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•Research article•

Novel SNP markers on ginsenosides biosynthesis functional gene for authentication of ginseng herbs and commercial products

WU Wen-Ru[1, 2Δ], CHENG Chun-Song[1Δ], CHENG Qi-Qing[1Δ], LAO Chi-Chou[1], CUI Hao[1], TANG Zi-Yu[1], OUYANG Yue[1], LIU Liang[1], ZHOU Hua[1, 3*]

[1] *State Key Laboratory of Quality Research in Chinese Medicine, Faculty of Chinese Medicine, Macau University of Science and Technology, Macao 999078, China;*
[2] *School of Chinese Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, Guangzhou 510006, China;*
[3] *Joint Laboratory for Translational Cancer Research of Chinese Medicine of the Ministry of Education of the People's Republic of China, Macau University of Science and Technology, Macao 999078, China*

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[ABSTRACT] *Panax ginseng* and *Panax quinquefolius* have similar bioactive components and morphological characteristics, but they are known to have different medicinal values, high-sensitive and accurate method is expected to identify the sources of ginseng products and evaluate the quality, but with a huge challenge. Our established UHPLC-TOF/MS method coupled with orthogonal partial least squares discriminant analysis (OPLS-DA) model based on 18 ginsenosides was applied to discriminate the sources of raw medicinal materials in ginseng products, and nested PCR strategy was used to discover 6 novel single nucleotide polymorphism (SNP) sites in functional dammarenediol synthase (*DS*) gene for genetic authentication of *P. ginseng* and *P. quinquefolius* for the first time. OPLS-DA model could identify the sources of raw ginseng materials are real or not. SNP markers were applied to identify ginseng fresh samples as well as commercial products, and proved to be successful. This established molecular method can tell exact source information of adulterants, and it was highly sensitive and specific even when total DNA amount was only 0.1 ng and the adulteration was as low as 1%. Therefore, this study made an attempt at the exploration of new type SNP marker for variety authentication and function regulation at the same time, and the combination of chemical and molecular discrimination methods provided the comprehensive evaluation and authentication for the sources of ginseng herbs and products.

[KEY WORDS] *Panax ginseng*; *Panax quinquefolius*; UHPLC-TOF/MS; Dammarenediol synthase; Nested PCR; SNP
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Introduction

Ginseng is a well known and commonly used traditional herbal medicine, *Panax ginseng* (Asian ginseng) and *Panax quinquefolius* (American ginseng) are currently two major medicinal plants referred to ginseng, and their products have attracted worldwide consumption, including herbal medicines, health care products and other formulations [1-4]. They are closely related species from the same genus, with similar morphological characteristics and chemical components, sometimes, in order to reduce the production cost or just by mistake, Asian ginseng is adulterated with American ginseng in commercial ginseng products [5]. When they are processed from raw herbs into products, discrimination becomes more difficult and remains to be not an easy task. However, they have different medicinal values, Asian ginseng is used in "yang-deficient" condition, whereas American ginseng is used in "yin-deficient" condition [6], which may cause harm for drug safety and accuracy. Therefore, a high-sensitive method is expected to authenticate the sources of raw medicinal materials in ginseng products to safeguard public health and consumers' rights.

Although many studies have been made to discriminate *P. ginseng* from *P. quinquefolius*, the reported chemical method encounters the problem that ginsenosides played as authentication markers may be affected by commercial processing [7]. In our lab's previous study, an approach that based on nearly 20 ginsenosides detected by UHPLC-TOF/MS coupled with the orthogonal projection to latent structure-dis-

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[*]**Corresponding author]** E-mail: hzhou@must.edu.mo
[Δ]These authors contributed equally to this work.
These authors have no conflict of interest to declare.

criminant analysis (OPLS-DA) model has been established for authenticating four ginseng varieties [8]. But when different ginseng varieties adulterate in the products, the chemical method may only give clues what can be the possible adulterants, while genetic markers are further needed to provide a strong evidence for authentication and discrimination [9].

As a growing number of molecular biology techniques have been applied to identify Chinese medicine varieties, most of studies chose non-functional genes as targets, such as ITS2 [10], ETS [11] and ESTs [12]. Some DNA molecular markers are related to phytochemical biosynthesis [13]. If we choose these specific markers related to the content of bioactive ingredients, we can accurately determine the authenticity and quality at the same time. SNP is one of specific molecular markers, which is usually used in authentication. Although identifying medicinal species of *Panax* genus by SNP markers in ITS2 has been reported [14], there is no report about SNPs in functional genes for authentication of *Panax* genus. Dammarenediol synthase (*DS*) is the key functional enzyme to catalyze the cyclization of 2, 3-oxidosqualene to dammarenediol II, the first step of skeleton formation for the most bioactive ginsenosides [15, 16]. Based on our previous study, specific SNPs in *DS* gene were associated with the accumulation of active components ginsenosides Rf, Rg1 and F1, moreover, one of the SNPs might play a role in influencing ginsenosides content level [17]. So the discovery of the SNPs in *DS* gene not only provides the genetic markers for authentication, but may also have a relationship with biosynthesis of ginsenosides and regulation of other relevant regulatory genes, which has an effect on quality evaluation.

This study detected 18 ginsenosides of 11 ginseng products to authenticate their sources of raw medicinal materials by our established UHPLC-TOF/MS method coupled with OPLS-DA model, and also found 6 novel SNP markers in functional *DS* gene for genetic identification of *P. ginseng* and *P. quinquefolius* for the first time. These SNP markers were successfully applied to authenticate ginseng fresh samples as well as their commercial products. And it can give source information of adulterants, which the chemical method is not good at. This is a new attempt to explore new type of SNP markers for variety authentication and function regulation at the same time, and it can work in coordination with chemical method to give accurate and comprehensive analysis of the medicinal material sources for ginseng products.

Materials and Methods

Samples used in this study

Different landraces (cultivars) of *P. ginseng* and *P. quinquefolius* were collected in Jilin and Liaoning Provinces, China, and were placed in −80 °C after cleaning and classification. Commercial products of *P. ginseng* and *P. quinquefolius* were purchased from local pharmacies in Guangdong Province, China. All samples in this study were performed the morphological identification by Dr. ZHANG Zhi-Feng (Faculty of Chinese Medicine, Macau University of Science and Technology), and the detailed samples information is shown in Tables 1−2.

Chemicals and reagents

The 18 chemical reference substances (CRSs, purity > 98%, HPLC-DAD) of ginsenoside Rg1, Re, Rf, S-Rh1, Rg2, Rb1, R-Rh1, Rc, F1, Ro, Rb2, Rb3, Rd, F2, S-Rg3, R-Rg3, notoginsenoside R1 and pseudoginsenoside F11 were obtained from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China). Acetonitrile (Mass grade) and formic acid (Mass grade) were purchased from Sigma-Aldrich (Steinheim, Germany). The ultrapure water was prepared by the Milli-Q water purification system (Millipore, Bedford, MA, USA).

Chemical analysis of ginseng merchandises

The 18 ginsenosides mentioned above were detected to distinguish the sources of 11 tested ginseng products. The determination was performed on an Agilent 1290 UHPLC coupled with Agilent 6230 accurate mass time-of-flight mass spectrometer system (Agilent Technologies, Palo Alto, CA,

Table 1 The information of the collected ginseng herb samples used in this study

No.	Name	Locality	Growing years	Tissue
1	*P. quinquefolius*	Fusong, Jilin, China	4	Root
2	*P. quinquefolius*	Fusong, Jilin, China	4	Root
3	Biantiao	Ji'an, Jilin, China	6	Root
4	Damaya	Changbai, Jilin, China	6	Root
5	Fuxingyihao	Fusong, Jilin, China	6	Root
6	Damaya under forest	Fusong, Jilin, China	6	Root
7	Damaya under forest	Fusong, Jilin, China	10	Root
8	Shizhu	Kuandian, Liaoning, China	6	Root
9	Shizhu	Kuandian, Liaoning, China	10	Root

Note: All samples were deposited in the state key laboratory of quality research in Chinese medicines (Macau University of Science and Technology)

Table 2 The list of merchandises collected from the market used in this study

No.	Name	Source	Classification	Amount
1	Ginseng bolus I	Pharmacy of Guangzhou	Chinese patent drug	30 g
2	Ginseng bolus II	Pharmacy of Guangzhou	Chinese patent drug	20 g
3	Ginseng bolus III	Pharmacy of Guangzhou	Chinese patent drug	20 g
4	American ginseng tea I	Taobao online shop in China	Health care products	20 g
5	American ginseng tea II	Taobao online shop in China	Health care products	100 g
6	Red ginseng tea I	Taobao online shop in China	Health care products	30 g
7	White ginseng tea	Taobao online shop in China	Health care products	30 g
8	Red ginseng tea II	Taobao online shop in China	Health care products	150 g
9	American ginseng tablet	Taobao online shop in China	Health care products	50 g
10	Red ginseng drink	Taobao online shop in China	Health care products	14 g
11	American ginseng tea III	Taobao online shop in China	Health care products	60 g

Note: In order to protect the manufacturers' identities, sample sources were described as their preparation names

USA) and an ACQUITY UPLC BEH shield RP_{18} column (2.1 mm × 100 mm id, particle size 1.7 μm). The raw data of the UHPLC-TOF/MS were processed by Agilent MassHunter Qualitative Analysis B.06.00 (Agilent Technologies, Palo Alto, CA, USA). 1 μL injection volume of sample was kept in an autosampler set at 10 °C. The column temperature was maintained at 40 °C. Water−acetonitrile (both the aqueous and organic phase containing 0.1% formic acid) at a flow rate of 0.35 $mL \cdot min^{-1}$ with a gradient program as follow: 25%−43% A (0−14 min), 43%−65% B (14−18 min), 65%−80% B (18−20 min), 80%−25% B (20−22 min), 25% B (22−25 min). The ESI iron source parameters were as follows in negative ionization mode: Gas temperature: 325 °C, Gas Flow: 8.0 $L \cdot min^{-1}$, Nebulizer: 35 psi, Sheath Gas Temperature: 350 °C, Sheath Gas Flow: 11.0 $L \cdot min^{-1}$, Cap: 3500 V, Nozzle Voltage: 1000 V, Fragmentor: 175. EIC mode was used to extract the target iron. The offline tuning of the TOF/MS was done before the detection of the samples using the reference material solution from Agilent Technologies. The accurate mass was tuned in the small molecules mass range that the *m/z* was < 1700. The online tuning was on process during all the run of the samples by automatic injection of the reference material from Agilent Technologies into the MS system.

OPLS-DA was performed by SIMCA version 13.0 (MKS Umetrics AB, Umeå, Sweden). 34 white ginsengs, 23 red ginsengs, 30 *P. notoginseng* and 21 *P. quinquefolius* that were collected in our previous study, were used to establish OPLS-DA model in this study [8]. After content data normalization, 11 ginseng products were detected and analyzed with this model data together.

DNA extraction, PCR amplification of DS region and gel electrophoresis

DNA was extracted by DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). Ginseng roots were ground into powder, and ginseng commercial products, like tea and bolus, were used directly in DNA extraction procedure. For DNA extraction interfered with caramel, methanol was used as the extraction solvent instead of the ethanol to optimize the method based on our previous study [18].

Primer pair P1/P2 was designed according to the conserved region of *P. ginseng* and *P. quinquefolius DS* genes from GenBank (Accession number: AB265170, GU183405, JN596111), and a fragment of about 750 bp length was obtained. Primers were designed using Primer3 (http://frodo.wi.mit.edu/primer3/) and Primer Premier 5.0 software (PREMIER Biosoft International, CA, USA) and were synthesized by BGI (Guangzhou, China).

Each 50 μL PCR mixture contained 25 μL of 2 × Premix PCR PrimeSTAR HS DNA polymerase (TaKaRa Bio, Ohtsu, Japan), 10 ng of template DNA, 0.2 $\mu mol \cdot L^{-1}$ of each primer. PCR runs were performed by a Veriti® 96-well Thermal cycler (Applied Biosystems, Foster City, CA, USA) with the programs as follows: pre-denaturation at 94 °C for 2 min, 35 cycles of 98 °C for 10 s, 64 °C for 10 s, and 72 °C for 45 s, and a final extension at 72 °C for 5min. PCR products were analyzed *via* 1.0% agarose gel electrophoresis visualized by SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA) under UV.

Determination of ginseng DS gene SNP locus

Sequencing of PCR products amplified by primer pair P1/P2 was done by BGI (Shenzhen, China). Then the sequences were analyzed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and DNA sequence alignment was performed using the sequences present in GenBank by DNAMAN 7.0 (Lynnon Biosoft, Quebec, Canada), to explore SNPs of *DS* gene among the different samples.

Molecular authentication of P. ginseng and P. quinquefolius by nested PCR strategy

Molecular authentication of *P. ginseng* and *P. quinquefo-*

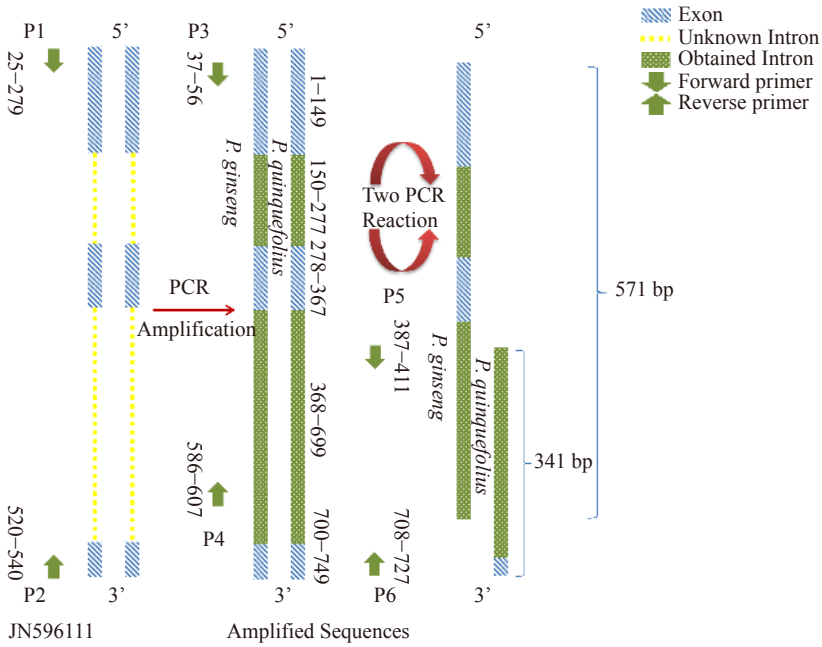

Fig. 1 The nested PCR strategy flowchart and its specific primers position

lius was performed under the nested PCR strategy (Fig. 1). The products of the first round PCR, carried out by P1/P2, were diluted 100 times to be the template for the second round PCR. The second round nested PCR was carried out in 2 PCR tubes, one used the primer pair P3/P4, and the other one used primer pair P5/P6. These 2 allele-specific primer pairs, P3/P4 and P5/P6, were designed respectively to generate different products of *P. ginseng* and *P. quinquefolius*. The allele-specific primers P4 and P5 were designed based on the SNP sites detected in *DS* intron region. Additionally, mismatches were introduced deliberately *via* the substitutions of G for T in primer P5 in order to ensure required allelic specificity. All the primers used in this study are shown in Table 3.

The nested PCR cycle profile was as follows: pre-denaturation at 98 °C for 30 s, 35 cycles of 10 s at 98 °C, 10 s at 66 °C for P3/P4 or 10 s at 63 °C for P5/P6 respectively, and 10 s at 72 °C, with a final extension of 72 °C for 7 min. The annealing temperature was optimized by using gradient PCR range from 64 to 67 °C for P3/P4 and range from 62 to 65 °C for P5/P6. Each 50 μL PCR mixture contained 25 μL of 2 × TransStart FastPfu PCR SuperMix (TransGen Biotech, Beijing, China), 1 μL of template DNA, and 0.2 μmol·L^{-1} of each primer. PCR products were analyzed *via* 1.0% agarose gel electrophoresis visualized by SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA) under UV. And the target fragments amplified by primer pairs P3/P4 and P5/P6 of representative samples were sequenced by BGI (Shenzhen, China).

Table 3 The primers used in this study

Primer name	Sequence (5′–3′)	Length (bp)
P1 (DSF272)	ACCGCCGTTGAGA TTAGATGA	21
P2 (DSR574)	ACTGACCCAATCA TCGTGCTG	21
P3 (DF61)	ATGCAGTTACAAC CGCTGTG	20
P4 (DR629G6-1)	TGTTTGTTACTCCC TCCGT***G***C***C***	22
P5 (DF412Q-1)	CCATTCGTTCCATA ATATTTGAT***CG***	25
P6 (DR751)	TATAGGATCCCCA TCCACCA	20

Note: Bold underlined nucleotide is the additional mismatch introduced *via* substitution of G for T. Italics nucleotides are the SNPs

In order to test the method's sensitivity and specificity, experiments were carried out on different DNA template amount of *P. quinquefolius* and *P. ginseng* and DNA mixture samples contained 1%, 2%, 5%, 10%, 20%, 50%, 80%, 90%, 95%, 98%, 99% amount of *P. quinquefolius* or *P. ginseng* respectively. After the method being proved to work in the authentication of Asian and American ginseng herb samples, it was applied to authenticate the 11 ginseng products.

Results

Chemical analysis with the OPLS-DA model

In the established OPLS-DA model of the four ginseng herbs (34 white ginsengs, 23 red ginsengs, 30 *P. notoginseng* and 21 *P. quinquefolius* samples), a clear separation of the four varieties was observed using 18 ginsenosides as variables. As shown in Fig. 2, the notoginseng and the American ginseng were respectively located at the end of the *t*[1] and *t*[2] direction. And the white and red ginseng stayed close to each other but could be roughly separated. So it showed that, there exhibited difference between the white ginseng and red

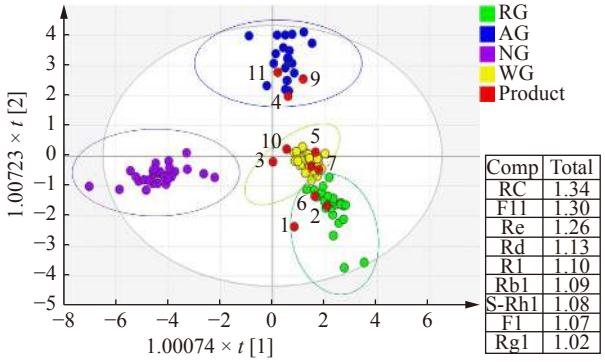

Fig. 2 The discrimination of the 11 ginseng products by using the constructed OPLS-DA model (containing 108 samples of the four ginseng herbs). 1, 2 and 3 were the ginseng boluses; 4, 5 and 11 were the American ginseng teas; 6, 8 and 10 were the red ginseng teas and drink; 7 was the white ginseng tea; and 9 was American ginseng tablet. All the OPLS-DA discriminations used three predict components and five orthogonal components

ginseng, but not as significant as the difference observed in the notoginseng and the American ginseng. Because the white ginseng and the red ginseng were from the same species, but had different treatment.

As the constructed model discriminated the herb origin successfully especially in our previous report [8], it was further employed to discriminate the herb origin in 11 deeply processed products. The detailed information of these 11 products is shown in Table 2. The 1st, 2nd and 6th samples were clustered into red ginseng area, the 3rd, 5th, 7th, 8th and 10th samples were clustered into white ginseng area, the 4th, 9th and 11th samples were clustered into American ginseng area, and no sample was clustered into *P. notoginseng* area. The chemical analysis results showed that adulteration could be identified for the most tested ginseng herb products except the 5th sample. The source of 5th sample was detected to be from ginseng, not from American ginseng. Chemical analysis results only showed that the source of 5th sample should not totally be American ginseng, but it cannot tell us whether it composed only by *P. ginseng* or composed of *P. ginseng* and *P. quinquefolius* together. Furthermore, more data is needed to provide source information, and genetic markers could be potential one to provide genetic evidence.

Ginseng DS gene fragment amplification and SNP loci confirmation

Ginseng *DS* gene fragment was obtained by the primer pair P1/P2, the PCR products of multiple samples were sequenced, and multiple sequences alignment analysis of these sequences was made to confirm 6 SNP sites. The PCR product length was measured 749 bp, and the BLAST result showed this fragment contains 2 introns of 128 bp and 332 bp as shown in Fig. S1. Two specific primers, P4 (to *P. ginseng*) and P5 (to *P. quinquefolius*), were designed on the SNP sites (Fig. 3). The relative positions and the expected sizes were showed that the cultivar-specific amplicons of *P. ginseng* should be 571 bp, and *P. quinquefolius* should be 341 bp in nested PCR system.

Allele-specific PCR was sensitive to annealing temperature. The annealing temperature for primer pair P3/P4 was tested from 64 to 67 °C. 66 and 66.5 °C were appropriate annealing temperature for authentication (Fig. 4A). In order to use widely, 66 °C was selected to be the best annealing temperature. Meanwhile, the annealing temperature for primer pair P5/P6 was scanned from 62 to 65 °C, the PCR products could generate the specific fragments under annealing temperatures from 62 to 63.5 °C (Fig. 4B). In order to ensure required allelic specificity, 63 °C was selected as the best annealing temperature.

The PCR products amplified by specific primer pair P3/P4 and P5/P6 were also sequenced, and the sequencing results verified the primer pairs can obtain the accurate sequence fragments of *P. ginseng* and *P. quinquefolius* respectively, so they can be successfully applied to identification.

The sensitivity and specificity test of nested PCR

Sensitivity test results showed that both *P. ginseng* and *P. quinquefolius* can be identified, when their DNA template amount is from 0.1 ng to 10 ng (Fig. 4C). Specificity test results were shown in Fig. S2−S3, it successfully detected that the intentional adulteration of *P. ginseng* and *P. quinquefolius* was as low as 1% at 10 ng level of total DNA respectively. These results illustrated that our molecular authentication method is sensitive and specific enough when the DNA is as low as 0.1 ng.

Molecular authentication of P. ginseng and P. quinquefolius

As shown in Fig. 5A, all the *P.* ginseng cultivars collected from different areas (in Table 1), produced specific amplicons of 571 bp, whereas the *P. quinquefolius* cultivars generated amplicons of 341 bp. Thus, these two species could be clearly distinguished and identified.

In order to verify the feasibility of this method to ginseng products in the market (in Table 2), 11 commercial ginseng products were tested (Fig. 5B). All samples had the specific electrophoretic band respectively. The SNP markers PCR results of all the products were consistent with commodity description except two American ginseng tea II and III, 5th and 11th samples. Their PCR results showed that they were *P. quinquefolius* mixed with *P. ginseng*. The above chemical analysis has also detected a problem on the 5th sample, which only proved the source of 5th sample is not American ginseng, but it cannot tell us its composition of raw materials or adulterants. Furthermore, the chemical analysis results didn't detect the adulteration of 11th sample while the genetic SNP markers found it. So the nested PCR method based on SNPs in *DS* gene was highly sensitive to detect trace adulteration in ginseng products, which might be easily ignored by chemical analysis method, and its feasibility and effectiveness were also confirmed. Therefore, we were able to conclude that nested PCR can be used to specifically identify *P. ginseng* and *P. quinquefolius*.

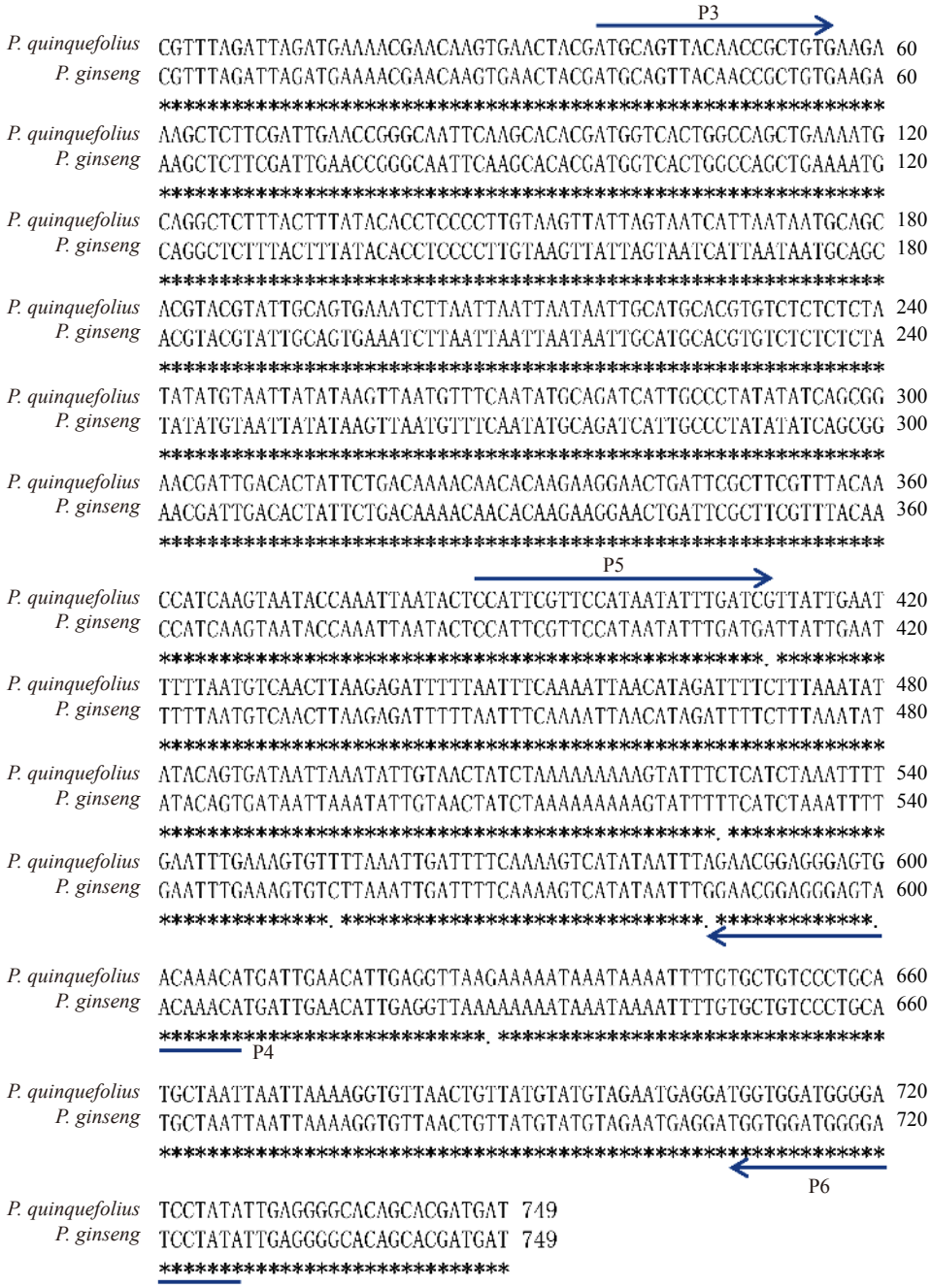

Fig. 3 SNP loci and specific primers design sites on *P. ginseng* and *P. quinquefolius DS* fragments

Discussion

Research strategy of SNPs of functional genes in medicinal plants

Molecular identification method before are mainly focused on proving it to be *P. ginseng* or *P. quinquefolius*, but it cannot distinguish them when they are mixed up [19]. There are reports about the molecular identification methods using 18S, ITS and *mat*K [14, 20, 21], which are easy to evolve and mutate.

At present pharmaceutical botany, herb genome program is still in progress [22]. Although some wide-genome data of medicinal plants have been reported, most of the medicinal plants are still lacking whole-genome data, so it's difficult to get objective genetic information. In this study, we don't need to know the genome information, and the cDNA sequence information was just needed to design the segmented primers. Specific and strong representative SNPs were discovered by the steps of gene amplification, sequencing and alignment. Then we can get rich and accurate SNP information, and it's a more economical process way to develop molecular identification markers in functional genes than to sequence the whole genome.

The importance of functional gene SNP studies

Because of low mutation rate, SNP in *DS* gene could be

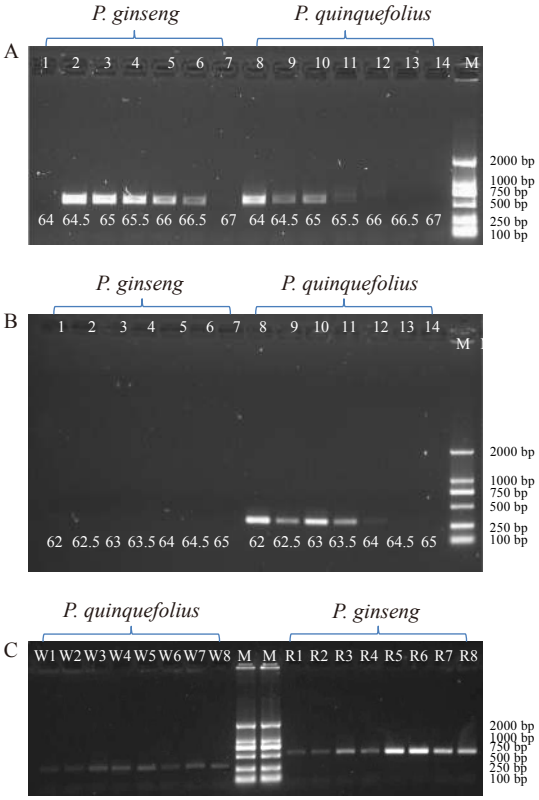

Fig. 4 PCR products of specific primers under different annealing temperatures and different amount. (A) PCR products of primer pair P3/P4 under different annealing temperatures. Lanes 1−7: *P. ginseng* PCR products from 64 to 67 °C. Lanes 8−14: *P. quinquefolius* PCR products from 64 to 67 °C. (B) PCR products of primer pair P5/P6 under different annealing temperatures. Lane 1−7: *P. ginseng* PCR products from 62 to 65 °C; Lane 8−14: *P. quinquefolius* PCR products from 62 to 65 °C. (C) PCR products of different amount of *P. ginseng* and *P. quinquefolius*. Lanes W1−W8: PCR products of 0.1, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 ng of *P. quinquefolius*. Lanes R1−R8: PCR products of 0.1, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 ng of *P. ginseng*. Lane M: D2000 DNA ladder

identification marker with high stability. As one of our research directions is to find the SNPs closely linked to chemical ingredients through correlation analysis of SNP and ginsenosides, in our previous study, SNPs in *DS* had a close relationship with the accumulation of bioactive ginsenoside Rf, Rg1 and F1 [17]. It shows huge potential for SNPs in functional gene to be candidate markers for rapid identification, quality assessment, and breeding selection, without positive standard sample in the detection process. It is also possible to establish a database of molecular markers in functional genes, which may provide more information and evidence for medicinal plants authentication and qualitative evaluation of relevant active ingredients.

A combination of chemical and molecular analysis gives a comprehensive authentication for ginseng products

In our study, 11 ginseng products were tested by chemical and molecular methods. For chemical method, it can evaluate the source of ginseng products to tell whether it matches the commodity description by the established model of ginsenosides content, but it cannot exactly tell the information of adulterants. For molecular method, it can both detect the adulteration and uncover the adulterant. In the first round PCR, molecular analysis can tell whether the sample contains ginseng herbs, and in the second round PCR, it can discover adulteration. Chemical method gives more detailed information than molecular method about bioactive ginsenosides to make sure there are accurate and enough effective components for health care and treatment. Molecular method proves to be faster and more sensitive than chemical method, which needs less time and data to detect and analyze. In addition, molecular way can also obtain gene information to evaluate medicinal materials quality. To select chemical or molecular method depends on our purposes, and the combination of chemical and molecular methods can provide comprehensive evaluation for ginseng products.

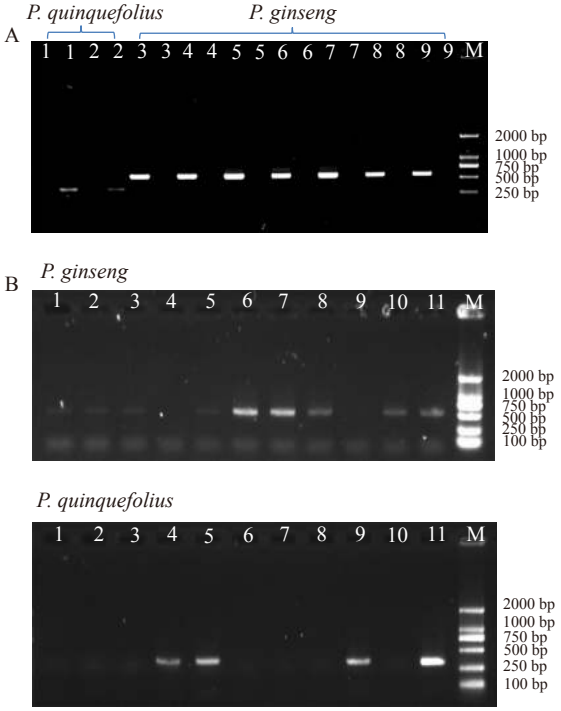

Fig. 5 Genetic authentication results of ginseng herbs and products samples based on *DS* gene SNP markers by nested PCR. (A) Electrophoresis results of PCR products from ginseng herbs. Lanes 1−2: *P. quinquefolius*, Lane 3: Biantiao, Lane 4: Damaya, Lane 5: Fuxingyihao, Lanes 6−7: Damaya under forest, Lanes 8−9: Shizhu. (B) Electrophoresis results of PCR products from ginseng products. Lane 1−3: ginseng bolus I−III, Lane 4, 5 and 11: American ginseng tea I, II and III, Lane 6 and 8: Red ginseng tea I and II, Lane 7: White ginseng tea, Lane 9: American ginseng tablets, Lane 10: Red ginseng drink. Lane M: D2000 DNA ladder

SNP markers provide effective integration of authenticity and quality evaluation

Although a growing number of molecular biology techniques have been applied to identify Chinese medicine variet-

ies, most of studies chose non-functional gene as target, such as ITS2 [10], ETS [11], and ESTs [12]. These methods can identify the authenticity of medicines, but cannot evaluate their quality. It is worth mentioning that there are technical challenges in our mentioned identification method about identifying the hybrid products of Asian ginseng and American ginseng. However, our method provides a rapid detection technique for identifying hybrid seedlings, which may effectively prevent the threatening of large-scale hybrid seedlings in the future. Furthermore, SNPs in functional gene coding regions may lead to morphological changes [23, 24], but SNPs in the noncoding genes cannot, thus the strong genetic stability in functional genes makes the test more accurate. In particular, as guanine is the most easily oxidized base among the four DNA bases, and *DS* gene has lower proportion of guanine than ETS and ITS genes, single-stranded DNA (ssDNA) of *DS* gene should have more resistance to oxidative damage and isomerization, which is shown in Fig. 6 [25]. Theoretically, when testing the DNA from samples treated with various preparation processes, identification by *DS* gene may have higher expectation of success.

Published study has found that SNP markers are phytochemical biosynthesis-related [26]. Compared with the previously developed SNP markers, the SNP markers in functional gene *DS* was first used to identify the ginseng plants and ginseng merchandises successfully, which also has a potential relationship with the content of active ingredients and pharmacological efficacy. It offers a new idea to establish a method for faster and more accurate molecular identification of Chinese herbal medicines, and also provides a solid foundation to further explore the quality assessment through functional genes, as well as gives more accurate evidence to ensure the safe and effective use of Chinese medicines for diseases treatment and health caring purposes.

Conclusion

In this study, 6 novel SNP markers were found in *P. ginseng* and *P. quinquefolius DS* gene, and they were used to authenticate *P. ginseng* and *P. quinquefolius* fresh samples and their commercial products successfully. This was a first attempt to explore new type of SNP markers in functional genes related to bioactive components ginsenosides biosynthesis, which could realize variety authentication and function regulation at the same time. Chemical method using OPLS-DA model based on 18 ginsenosides could also be applied to identify the sources of raw ginseng materials, while molecular method using SNPs showed higher sensitivity and specificity than it. The combination of chemical and molecular discrimination methods provided the comprehensive evaluation and accurate authentication of the sources for ginseng herbs and products.

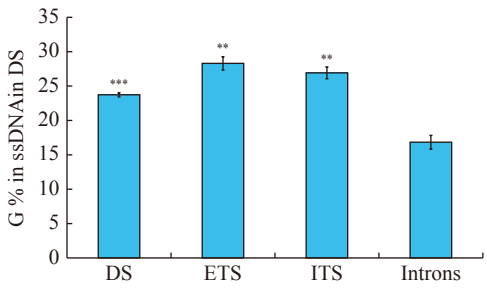

Fig. 6 The proportion of guanine in *DS*, ETS and ITS ssDNA. Data are expressed as mean ± SD ($n > 6$), $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* Introns

Supporting Information

Supporting information of this paper can be requested by sending E-mails to the corresponding author.

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