Research Article

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Detection of *Panax quinquefolius* in *Panax ginseng* using 'subtracted diversity array'

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Abstract

BACKGROUND: Food adulteration remains a major global concern. DNA fingerprinting has several advantages over chemical and morphological identification techniques. DNA microarray-based fingerprinting techniques have not been used previously to detect adulteration involving dried commercial samples of closely related species. Here we report amplification of low-level DNA obtained from dried commercial ginseng samples using the Qiagen™ REPLI-g® Kit. Further, we used a subtracted diversity array (SDA) to fingerprint the two ginseng species, *Panax ginseng* and *Panax quinquefolius*, that are frequently mixed for adulteration.

RESULTS: The two ginseng species were successfully discriminated using SDA. Further, SDA was sensitive enough to detect a deliberate adulteration of 10% *P. quinquefolius* in *P. ginseng*. Thirty-nine species-specific features including 30 *P. ginseng*-specific and nine *P. quinquefolius*-specific were obtained. This resulted in a feature polymorphism rate of 10.5% from the 376 features used for fingerprinting the two ginseng species. The functional characterization of 14 *Panax* species-specific features by sequencing revealed one putative ATP synthase, six putative uncharacterized proteins, and two retroelements to be different in these two species.

CONCLUSION: SDA can be employed to detect adulterations in a broad range of plant samples.

Keywords: subtracted diversity array; suppression subtractive hybridization; DNA fingerprinting; *Panax ginseng*; *Panax quinquefolius*; adulteration

INTRODUCTION

Panax ginseng (Chinese ginseng) and *P. quinquefolius* (American ginseng) of the family Araliaceae are important functional foods. These two ginsengs are used for disease treatment.[1] *P. quinquefolius* is widely used to regulate blood sugar levels,[2] whereas dried roots of *P. ginseng* are used to treat stress[3] and fatigue.[4] The dried commercial roots of these two species are highly similar in morphological appearance.[3] Therefore, these two ginseng products are often substituted for each other in the market. Since *P. ginseng* is more expensive than *P. quinquefolius*, people deliberately mix them to fetch a higher income in Korea.[5] Consequently, the illegal practice of disguising *P. quinquefolius* as *P. ginseng* has become a common problem in recent years.[3] Adulteration like this often dramatically reduces the efficacy/value of *P. ginseng*. Therefore, accurate identification procedures are needed for discriminating these two ginsengs to prevent such instances in future.

Classically, medicinal plants were identified by morphological characteristics. However, as it is difficult to distinguish between certain species purely based on morphology, chemical and molecular identification techniques were developed to complement morphological identification. A limitation of chemical analysis techniques is that the chemical composition of these plants varies with environmental effects such as harvest season, plant origin and drying procedure.[6] Species identification using genetic diversity is more reliable as genomic information is more specific and does not readily change with environmental factors.[7,8] Therefore, DNA-based fingerprinting, e.g. random amplified polymorphic DNA (RAPD) or random-primed polymerase chain reaction (RP-PCR),[9,10] amplified fragment length polymorphism (AFLP)[1] and PCR restriction fragment length polymorphism (PCR-RFLP)[3,11] were developed to discriminate *P. ginseng* species. However, all these techniques are PCR-based and require agarose gel electrophoresis, which is a time- and labour-intensive process. Therefore they are not suitable for large-scale genotyping operations.[12,13] Further, damaged DNA, such as that obtained from a dried plant, can affect PCR-based identification techniques.[14,15]

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Recently, high-throughput microarray-based techniques have been used to successfully discriminate closely related species.[13,16] There are some good reviews that discuss the authentication of herbal species using various fingerprinting techniques.[17,18] Besides, an overview of some recent patents that cover the extraction of DNA from medicinal materials, the amplification of DNA using improved reaction conditions, the generation of DNA sequences and fingerprints, and the development of high-throughput authentication methods is available.[19] However, none of those have been employed to fingerprint the adulteration of dried plant tissues that are commercially sold. Previously, we reported a novel sequence-independent microarray, the subtracted diversity array (SDA), that successfully differentiated the six main clades of angiosperms[20] and correctly clustered nine flowering plants at the family level.[21] This technique was found to be superior to other sequence-independent microarrays like diversity array technology (DArT™) and suppression subtractive hybridization (SSH)-based arrays, because it does not require multi-sequencing and multi-SSH. The current study first investigates the sensitivity of SDA to fingerprint *P. ginseng* and *P. quinquefolius* from dried roots (as commercially sold). Secondly, we test the sensitivity of this array to detect a deliberate adulteration of *P. quinquefolius* DNA in *P. ginseng* DNA.

MATERIALS AND METHODS

Dried commercial samples

Dried roots of *P. ginseng* and *P. quinquefolius*, as they were commercial sold, were purchased from Min Wei Huang TCM Clinic in Melbourne, Victoria, Australia. The Chinese pharmacist verified all the specimens. These herbal samples have been deposited at the Medicinal Plant Herbarium, Southern Cross University, Lismore, NSW, Australia. Their voucher numbers are PHARM10011 (*Panax quinquefolius*) and PHARM10012 (*Panax ginseng*).

Genomic DNA preparation

Genomic DNA was extracted from the tissues of all specimens using the modified Cetyltrimethyl Ammonium Bromide (CTAB) method.[22] Briefly, 1 g dried herbal plant sample was rinsed sequentially with double-distilled water and 70% ethanol for 3 min each before pulverization in liquid nitrogen. The powdered sample was suspended in DNA washing buffer (250 mmol L^{-1} Tris-HCl (pH 8.0), 50 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 250 mmol L^{-1} NaCl) at 0 °C for 10 min. The solution was centrifuged at 7000 rpm for 10 min, and the pellet was subsequently resuspended with 15 mL DNA extraction buffer (100 mmol L^{-1} Tris–HCl (pH 8.0)), 20 mmol L^{-1} EDTA (pH 8.0), 1.4 mol L^{-1} NaCl, 2% polyvinyl pyrrolidone, 3% CTAB). The mixture was incubated at 55 °C for 30 min with occasional shaking and subsequently centrifuged at 7000 rpm for 10 min. The supernatant was extracted using the same volume of chloroform–isoamyl alcohol (24 : 1). The mixture was then centrifuged at 7000 rpm for 10 min at room temperature. This extraction process was repeated twice. The upper aqueous portion was precipitated with 2.5 volumes of ice-cold absolute ethanol, 0.1 volume of 7.5 mol L^{-1} ammonium acetate and 1.4 volumes of 5 mol L^{-1} sodium chloride at −20 °C for 60 min. After centrifugation at 9000 rpm for 25 min, the DNA pellet was washed with 70% ethanol, air dried, and suspended in 200 µL TE buffer (10 mmol L^{-1} Tris-HCl, pH 8.0, 1 mmol L^{-1} EDTA, pH 8.0). Subsequently, 2 µL RNase (1 mg mL^{-1}, Fermentas, Burlington, Ontario, Canada) was added, the solution was incubated at 37 °C for 15 min and transferred to a mini spin column (Qiagen, Sydney, Australia), followed by centrifugation at 8000 rpm for 1 min. Finally, 100 µL elution buffer (Qiagen) was transferred to the mini spin column and followed by centrifugation at 8000 rpm for 1 min to elute the DNA.

Genomic DNA amplification

Considering that the quantity of genomic DNA isolated from the dried root samples was too low to be used directly for target preparation, the Qiagen™ REPLI-g® Kit was employed to amplify the whole genomic DNA. This commercial kit contains DNA polymerase, buffers, and reagents for whole-genome amplification from small samples using multiple displacement amplification (MDA).[23] 50 ng DNA was used as template and subsequently amplified according to the manufacture's guidelines (Qiagen™ REPLI-g® Mini/Midi Kit manual). The amplified DNA samples were stored at 4 °C prior to use. The genomic DNA was quantified reading the absorbance at 260 nm and its purity was determined by the ratio of absorbance at 260 nm and 280 nm. Integrity of DNA was verified by 1.5% (w/v) agarose gel electrophoresis.

Construction of subtractive diversity array (SDA)

Construction of the SDA array was detailed in a previous study.[20] Briefly, the genomic DNA pool of five representative non-angiosperm species was subtracted from the genomic DNA pool of 49 representative angiosperm species. The subtraction was performed using a PCR-Select™ cDNA Subtraction Kit (Clontech, Mountain View, CA, USA) with some modifications. 376 subtracted fragments were cloned and PCR amplified for microarray printing. Four positive controls consisting of 'housekeeping' cDNA sequences sourced from a cDNA library of the angiosperm *Cicer arietinum* were also included.[24] Negative controls consisted of printing buffer, nested PCR primers 1 and 2R (Clontech) and pGEM®-T Easy vector (Promega, Fitchburg, WI, USA) double-digested with *Alu*I and *Hae*III. The SDA with these 384 features (376 subtracted fragments and eight controls) was printed in a 32 × 12 format as described.[20]

Target preparation and DNA hybridization

Target preparation and DNA hybridization were performed as described by us previously, with slight modifications.[20] Briefly, 1.5 µg DNA from each ginseng sample was digested with 5 units of *Alu*I and *Hae*III (Fermentas, Ontario, Canada) at 37 °C overnight, purified with the Qiagen® PCR Purification Kit, and subsequently labelled with biotin using the DecaLabel™ DNA Labeling Kit (Fermentas) according to the manufacturer's guidelines. The SDA slide pre-hybridization, hybridization of biotin-labelled targets, and detection using FluroLink™ Streptavidin-Cy3 (Amersham Pharmacia, Buckinghamshire, UK) kit was performed as described.[20]

Quality control

The hybridization pattern was determined by scanning each slide in a ScanArray Gx microarray (PerkinElmer, Waltham, MA, USA) at 543 nm. The slides were scanned at a resolution of 20 µm and 60% photomultiplier gain. After scanning, signals of the features were quantified using an 'easy quant', in which the 'lowess' (locally weighted linear regression) normalization method was chosen. Then, to facilitate uniform comparison between species, the signal data were transformed into binomial data. The signal intensities

of the features were calculated manually by removing those of the background. The results were subsequently used to calculate the signal-to-background ratios.

Spots with a signal-to-background ratio of ≥ 2.0 were considered to represent positive spots, and their values were converted to one. Spots with a signal-to-background ratio of <2.0 were considered based on their values converted to zero. Only those features with 'good' spots of consistent classification (positive or negative) in all six replicates of each hybridization were accepted for data analysis. Subsequently, the six technical replicates for each spot were combined as either a positive (1) or negative (0) spot.

Statistical analysis

As mentioned previously, a total six replicates, including three technical replications (three subarrays with the same target) for each of the two biological replications (separately synthesized target) were used for each sample. The resulting binary data were examined using SPSS v.15.0 (SPSS Inc., Chicago, IL, USA) for phylogenetic relationships by constructing a dissimilarity dendrogram using Pearson correlation and hierarchical cluster analysis with between-groups linkage. The dendrogram showing the phylogenetic relationships among the ginseng species was subsequently compared to that predicted by the Angiosperm Phylogeny Group II (APG II) classification.[25] The APG II system was constructed using conventional morphological information, and recently has been reinforced by gene sequencing and PCR-based tools.

DNA sequencing

One hundred and fifty-two DNA clones were randomly selected. The DNA clones with inserts of between 250 and 1000 bp were amplified by PCR reaction in a 25 μL system as follows: 2.5 μL 10× PCR buffer, 0.5 μL deoxynucleoside triphosphate (dNTP) mix (10 mmol L^{-1} of each dNTP), 1.25 μL of 50 mmol L^{-1} $MgCl_2$, 0.5 μL of 10 μmol L^{-1} T7 and SP6 primer (Clontech) each, and 0.125 μL *Taq* DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). The amplification program began with an incubation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, and extension at 72 °C for 45 s, followed by a final extension cycle of 10 min at 72 °C. Subsequently, all amplified products were subjected to single-pass sequencing from the 5′ end of the vector using BigDye™ Terminator chemistry (Applied Biosystems, Scoresby, Australia) according to the manufacturer's instructions. Sequencing was conducted by the Macrogen Company (Seoul, Korea) and Micromon DNA sequencing (Monash University, Melbourne, Australia).

Sequence analyses

Poor-quality sequence reads were removed manually. Each independent DNA sequence was generated by deleting the adapter and primer sequences (size of length is over 50–60 bp). Functional characterization was performed using BlastX by comparing each sequence with existing entries in the SwissProt® and SpTrEMBL® databases. Database hits were ranked by the expectation (*e*) value. A hit was regarded as a 'significant match' to the input sequence if $e \leq 1 \times 10^{-10}$. DNA sequences were regarded as a 'good' match if the e value was in the range $1 \times 10^{-5} \geq e > 1 \times 10^{-10}$, or a 'poor' match when $0.02 \geq e > 1 \times 10^{-5}$. DNA sequences were characterized as 'unknown' if *e* values were <0.02. DNA sequences were described as 'no hits' if no match was found in the databases.

Automatic flagging eliminated empty spots. Spots with a signal-to-background ratio of ≥ 2.0 were considered to represent positive spots, and their values were converted to one. Spots with a signal-to-background ratio of <2.0 were converted to zero. Only those features with 'good' spots of consistent classification (positive or negative) in all six replicates of each hybridization were accepted for the data analysis. Subsequently, the six replicates for each spot were combined as either a positive (1) or negative (0) spot. The polymorphic features were determined by comparing the values of the same features in the hybridizations of the dried herbal plants. A feature was classified as 'non-polymorphic' if their values were consistent for all hybridizations, whereas it was classified as 'polymorphic' if the values varied.

Discrimination of a deliberate adulteration

1.35 μg DNA isolated from *P. ginseng* was deliberately contaminated with 0.15 μg DNA isolated from *P. quinquefolius*, producing a 1.5 μg sample with a 9 : 1 purity ratio. The DNA was double-digested, labelled, and subsequently hybridized with the SDA. The hybridization pattern was subsequently compared with those of the two ginsengs.

RESULTS AND DISCUSSION

Amplification of DNA

The Qiagen™ REPLI-g® Midi Kit successfully amplified the small quantities of DNA isolated from the dried ginseng roots, which was evidenced by the increased intensity of EtBr-stained bands. A260/A280 absorbance ratios of 1.91 and 1.83 indicated that these amplified DNA samples were of high purity. The successful restriction digestion and clear hybridization patterns (Fig. 1) indicated that the final amplified DNA was of sufficiently high quality for the fingerprinting experiment. The successful fingerprinting in this study provides a new method for target or probe preparation for microarray-based fingerprinting of dried plant tissues.

Polymorphism between ginseng species

SDA generated a polymorphism rate of 10.5% from the fingerprinting of the two closely related *Panax* species. After removing 11 flagged features from the data analysis, 39 species-specific features, including 30 *P. ginseng*-specific and nine *P. quinquefolius*-specific features, were obtained (Fig. 1 and Table 1), and this resulted in a feature polymorphism of 10.5% for this fingerprinting. This polymorphism rate is similar to that of the 3–17%[13,16,26,27] reported in previous DArT™ studies, but is lower than that in the previous SDA study.[20,21] However, the current fingerprinting was performed at a different level from the SDA-based fingerprinting of angiosperms.[20,21] This technique was used to discriminate the six main clades of angiosperms and species representing different families, while the present fingerprinting was used to discriminate closely related species belonging to the same genera. Also, those authors used fresh leaf tissues as materials[20,21] whereas this study reports fingerprinting using dried root samples. The DNA recovered from dried root tissues is of poor quality and integrity compared with that obtained from fresh leaves, thus affecting optimal hybridization.

Sequencing results

Fourteen species-specific features (Table 2) were sequenced, including 11 *P. ginseng*-specific and three *P. quinquefolius*-specific features. Sequences of nine features significantly matched

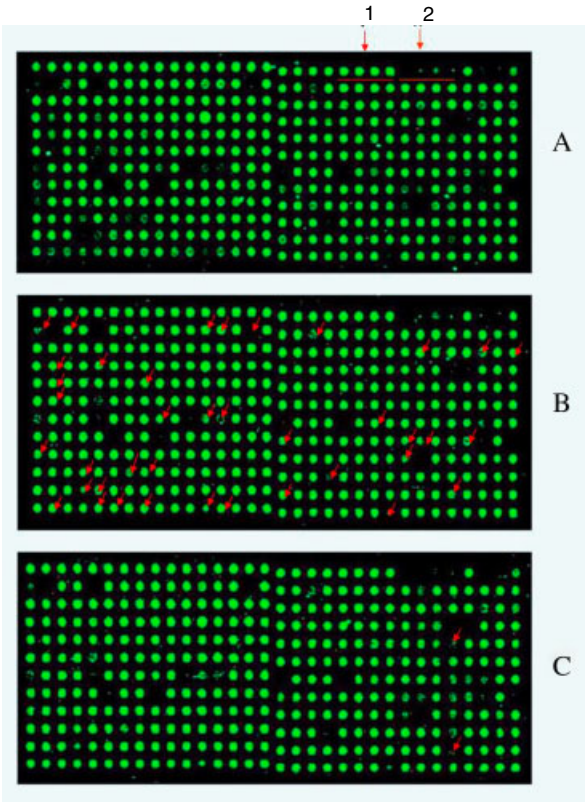

Figure 1. Hybridization patterns of *Panax* species obtained using SDA. (A) *Panax quinquefolius* (1, positive controls; 2, negative controls). (B) *Panax ginseng* (39 polymorphic). (C) *Panax ginseng* DNA with 10% *Panax quinquefolius* DNA (two polymorphic features are marked).

to the entries in the existing databases, with one identified as 'ATP synthase subunit beta (EC 3.6.3.14) (fragment)', six matched to 'putative uncharacterized protein', while the other two were retroelements i.e. 'putative retroelement integrase' and 'retrotransposon protein (putative, Ty3-gypsy subclass)'. Additionally, one sequence poorly matched to 'polyphenolic adhesive protein 1-like protein', three obtained 'no hits' in the databases, while the remaining one was classified as 'unknown'. The new sequences reported in the present study will contribute to the existing entries in the database. Also, these sequences may be suitable for developing sequence-characterized amplified region (SCAR) markers to facilitate the quick discrimination of the two ginseng species in future studies.

Detecting deliberate adulteration of *P. ginseng*

We compared the hybridization patterns of the *P. ginseng*, *P. quinquefolius* and the deliberately adulterated sample (9 : 1 proportion of *P. ginseng* and *P. quinquefolius* DNA). The deliberately adulterated sample could be successfully discriminated from the two ginseng samples. Thirty-nine species-specific features (Fig. 1) between the hybridizations of the two *Panax* species contributed to the differentiation of *P. ginseng* and *P. quinquefolius* (Fig. 2). From these, the pure *P. ginseng* DNA sample and the deliberately adulterated sample could be discriminated because of two polymorphic features (Fig. 1). Therefore, the sensitivity of the SDA was not only efficient for differentiating the two *Panax* species but also for differentiating a small level of adulteration (10% DNA) of *P. quinquefolius* in the pure DNA of *P. ginseng*.

Table 1. Average fluorescent intensity of polymorphic features discriminating the two ginseng species

Clones	*Panax ginseng*	*Panax quinquefolius*	Specific for
A3	4.44	1.84	*P. ginseng*
A6	3.41	1.57	*P. ginseng*
A8	4.39	1.89	*P. ginseng*
A10	4.17	1.63	*P. ginseng*
A21	4.83	1.77	*P. ginseng*
A29	1.52	2.17	*P. quinquefolius*
A43	1.99	3.23	*P. quinquefolius*
A53	4.76	1.86	*P. ginseng*
A115	0.65	2.34	*P. quinquefolius*
A117	3.45	1.99	*P. ginseng*
A125	2.84	1.98	*P. ginseng*
A129	2.97	1.68	*P. ginseng*
A131	3.65	1.68	*P. ginseng*
A145	4.82	1.89	*P. ginseng*
A149	4.83	1.993	*P. ginseng*
A153	3.65	1.49	*P. ginseng*
A154	4.40	1.70	*P. ginseng*
A155	4.99	1.98	*P. ginseng*
A157	3.71	1.99	*P. ginseng*
A167	4.32	1.86	*P. ginseng*
A169	4.22	1.82	*P. ginseng*
A171	4.83	1.99	*P. ginseng*
A176	3.38	1.50	*P. ginseng*
A193	3.64	1.60	*P. ginseng*
A197	4.16	1.73	*P. ginseng*
A206	3.17	1.48	*P. ginseng*
A212	2.89	1.69	*P. ginseng*
A215	4.47	1.823	*P. ginseng*
A244	0.02	9.85	*P. quinquefolius*
A245	0.58	5.99	*P. quinquefolius*
B19	1.52	4.83	*P. quinquefolius*
B72	3.01	1.80	*P. ginseng*
B83	3.83	1.95	*P. ginseng*
B116	2.48	1.66	*P. ginseng*
B131	1.80	3.70	*P. quinquefolius*
B136	1.20	2.63	*P. quinquefolius*
B199	2.73	1.96	*P. ginseng*
B224	3.64	1.74	*P. ginseng*
B240	1.05	2.43	*P. quinquefolius*

Interestingly, the two ginseng species investigated here were not included while doing the original DNA subtraction reaction to develop the SDA.[20] In previous studies, SDA correctly clustered the medicinal species used in the initial genomic DNA pool.[20] In the present study, *P. ginseng* and *P. quinquefolius* (both not used in the creation of the angiosperm pool) were successfully fingerprinted, indicating that SDA may be suitable for extensive fingerprinting of medicinal and functional food species.

Advantages of SDA

Use of SDA provided a number of advantages compared with previous reported sequence-independent microarrays. Suppression subtractive hybridization (SSH) has been previously employed to construct a microarray to fingerprint plants.[28,29] However, these SSH-based arrays were costly and labour intensive because they employed multiple SSH (all species to be discriminated were

Table 2. Identities of species-specific features for the two *Panax* species revealed by SDA

Clone	Length	Specific for species	Accession number for the Genbank	Matching entry (in SwissProt + SpTrEMBL)	Description	*e* value
B240	353	*P. ginseng*	HM038452	Q8S8M1_ARATH	Putative retroelement integrase	5×10^{-20}
A215	374	*P. ginseng*	HM038449	A8NMG1_BRUMA	Putative uncharacterized protein	6×10^{-17}
A167	729	*P. ginseng*	HM038447	Q2R3T2_ORYSJ	Retrotransposon protein, putative, Ty3-gypsy subclass	1×10^{-22}
B83	300	*P. ginseng*	HM038451	A7YXM6_9ALVE	Polyphenolic adhesive protein 1-like protein	0.0002
A117	247	*P. ginseng*	HM038446	Q3HKA5_TOBAC	Putative uncharacterized protein	2×10^{-21}
A3	404	*P. ginseng*	HM038441	A5BK92_VITVI	Putative uncharacterized protein	4×10^{-18}
A8	339	*P. ginseng*	HM038443	Q9RXQ4_DEIRA	Putative uncharacterized protein	8×10^{-19}
A6	292	*P. ginseng*	HM038442	File contains no hits		
A21	249	*P. ginseng*	HM038444	File contains no hits		
A29	271	*P. ginseng*	HM038445	File contains no hits		
A171	247	*P. ginseng*	HM038448	Q3HKA5_TOBAC	Putative uncharacterized protein	2×10^{-22}
A212	374	*P. ginseng*	HM038450	A8NMG1_BRUMA	Putative uncharacterized protein	6×10^{-17}
A43	338	*P. quinquefolius*	HM038453	A7SHI0_NEMVE	Predicted protein	Unknown
B131	173	*P. quinquefolius*	HM038454	Q9XQZ3_9MAGN	ATP synthase subunit beta (EC 3.6.3.14) (fragment)	4×10^{-98}

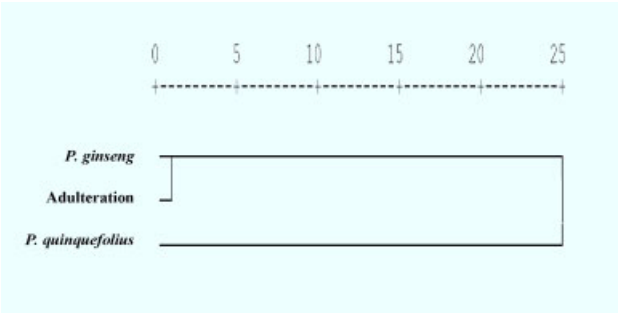

Figure 2. Hierarchical dendrogram for hybridization patterns of ginseng species (with and without adulteration) using signal intensity mean with threshold ratio 2.0. The steps of the dendrogram show the combined clusters and values of distance coefficients at each step; the values have been rescaled to numbers between 0 and 25, preserving the ratio of the distances between the steps.

subtracted from each other). Diversity array technology (DArT™) has been used to find species-specific spots between closely related species.[26,27] However, the use of DArT™ arrays has been limited to studying the species used to generate the arrays.[30,31] In comparison, our SDA employed a single SSH involving two pools of species representing angiosperms and non-angiosperms.[20] This broad subtraction employed in SDA made it possible to differentiate between closely related species that were not even used in the subtraction pooling. This feature of SDA makes it considerably more efficient and cost effective compared with previous sequence-independent microarrays. Further, the use of frequent cutting restriction enzymes like *Alu*I and *Hae*III that recognize 4 bp sequences has an added advantage compared to the 6 bp cutters used to generate DArT™ arrays. The 4 bp cutters generated an increased number of shorter fragments compared with 6 bp cutters, resulting in an increased polymorphism rate.[21]

Sensitivity of SDA compared to previous ginseng fingerprinting methods

The sensitivity of this SDA cannot be directly compared with previous techniques used to fingerprint ginseng samples. The possible reasons are that different techniques and contaminants have been used in these studies. For instance, a small level of adulteration (10%) of *P. ginseng* as a contaminant in *P. quinquefolius* samples was detected using the RFLP profiles.[32] Considering that the SDA in the present study was used to fingerprint the opposite adulteration, i.e. a *P. ginseng* DNA sample contaminated with 10% *P. quinquefolius* DNA, valid sensitivity comparisons between the techniques used to differentiate adulterations in these studies cannot be made.

Even though a limited number of polymorphic features were obtained, the sensitivity of SDA was sufficient for fingerprinting the two ginseng species from dried root tissues. Moreover, it successfully detected a 10% adulteration of pure *P. ginseng* DNA samples. We did not test any lesser amount of adulteration to check the limit of sensitivity of this array. However, the number of polymorphic features is predicted to be decreased when a lower level of substitution is used, and this may lead to an insufficient discrimination of the substitution. Therefore, we propose that the sensitivity of SDA may be limited to detecting 10% adulteration of *P. ginseng* samples.

CONCLUSIONS

The quality of DNA extracted from dried root tissues and amplified using the Qiagen™ REPLI-g® Kit was sufficient for microarray

fingerprinting of the two ginseng species. The sensitivity of SDA was sufficient to detect even a low level (10%) of *P. quinquefolius* DNA in *P. ginseng* DNA. Further work is required using SDA to differentiate a greater range of closely related species and samples from the same species derived from different locations and processed and/or stored under different conditions in order to more accurately evaluate the utility of this method for commercial application in ginseng quality control. To do this, some improvements in the method in order to increase the sensitivity and discrimination level of SDA may be necessary.

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