.0250	0.0852	0.7732	0.1467
SD	0.0386	0.0057	0.0329		
PH	0.0534	0.0295	0.0239	0.4468	0.6190
SD	0.0185	0.0066	0.0119		
PI	0.0614	0.0227	0.0387	0.6296	0.2941
SD	0.0229	0.0052	0.0177		
PJ	0.0864	0.0273	0.0591	0.6842	0.2308
SD	0.0309	0.0061	0.0248		
PK	0.2478	0.2058	0.042	0.1696	2.4476
SD	0.0312	0.022	0.0092		

*: *Ht* = Total gene diversity; *Hs* = Gene diversity within population; *Gst* = The coefficient of gene differentiaton; *SD* = Standard deviation; **Nm* = estimate of gene flow from Gst or Gcs. E.g., *Nm* = 0.5(1 - Gst)/Gst; See McDermott and McDonald, Ann. Rev. Phytopathol. 31:353-373 (1993).

Table 5. The Nei's genetic similarity (above the diagonal) and the genetic distance D (below the diagonal).

pop	PA	PB	PC	PD	PE	PF	PG	PH	PI	PJ	PK
PA	****	0.9311	0.9206	0.8779	0.8779	0.8555	0.8111	0.8593	0.882	0.8934	0.786
PB	0.0714	****	0.9499	0.9551	0.9551	0.9250	0.8472	0.8999	0.888	0.8837	0.809
PC	0.0827	0.0514	****	0.9345	0.9345	0.8941	0.8345	0.9222	0.904	0.8877	0.826
PD	0.1306	0.0459	0.0678	****	1.0179	0.9611	0.8494	0.8963	0.881	0.8536	0.825
PE	0.1303	0.0459	0.0678	−0.017	****	0.9611	0.8494	0.8963	0.881	0.8536	0.825
PF	0.1561	0.078	0.1119	0.0397	0.0396	****	0.8732	0.8673	0.871	0.8390	0.834
PG	0.2094	0.1658	0.1809	0.1632	0.1633	0.1355	****	0.8810	0.842	0.8254	0.824
PH	0.1517	0.1055	0.0810	0.1095	0.1097	0.1424	0.1267	****	0.943	0.9089	0.852
PI	0.1247	0.1179	0.1007	0.1267	0.1266	0.1372	0.1711	0.0580	****	0.9536	0.824
PJ	0.1127	0.1237	0.1192	0.1583	0.1583	0.1756	0.1919	0.0955	0.047	****	0.807
PK	0.2408	0.2114	0.1902	0.1921	0.1924	0.1811	0.1934	0.1594	0.193	0.2138	****

Table 6. The genetic variation among populations.

Population	Total number of loci	PPB	na*	ne*	H*	I*
Wenshan	110	70	1.7	1.3182	0.1965	0.3063
Guangxi	98	81.82	1.8182	1.396	0.2442	0.3782
YELLOW	133	50.91	1.5091	1.1774	0.1166	0.1893
PURPLE	102	37.27	1.3727	1.1585	0.1029	0.1633
stipuleanatus	118	79.6	1.8	1.4168	0.2544	0.3896

*: PPB = The percentage of polymorphic loci is; *na = Observed number of alleles; *ne = Effective number of alleles [Kimura and Crow (1964)]; *h = Nei's (1973) gene diversity; *I = Shannon's Information index [Lewontin (1972)].

(0.3782) and the Nei's genetic diversity index (0.2442) of Guangxi population were highest; the ones of Wenshan population were next to Guangxi population. And PPB (37.27), alleles number (1.3727), Shannon index (0.1633) and the Nei's genetic diversity index (0.1029) of purple *panax notoginseng* were minimum value in all populations, shows the amount of genetic variation is the lowest.

Based on the proportion of genetic diversity level in Ht, Ht-Hs and Gst, we know that species genetic differentiation was 0.5182. There was 48.12% molecular variation existed within populations, and more molecular (51.82%) variation existed between populations. In different population level, there was only 16.96% molecular variation existed among populations, and 83.04% one existed within populations in Guangxi's populations. There was 21.37% molecular variation existed among populations and 78.63% molecular variation existed in population in yellow fruit *notoginseng*. There was 14.76% molecular variation existed among populations, and 85.24% existed in population in *Panax stipuleanatus.* In the five populations, the genetic differentiation mainly existed among populations. Based on Wright (1931) [2], the migration number of every populations (Nm^*) was over 1, which suggested that there existed sufficient genetic exchanges to prevent the genetic differentiation made by genetic drift among population. The sufficient genetic exchange number was inversely proportional to genetic differentiation level among populations in all populations and the genetic differentiation was lowest, except Wenshan's population (**Table 7**).

The analysis results of genetic distance showed that the genetic distance of every populations ranged from 0.137 to 0.259 (**Table 8**). The distance ranged from *Panax notoginseng* to *Panax stipuleanatus* was farthest, which was over 0.5154. And genetic distance of *Panax notoginseng* cultivated in Wenshan was close to Guangxi one. The distance of yellow fruit *Panax notoginseng* was close to purple fruit *Panax notoginseng* which was 0.5001.

And The cluster analysis using UPGMA method showed that Wenshan's *Panax notoginseng* and Guangxi's classified one group, yellow fruit *Panax notoginseng* and purple fruit *Panax notoginseng* classified one group, *Panax notoginseng* was Separate clustering (**Figure 6**).

4. Discussion

Intraspecific genetic variation decides the evolution trend of species [3]. The more genetic diversity, the more extensive evolution [4]. So it is the basic for sustainable use of genetic resources that maintain the intraspecific

Table 7. The genetic diversity analysis of populations.

Population	Ht	Hs	Gst	Nm^*
Wenshan	0.1963	0.0946	0.5182	0.4649
SD	0.0325	0.0099		
Guangxi	0.2478	0.2058	0.1696	2.4476
SD	0.0312	0.022		
Yellow fruit	0.1181	0.0929	0.2137	1.8393
SD	0.0235	0.0137		
Purple fruit	0.0899	0.071	0.2197	1.8712
SD	0.0224	0.0145		
stipuleanatus SD	0.2549	0.2173	0.1476	2.887
	0.0301	0.0227		

*: *Ht* = Total gene diversity; *Hs* = Gene diversity within population; *Gst* = The coefficient of gene differentiaton; *SD* = Standard deviation; **Nm* = estimate of gene flow from Gst or Gcs. E.g., Nm = 0.5(1 - Gst)/Gst; See McDermott and McDonald, Ann. Rev. Phytopathol. 31:353-373 (1993).

Table 8. The Nei's genetic similarity (above the diagonal) and the genetic distance D (below the diagonal).

pop	Wenshan	Guangxi	Pingbian	Purple fruit	Yellow fruit
Wenshan	****	0.8720	0.7718	0.8598	0.8710
Guangxi	0.1370	****	0.8327	0.8320	0.8491
Pingbian	0.2590	0.1831	****	0.7667	0.7729
Purple fruit	0.1511	0.1839	0.2656	****	0.9674
Yellow fruit	0.1381	0.1636	0.2576	0.0331	****

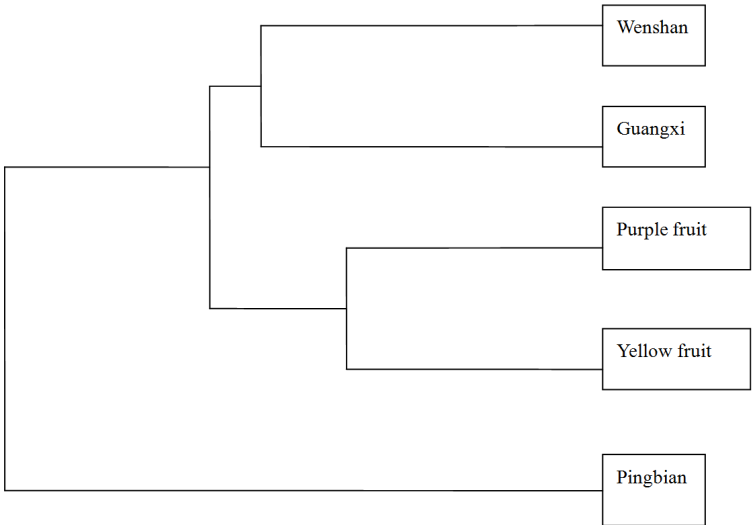

Figure 6. The UPGMA clustering dendrogram of populations.

genetic variation. The results of DALP analysis showed that *Panax notoginseng* and its allied species were abundant in genetic diversity which had strong evolution potential. Based on Ruozhu QU [5], the stable group had high Gst value, but the unstable one had low Gst value. The genetic differentiation index of Wenshan's populations and Guangxi's ones were over 0.3439, which showed that more genetic variation existed among populations, so they had great genetic differentiation and stable population. Many researches showed that the genetic diversity of endangered species was lowed, and the lack of diversity was the important reason to endanger [6]-[11]. So for *Panax notoginseng*, we must protect its genetic diversity at first, to build the resource garden and breed good varieties. The study provides basic information for resource pretection and sustainable use of *Panax notoginseng*.

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