JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of 3′ Untranslated Region (UTR) Sequence-Based Amplified Polymorphism Analysis in the Rapid Authentication of *Radix astragali*

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Radix astragali (root of *Astragalus membranaceus*) is an important traditional Chinese medicine. It has been used as a tonic herb for thousands of years in China. The water extract of the roots has a wide range of immunopotentiating effects and has been proven to be efficacious as an adjunct cancer therapy. Authentication of the herbal plant is routinely required for general practice in the field of herbal medicine. To facilitate rapid identification of numerous varieties of *Radix astragali* that are circulating on the herb markets, a rapid molecular genetic method, named 3′ untranslated region (3′ UTR) sequence-based amplified polymorphism (UAP), has been developed. A cDNA library was first built from transcripts of an authentic *A. membranaceus* species. Several cDNA clones specific to *A. membranaceus* were identified through subtractive hybridization of the *A. membranaceus* cDNA library with *Arabidopsis* total cellular RNA. On the basis of these cDNA sequences of the 3′ untranslated region (3′ UTR) of selected cDNA clones, a Polymerase Chain Reaction (PCR) was performed on genomic DNAs of the dry roots of several putative *A. membranaceus*. PCR fragment length polymorphism was found between *A. membranaceus* and its relatives. By using this method, it was possible to differentiate the authentic *A. membranaceus* root from those putative ones obtained from herbal medicine markets. To the authors' knowledge, this is the first paper applying UAP in the authentication of traditional Chinese medicine plants.

KEYWORDS: 3′ UTR; UAP; authentication; *Radix astragali*; huang qi root

INTRODUCTION

Astragalus membranaceus is a member of the Leguminosae family. Its root (huang qi root or milkvetch root) has been used as a tonic herb in China for thousands of years. According to the theory of traditional Chinese medicine (TCM), the water extract of dried huang qi root is able to boost immunity, to delay the aging process, and to invigorate body energy. Phytochemical analysis has shown that this root in fact contains more than 40 different saponins, such as astragaloside, as well as flavonoids, amino acids, polysaccharides, and multiple trace minerals. These water extracts have been demonstrated to have broad anti-inflammatory effect (*1*−*3*). These medical research results clearly show that the extract of *Radix astragali* can enhance the motility of human spermatozoa (*4*), stimulate the release of growth hormone in pituitary cell culture (*5*), and delay senescence in human cells (*6*). Further clinical studies also indicated that *R. astragali* has cardioprotective effects (*7*). Recently, it has been considered as an alternative remedy for cancers (*8*−*11*), such as prostate carcinoma (*12*), lung cancer (*13*), and liver cancer (*14*) and even for AIDS and smoking cessation (*15*).

Given its broad medicinal use, huang qi root has become an important commodity in herb markets. Generally speaking, the authentic huang qi roots of medicinal use are normally processed from two legume cultivars, *Astragalus membranaceus* (Fisch.) Bunge and *Astragalus membranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge) (*16*). In contrast, adulterant huang qi roots are derived from closely related legumes or from other plant roots that have a close morphological resemblance to huang qi root. These adulterants, escalating in both domestic and intentional herbal markets, will severely curtail the efficacy of *R. astragali* in medicinal practices. To eradicate this problem, chemistry approaches, such as thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC), and gas chromatography (GC), have been developed in the past (*17*−*19*).

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10.1021/jf051334g CCC: $30.25
Published on Web 10/04/2005

To make the authentication process more efficient and reliable, DNA-based authentication methods including RAPD were later introduced (*20*, *21*). These molecular methods basically relied on the rapid isolation and sequencing of DNA fragments encoding ribosomal RNA genes such as the 5S rRNA spacer, internal transcribed spacer (ITS), or 18S rRNA (*22*−*24*). However, these methods become problematic if two closely related cultivars are subjected to analysis.

To circumvent the problem, we propose to study the DNA sequence variations that occur at the 3′ untranslated region (UTR) of a gene among *A. membranaceus* cultivars. The genetic variations at the 3′UTR have been reported for many genes from various organisms (*25*−*30*). Russell (*31*) once observed a sequence duplication at the 3′UTRs of the *BoLA-DQB* gene, and it was suggested that resolution of allele-specific features may be found from the analysis of these noncoding and flanking DNA sequences. These allele-specific DNA variations at 3′UTR may result in distinct PCR amplification profiles among species, subspecies, and cultivars. Therefore, the 3′UTR of a gene should be useful in establishing the polymorphism among a group of *A. membranaceus* cultivars, which in turn can be utilized in the authentication of dried huang qi roots. To evaluate the practicability of this approach, several cDNA clones were first isolated from a cDNA library of *A. membranaceus* (Fisch.) Bunge and sequenced completely or partially. The 3′UTR sequences of these randomly selected cDNA clones were used as molecular markers to establish the 3′UTR-based amplified polymorphism (UAP) among these putative *R. astragali* roots purchased from herb markets. Our results confirmed that UAP is a promising authentication method, which may be applied in the large-scale and rapid authentication of plant and animal materials for TCM use.

MATERIALS AND METHODS

Plant Materials. *A. menbranaceus* (Fisch.) Bunge. (Fam. Leguminosae) was obtained from Nanjing Botanical Garden of Jiangshu Province; *A. membranaceus* (Fisch.) Bunge. var. *mongholicus* (Bunge.) Hsiao was from the herb garden of the Beijing Institute of TCM. Four commercially available putative *R. astragali* dry roots were obtained from Nanjing, Jiangshu Province, Inner Mongolia; Litan, Sichuan Province; and Linqiu, Shanxi Province, respectively.

Construction of cDNA Library and Identification of *A. membranaceus*-Specific cDNA. Total cellular RNA of *A. membranaceus* was extracted from the fresh roots using guanidinium isothiocyanate lysis buffer and cesium chloride ultracentrifugation (*32*), and the cDNA library was constructed by Invitrogen (Carlsbad, CA) in plasmid pCMV-SPORT6.1 and stored in DH10B-TonA cells.

Arabidopsis RNA was extracted using a RNeasy Mini Kit (QIAGEN, Valencia, CA), and its cDNA was prepared by using a SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA) and labeled by using the random primed DNA labeling method (*33*). The cDNA probes were then purified through a QIAquick Nucleotide Removal Kit (QIAGEN) and hybridized to an *A. membranaceus* cDNA library (*32*). The cDNA clones that failed to be hybridized by *Arabidopsis* total cellular RNA-dependent cDNA probes were selected. Consequently, the cDNA inserts in these recombinant clones were sequenced from both directions. Northern blot analysis was carried out as described (*34*). After mRNA was reverse transcribed using the SuperScript First-Strand Synthesis System (Invitrogen), real time (RT)-PCR was performed using the following cycling profile: 35 cycles of 94 °C for 1 min, 56−60 °C for 1 min, and 72 °C for 1 min. Primers are listed in **Table 1**.

Genomic DNA Extraction, PCR Amplification, and Sequencing. Genomic DNA was extracted from the ground powder of *R. astragali* by using a DNasey Plant Mini Kit (QIAGEN).

The PCR amplification of the *A. membranaceus* genomic DNA was performed in a 25 μL reaction mixture containing 30 ng of genomic DNA, 1× PCR buffer, 0.04 mM dNTPs, 1.5 mM $MgCl_2$, 5 pmol of primers, and 1 unit of Taq DNA polymerase using the following cycling profile: 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 56−60 °C for 1 min, and 72 °C for 1 min. Following amplification, the products were resolved on 1.5% agarose gel with ethidium bromide and visualized and photographed under UV illumination. Primers and annealing temperature used in different fragment amplification are listed in **Table 1**.

Table 1. Primers and Annealing Temperature Used in 3′ Untranslated Region Sequence-Based Amplified Polymorphism of *A. membranaceus*

clone	primer sequence (5′–3′)	annealing temp (°C)
AM12	AAA TAC CTG GTA AAA GGG AGA GAG AA TGA GGC GGT ACC ACG AGT TGA AGG	56
AM19	GAA TGA AAT TCC TCT ATT AAT GG GGA CCC CAA GAG ATG GCA TCG CC	60
AM22	AAC TTC ATG TCA ACA TCA ACT TGG TTG GGG TTC TTT GCA TCT CTG G	56
AM47	GCA AGC TTT GGG CAA GCC TC CTT CAA TGC GTG GTT CAT CTC C	56
AM48	CAA CAT TTT CAG TAA GAT GAC GC GGT GTT TAA GTT CAT CCT TGA AG	60
AM56	TAA ATA CAA CAC TTC TTT CAG TGA CCG AGC CCT TGG ATC CTA G	58
AM77	TTA ACG CCA TCA CAA CGT CC CGC ACG CTT GCC AAC TGG TCG	60
AM79	TTA AAC TTG AAA GAT AAG CAA C AGC CAG GAG TTT CGG AGC TGC TG	60

The *A. membranaceus* ITS region was amplified using a pair of primers: ITS1, 5′ CAC ACC GCC CGT CGC TCC TAC CGA 3′; ITS2, 5′ ACT CGC CGT TAC TAG GGG AA 3′ (*35*). These correspond to the conserved regions of plant 18S and 26S rDNA, respectively. The ITS band was excised from agarose gel, purified using GENECLEAN III (BIO 101, La Jolla, CA), and sequenced from both directions. The ITS sequences of root samples were analyzed by an Internet-based software called ClustalW (http://www.ch.embnet.org/software/ClustalW.html), and the phylogenetic tree was constructed using PHYLIP and displayed in Tree-View 1.6.6.

RESULTS AND DISCUSSION

Identification of *A. membranaceus*-Specific cDNA Fragments. The *A. membranaceus* cDNA library was first hybridized with the radioactively labeled *Arabidopsis* cDNA probes. It was believed that *A. membranaceus* cDNA clones, having lower homology to *Arabidopsis* cDNAs, would not hybridize to probes. The hybridization signals of these cDNA clones should be negative, and they were therefore considered to be *A. membranaceus*-specific cDNA clones. These cDNAs were then re-examined by rehybridization with the same probes. In contrast, those *A. membranaceus* cDNA clones of higher homology to *Arabidopsis* cDNAs were discarded. The resulting cDNA inserts in these *A. membranaceus*-specific cDNA clones were sequenced from both directions to reveal their genetic codes. Four of these cDNA sequences are shown in **Figure 1**. These sequences contain both the 3′ coding sequence and the adjacent 3′UTR (**Figure 1**).

To confirm whether these *A. membranaceus*-specific cDNA clones originated from the actively expressed mRNAs in *A. membranaceus* root, Northern blot analysis and RT-PCR were performed separately on the total cellular RNA isolated from *A. membranaceus* root tissues. The transcript level of cDNA clones, *AM47* and *AM48*, was detected by Northern blot analysis (**Figure 2A**a,b), whereas the gene expression of *AM77* and *AM79* was detected by RT-PCR analysis (**Figure 2B**a,b). These data confirmed that these *A. membranaceus*-specific cDNA clones were made from actively expressed mRNAs.

A AM47

ACAGAGATTGATATGACGAAGGGAGTAATGGATGAATCGTTCAAGAGATCAGGGTCCGTACCATTCCAATGGGAAGTTC
AGCCTGGGGTTCCCAAGTCATACGCCCCACCACCATCTCCGTCACCAACAAATACAAAGCTTAGGCCGCCACCATCATC
TCCGTCTCATGGATTGACAGCTCCAAGTCGATCAGATATCGGTATTCTTAGTCCACGGAAACCGCGGCGACCATCACGT
GGTTGCTTCCCAGCGGCGGCGTCTTTCCTGGGTAGCAGCAGTAAGCGTAAGGCTGACTTGGATTTTGGCGGTGGCTCT
GATTCCCGCTCATCTAGCTCAGCACAGAATTCTCCCT**CTTCAATGCGTGGTTCATCTCC**ACCACCAGCCTCCGATGTTG
AGTGGGCAGCTTTCGGCCTTTTCTGAAGCCTATTTCGTTGCTTATGGCATATATATGTACAGACTACAGAGATCAAGAGA
TACAGTACGATTTTTCAAATCACGCTACTGTGTGTTCCGTGCCACGCACAGGTCTTAGAATTTTAAATGTGGGATGCATT
ACATATTTTTGACCTGTATTTTTTATTTTATTTTTGGTAATCTCTCCAACTGTTTCTAGATCTAACTTTATTTTCTATTGTTTT
TTTTAATATTTAAT**GAGGCTTGCCCAAAGCTTGC**ATTTTTTTTCCCCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAA

B AM48

ATTTTATTTGGTTCATTTCTTGTTTCTATCGAGTTGATTGTTTCACTCTCTACAGTCATGGTACTCGCTGGAAAACTCAGTA
CAGAACTTGGCATCAAATCACCAGCTGCCAAATTCTTCAACCTCTTTGCAACAGAACTTCATAATGTTCAAAACCATTGTG
AAAGAGTGCATGGAACCAAGCTCCATGAAGGTGATCACTGGCATGGACCAGATTCGATTAAACACTGGACTTATGTCAT
AGACGGCAAAGTCCACCAATGTCTGGAAAAAATTGAGGATGTTGATGAACAAAACAAAAAAATAACTTACAGGGTCTTC
GGTGAAGATATTGATAAGCACTATAA**GGTGTTTAAGTTCATCCTTGAAG**TGATTGATAACAAGGGTACTGGTCATGATGC
TGTTAAATGGACTGTTGAATATGAGAAGCTCAACGAGGATATTGATCCTCCAAATGCCTACATGGATTACCTTAGCAAAT
GCACTAAAGACATTGATGCTAATCTTCTCAAGGCATAGATGGCTCCATATATATATATATATATATATATATATATATAT
ATATATATATATAAGTCCCAAAGAAAATAAGAAATAAGGAGTTATGGATTTGGCTTTGTGTGTAAGTGTTTATCTTTATAAT
TAGTGTGAAGTTTGGCATGTAAAATAATGCTTGTTTGCATCTGTAAGTTCTTCTTCATCATGTGTGTAATAAAT**GCGTCAT**
CTTACTGAAAATGTTGTCCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

C AM77

TGGTCCGTTGGTTGTGTTTTGGCTGAGCTGCTGCTTGGACAGCCTTTATTCCCCGGTGAGAGTGGAGTTGATCAGCTTG
TTGAGATCATCAAGGTTCTGGGCACTCCTACAAGAGAAGAGATTAAATGCATGAATCCTAATTATACAGAATTTAAATTCC
CTCAAATTAAAGCGCATCCATGGCACAAGATCTTCCATAAGCGCATGCCCCCAGAAGCTGTTGATTTGGTATCAAGGCT
ATTGCAATACTCCCCAAACCTGCGGTGCCAAGCTTTAGATGCATTGACCCATCCTTTCTTTGATGAGCTTCGGGACCCAA
A**CGCACGCTTGCCAACTGGTCG**TTTCCTTCCACCACTATTTAATTTCAAACCTCATGAGCTGAAAGGAGTTCCAGCGGA
GACTTCGGTGAAATTGGTTCCAGAGCATGCAAGGAAGCAATGCCCATTTCTTGGCTTGTAATATGTCGTGAAATGTAACA
AAACTGCAAGTGTTGTTCCCATCTGAACGCTACGCGTTCATTCTATTTGATGATATGATATTTGTTAGTATCTTTGTTGTAT
TCGGTTGCCCTGTTGAAAGCAGATTTAGAGAGAATATATGCTACCCAACCCTCGATGGGTATTCAGAATACCCCATTTTC
CATATAACAGCAGATTGTAACATGCAATAGGAGATAACTTACAAGTGTCTGCGTTATCTAAATATTGTATCAGTATTTGTA
TTTGTTGAGACAATGATGGGTTGTTGGCTTAATTTAGTTAATGTATTT**GGACGTTGTGATGGCGTTAA**AAAAAAAAAAAAA
AAA

D AM79

ATGGGGAAGGACGTAGAAGTTGCAGAGCACGGTGAGTTCTCGGCAAAGGACTACACTGATCCACCCCCAGCACCTCTG
ATTGATATGGCAGAGCTGGCAAGTTGGTCTTTCTACAGAGCTATCATAGCCGAGTTCATCGCAACTCTTCTGTTTCTGTA
CATTACAGTGTTAACTGTAATTGGGTACAAGAGTCAAAGCGCGACGGACCAGTGTGGTGGTGTTGGTATTCTTGGTATA
GCCTGGGCTTTTGGTGGAATGATCTTTGTTCTTGTCTACTGCACTGCAGGAATTTCCGGGGGGTCATATTAATCCAGCTGT
GACATTCGGATTGTTCCTGGCAAGAAAAGTATCGCTGATTCGAGCAGTGATGTACATGGTGGCTCAGTGTTTGGGAGCC
ATATGCGGTGTAGGGTTCGTCAAGGCTTTCCAGAAAAGCTACTACGTCAAATTCGGTGGTGGTGCCAACGAGCTTGCG
GATGGTTACAACAAGGGCACTGGTTTAGCTGCCGAGATCATTGGGACCTTTGTTCTTGTTTACACTGTCTTCTCTGCAAC
AGATCCAAAGAGGAGTGCCAGAGACTCCCATGTTCCGGTATTGGCACCACTTCCTATTGGATTTGCAGTGTTCATGGTT
CACTTGGCCACTATCCCAATTACCGGCACCGGTATCAACCC**AGCCAGGAGTTTCGGAGCTGCTG**TAATTTACAACAAGG
ACAAGGCATGGGATGACCAGTGGATCTTCTGGGTTGGACCATTTGTTGGTGCTGCCATTGCCGCATTCTACCACCAATT
TGTTCTTAGAGCAGCTGCCATCAAGGCTCTCGGATCCTTCAGGAGCAATGCCCACAATTAATTTTCTGCCAGATTGTCAA
GTTTCCATTTTCCTTTGAAGAATAATGAACGAATGGACGGGTTTGTAAGGTTGTGTTTGTGTCCCTGCTGAGGTCTTCCC
CTTTGCCGCTAAATGGTTTCTCCTAGGGCTGCTGGGCAACCATTGAATTGTAGATACGGATCAAATTGGCAGATAAGGG
CTGCCCTCTGTTTATTTATTATGTATCCCTTTACTTAATCTT**GTTGCTTATCTTTCAAGTTAA**AAAAAAAAAAAAAAAAAAA
AAAAAAAAG

Figure 1. Sequences of four specific cDNA fragments of *A. membranaceus* identified through subtractive hybridization of its cDNA library with *Arabidopsis* cDNA probes. Accession numbers are AY888003, AY888004, AY888005, and AY903443 for AM47 (**A**), AM48 (**B**), AM77 (**C**), and AM79 (**D**), respectively. The 3′UTRs are highlighted in red, and the sequences used to design primers are underlined and in bold.

Further analysis of these cDNA sequences showed that some of them, like *AM47*, encode novel proteins. However, the rest of the cDNAs were found to encode proteins homologous to known proteins of other organisms. For example, *AM48*, *AM77*, and *AM79* encode the pathogenesis-related protein Betv I family, serine/threonine kinase, and plasma membrane intrinsic protein, respectively. Although *A. membranaceus* has a valuable pharmacological property and important clinical application, only a few cDNA sequences have thus far been reported for this herb. Our sequencing results on *A. membranaceus* cDNAs may be useful to the future study of the biological production of saponins in this herb.

Authentication of *R. astragali* Roots Using UAP. Based on the sequences of *A. membranaceus* cDNA clones, a total of eight pairs of primers were synthesized to amplify the 3′UTRs of the eight corresponding genes (**Table 1**). We predicted that DNA fragment length polymorphism should exist for these 3′UTR markers between *A. membranaceus* varieties of medicinal use and those putative ones. *A. membranaceus* and *A. membranaceus* var. *mongholicus* are two authentic *R. astragali* used

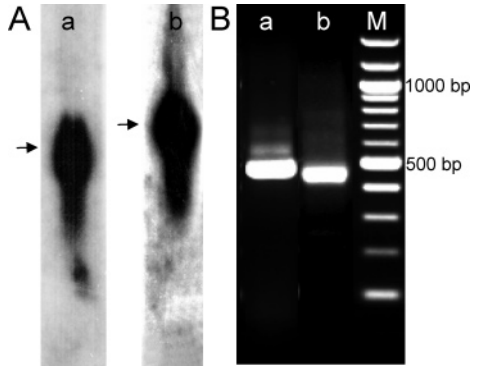

Figure 2. Detection of the *Astragalus* cDNA in root tissue by Northern blot analysis (**A**) or RT-PCR (**B**). Signals in the Northern blot corresponding to AM47 (**A**a) and AM48 (**A**b) are indicated by arrow, and the RT-PCR product of AM77 and AM79 is labeled a or b, respectively, in panel **B**. The marker is the 100 bp DNA ladder (New England).

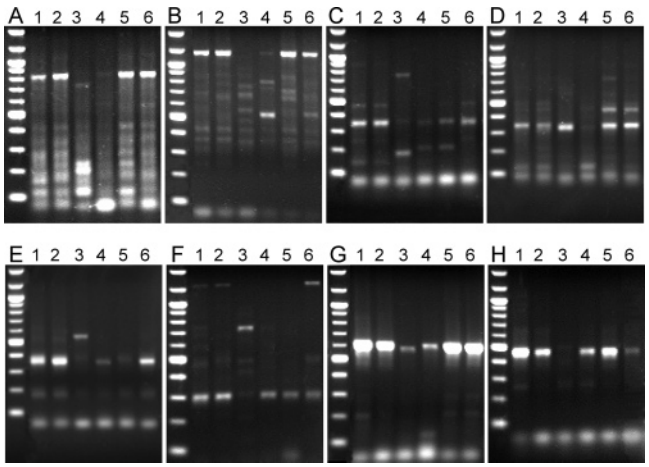

Figure 3. PCR authentication of *R. astragali* using UAP: (lanes 1–4) four commercial *R. astragali* from Nanjing, Jiangshu Province; Neimenggu Province; Litan, Sichuan Province; and Linqiu, Shanxi Province, respectively; (lane 5) *A. membranaceus*; (lane 6) *A. membranaceus* var. *mongholicus*. Primers were designed to amplified genomic DNA region corresponding to cDNA clones AM12 (**A**), AM19 (**B**), AM22 (**C**), AM47 (**D**), AM48 (**E**), AM56 (**F**), AM77 (**G**), and AM79 (**H**), respectively. The marker is the 100 bp DNA ladder (New England).

in herbal medicine. These two plants were employed in our experiments as the positive control. Our results from PCR amplification of genomic DNA samples isolated from both controls and four putative *R. astragali* roots that were purchased from markets showed that *A. membranaceus* and *A. membranaceus* var. *mongholicus* have identical PCR fragment profiles in six polymorphic markers, *AM12*, *AM19*, *AM47*, *AM48*, *AM77*, and *AM79* (lanes 5 and 6 of **Figure 3A**,**B**,**D**,**E**,**G**,**H**), whereas polymorphism exists in the other two markers, *AM22* and *AM56* (lanes 5 and 6 of **Figure 3C**,**F**). These data suggest that UAP polymorphic markers *AM22* and *AM56* are able to differentiate *A. membranaceus* var. *mongholicus* from *A. membranaceus* variety, although these two plants were once classified as the same variety by Hsiao (*36*) and Dong (*23*). The multiple weak DNA bands detected in PCR reactions of *AM12*- and *AM19*-derived primers (**Figure 3A**,**B**) may be due to the lower complexity of the 3′UTR. More interestingly, when the PCR analysis was performed on the commercially available putative *R. astragali* samples from Nanjing, Inner Mongolia, Litan, and Linqiu, as shown **Figure 3**, lanes 1, 2, 3, and 4, respectively, it was found that the Nanjing and Inner Mongolia samples have DNA fragment profiles identical to that of *A. membranaceus* var. *mongholicus* in all of the markers used (lanes 1, 2, and 6 of **Figure 3**), suggesting that they are authentic *A. membranaceus* var. *mongholicus* herb variety. In contrast, the sample from Litan has a DNA fragment length profile completely different from that of the authentic control *A. membranaceus* varieties (lanes 3, 5, and 6 of **Figure 3**), suggesting that the Litan root is an adulterant. In the case of the Linqiu sample, two of eight polymorhpic markers, *AM12* and *AM19*, showed a difference in DNA fragment profile (lanes 4−6 of **Figure 3A**,**B**), whereas the remaining six markers generated the same DNA fragment profile as *A. membranaceus* (lanes 4 and 5 of **Figure 3C**−**H**), suggesting that the Linqiu sample is most likely a variety closely related to *A. membranaceus*.

To show the superiority of the UAP method over the commonly used ITS sequence alignment method (*24*), the ITS regions of these six *R. astragali* were amplified and sequenced. Sequence alignment data indicated that the ITS regions of *R. astragali* from Nanjing, Inner Mongolia, and Linqiu are 100% identical to those of both *A. membranaceus* and *A. membranaceus* var. *mongholicus* control varieties, whereas a sample from Litan differs from that of *A. membranaceus* by 15 bp but shows 99% identity (3 bp difference, sequencing results are not shown here) to *Astragalus umbellatus* (*24*), supporting the conclusion that the so-called "huang qi" root from Litan is indeed an adulterant. These results confirm that our UAP method is a reliable method. At the same time, it is more rapid, inexpensive, and sensitive as compared to the previously used ITS sequence alignment method.

In the past decade, numerous molecular authentication methods have been tested to identify medicinal plants, such as amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR) (reviewed in ref *37*), and methods based on sequencing of ITS, conserved mitochondria, or chloroplast gene (*38*, *39*). An amplification of restriction enzyme digested fragments using adapter-specific primers, AFLP is reliable and robust in authentication, but it is also expensive and time-consuming and demands high-quality DNA, which is usually a challenge when powdered or preserved medicinal plants are being used. RAPD, on the other hand, is a relatively simple and inexpensive approach, but its reproducibility is not as good as we would hope for. ISSR also has similar limitations. The ITS sequence alignment-based authentication method is thus far a method of choice for the identification of plants among species and subspecies. The ITS DNA sequences from 115 species of *A. membranaceus* have been determined (*24*), and the phylogeny of *A. membranaceus* grown in China has been constructed on the basis of DNA sequences of ITS as well as the 5S rRNA spacer and 18S rRNA (*23*). Because of the high sequence conservation of targeted sequences, this method requires either DNA sequencing or restriction enzyme digestion of PCR products to identify the subtle nucleotide differences interspecies and intraspecies (*23*), which certainly require more steps than the UAP method. Additionally, the primers used to amplify the ITS region are also quite conserved among plants and fungus. It is possible that ITS of contaminating fungi could also be amplified and sequenced, which makes the ITS sequencing results more difficult to interpret. Sometimes, cloning of the PCR products may be required before DNA sequencing can be performed. In contrast to these disadvantages stated above, the UAP method shown in this paper is based on some gene regions relatively specific to plants, which, supposedly, can help to eradicate these problems. Under a relatively

stringent PCR condition (**Table 1**), the UAP method has been demonstrated to be able to provide polymorphism among intraspecies and closely related varieties of *R. astragali* using a unique set of markers (**Figure 3**). These genetic sequence markers will be useful in the future in a large-scale screening of herbs from herbal medicine markets.

ABBREVIATIONS USED

TCM, traditional Chinese medicine; 3′UTR, 3′ untranslated region; ITS, internal transcribed spacer; UAP, 3′UTR sequence-based amplified polymorphism; PCR, Polymerase Chain Reaction; AFPL, amplified fragment length polymorphism; RAPD. randomly amplified polymorphic DNA; ISSR, inter-simple sequence repeat.

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Received for review June 6, 2005. Revised manuscript received August 23, 2005. Accepted September 1, 2005. This work was supported by RGC Grants CMI01/02.SC04 and HKUST6276/03M awarded to N.L. and partially by Grant 2002CCC00500 from the Ministry of Science and Technology of China awarded to N.L. and B.X.

JF051334G