

## Utilization of ITS-Based DNA Bar code for Classification of Different *Panax* species in Vietnam

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### ABSTRACT

Several species in *Panax* genus have been identified and utilized as important traditional medicines in Vietnam for decades. Due to the varying degrees of scarcity and value of medicinal herbs, different *Panax* types are often blended to provide illegal benefits. Current morphology-based identification of *Panax* species is low accuracy. In this study, Internal Transcribed Spacer (ITS) regions of 23 *Panax* samples in Vietnam were amplified and sequenced. The obtained sequences were then searched for homology in NCBI Genbank to identify Latin names. The genetic relatedness of ITS sequences were then analyzed by phylogenetic analysis. The results show that 23 studied samples belong to four *Panax* species. The sequence alignment also reveals the distinct variation in ITS regions of *Panax* species in Vietnam in the comparison to ITS sequences of *Panax* species from other countries. The obtained results show high effectiveness of using ITS in distinguishing *Panax* species in Vietnam. Furthermore, the variation within this region could be developed into specific marker for authenticating ginseng-derived products.

**KEY WORDS:** DNA BARCODE, GINSENG, IDENTIFICATION, ITS, PANAX SPECIES.

### INTRODUCTION

Ginseng is the common name of the species in the *Panax* genus Araliaceae. This plant has been used as medicine for a long time as its outstanding medicinal properties include stimulating metabolism in the body, regulating blood sugar in the body, and protecting the activity of the central nervous system, counteract the formation and inhibit the growth of tumors, increase the body's resistance to diseases (Ali et al., 2012; Peng et al., 2012; Lee et al., 2015). High medicinal activity of ginseng is due to the plant's high ginsenoside content, it is also rich in other valuable ingredients such as anti-oxidant compounds, peptides, polysaccharides, and vitamins (Lee et al., 1995; Hong et al., 2012). So far, at least 17 *Panax* species have been reported worldwide (Zhang et al., 2000) and medicinal compounds are variable from species to species (Harkey et al., 2001; Liu et al., 2016, Hyeonah et al., 2021).

Currently, ginseng identification and classification is mainly based on morphological characteristic since it is straight forward to perform and feasible to carry out on the field at a low cost (Proctor et al., 2011; Hong et al., 2012). Nevertheless, significant limitations of this method have been addressed such as low number, complex inheritance pattern, and vulnerable to changes in the environment (Ahmedand and Mohamed, 2014). Consequently, ginseng authentication and cultivar identification based on this method are unreliable.

With the rapid development in molecular biology, DNA-based markers have been used intensively for characterizing the genetic relationships among plant species due to their fast, specific, reliable, and sensitive features (Shim et al., 2003; Serrone et al., 2006; Kim et al., 2016). Recently, the DNA barcode has been used intensively for classifying at the species level (CBOL Plant Working Group, 2009). This marker type has been applied to discriminate at species level for different organism such as plants, animals, fungi and bacteria (Hollingsworth et al., 2011; Barcaccia et al., 2016; Hu et al., 2019).

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Among several DNA barcode loci, Internal Transcribed Spacer (ITS) region has been proposed as a standard region for plant identification because the ITS region is highly conserved among interspecies but variable between inter Species and is easy to amplify even from limited DNA quantity (Chase et al., 2007; Giudicelli et al., 2015). Numerous researchers reported that discrimination power of ITS is higher in the comparison to other plastid markers (Muellner et al., 2011; Yang et al., 2012; Zhang et al., 2014). So far, several studies have exploited the ITS-based DNA barcode in the investigating the genetic composition and developing protocols to differentiate species in *Panax* genus. In 2011, a study in India showed the effectiveness of ITS in clustering analysis of *Panax assamicus* populations according to geographic locations (Panday and Ali, 2011).

In Saudi Arabia, Ali and colleagues applied ITS2 sequence to control ginseng-derived products (Ali et al., 2012). Bang and colleagues used ITS to differentiating and authenticating *Panax* species in Korea (Bang et al., 2015). More recently, this barcode region was also applied to characterize genetic diversity of 24 ginseng samples collected at Lai Chau province of Vietnam (Pham et al., 2018). The present study describes the effectiveness of ITS-based DNA barcode in classifying 23 *Panax* samples belong to different species collected in different geographical locations in Vietnam. The obtained data could be applied for classification, identification, and authentication activities in *Panax* plants of Vietnam.

Figure1: Map showing locations for collecting *Panax* samples.



## MATERIAL AND METHODS

**Sample collection:** Total of 23 *Panax* samples were collected from North to South of Vietnam, samples were collected from different sources such as *Panax* traders, research institutes, universities, and companies in different locations (Figure 1 and Table 1). Collected samples were then store in cool and dry condition and targeted for DNA extraction.

**DNA extraction:** DNA from *Panax* samples extracted by cetyltrimethyl ammonium bromide (CTAB) method followed Li et al. (2013). After extraction, DNA quality was examined by electrophoresis on 1% agarose then spectrophotometer (Optima SP 3000 nano UV-VIS, Japan) was used to determine DNA concentrations. The DNA samples were then kept at -20 °C freezer until use for PCR reactions.

Table 1. List of collected *Panax* samples used in this study.

No.	Sample ID	Local name	Collection location
1	TTB1	Tam that bac	Quang Nam province
2	TTB2	Tam that bac	Quang Nam province
3	TTH1	Tam that hoang	Lao Cai province
4	TTH2	Tam that hoang	Yen Bai province
5	TT1	Tam that	Quang Nam province
6	TT2	Tam that	Quang Nam province
7	TT3	Tam that	Quang Nam province
8	TT4	Tam that	Quang Nam province
9	TT5	Tam that	Quang Nam province
10	TT6	Tam that	Quang Nam province
11	TT7	Tam that	Quang Nam province
12	TT8	Tam that	Quang Nam province
13	TT9	Tam that	Quang Nam province
14	TT10	Tam that	Quang Nam province
15	SLC1	Sam Lai Chau	Lai Chau province
16	SLC2	Sam Lai Chau	Lai Chau province
17	SNL1	Sam Ngoc Linh	Ho Chi Minh city
18	SNL2	Sam Ngoc Linh	Quang Nam province
19	SNL3	Sam Ngoc Linh	Quang Nam province
20	SNL4	Sam Ngoc Linh	Quang Nam province
21	SVD1	Sam vu diep	Yen Bai province
22	SVD2	Sam vu diep	Lao Cai province
23	SVD3	Sam vu diep	Yen Bai province

**PCR reactions and DNA sequencing:** The composition of PCR reactions to amplify ITS region as follows: 7.5 µL 2X Mytaq Red Mix (Bioline, UK), 20 ng DNA, 0.2 µM of each primer (C26A 5' AGGAGAAGTCGTAACAAG3' and N-nc18S10 5' GTTTCTTTTCTCCGCT 3') (Wen and Zimmer, 1996), and PCR water for a final volume of 15 µL. The PCR cocktails were run in thermal cyclers SureCycler 8800 Thermal Cycler (Agilent, USA) with following conditions: initial denaturation at 94 °C for 2 minutes; then repeated by 35 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C, 50 seconds at 72 °C, and finally one minute at 72 °C to complete the reaction. The PCR products were visualized on gel electrophoresed and use 1 kb ladder (Bioline, UK) to determine amplification length.

Correct PCR products were sequenced by Sanger methods at Nam Khoa Company (Ho Chi Minh City, Vietnam). Each sample were sequenced for both sense and antisense

directions. Nucleotide sequences of both DNA strands were obtained and compared the forward and reverse sequence to ensure accuracy. The finally assembled sequences were submitted to NCBI GenBank to obtain accession number (Table 2) and then used for following analysis. Data analysis: The homology identification of each sequence were implemented by using Basic Local Alignment Tools (BLAST) function in NCBI (National Center for Biotechnology Information, USA) database. To evaluate the capacity of ITS based DNA barcode in distinguish *Panax* accessions from Vietnam and abroad, additional ten ITS sequences of related *Panax* species

retrieved from Genbank. All sequences were aligned using the Clustal method in Molecular Evolutionary Genetics Analysis (MEGA) 6 software (<https://www.megasoftware.net>). Evolutionary trees were constructed based on two methods consisting of Maximum Likelihood (ML) and Neighbour Joining (NJ) since they represent for discrete character methods and distance methods, respectively. For satisfactory reliability in phylogenetic construction, bootstrap value was set at 1000 replicates and bootstrap support was classified as Kress et al. (2002).

Table 2. Genbank accession numbers of samples and corresponding Latin names.

No.	Sample ID	Length (bp)	Accession number	Latin name	Percent identity (%)
1	TTB2	704	MZ149934	<i>Panax notoginseng</i>	100
2	TTB1	707	MZ149935	<i>Panax notoginseng</i>	99.86
3	TTH1	703	MZ149936	<i>Panax stipuleanatus</i>	99.72
4	TTH2	631	MZ149937	<i>Panax stipuleanatus</i>	99.68
5	TT1	705	MZ149938	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.43
6	TT2	702	MZ149939	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
7	TT3	702	MZ149940	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
8	TT4	702	MZ149941	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
9	TT5	702	MZ149942	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
10	TT6	702	MZ149943	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
11	TT7	701	MZ149944	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.43
12	TT8	702	MZ149945	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
13	TT9	702	MZ149946	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
14	TT10	702	MZ149947	<i>Panax japonicus</i> var. <i>bipinnatifidus</i>	99.57
15	SLC1	700	MZ149948	<i>Panax vietnamensis</i> var. <i>fuscidiscus</i>	99.86
16	SLC2	630	MZ149949	<i>Panax vietnamensis</i> var. <i>fuscidiscus</i>	99.52
17	SNL1	704	MZ149950	<i>Panax vietnamensis</i>	100
18	SNL2	701	MZ149950	<i>Panax vietnamensis</i>	99.71
19	SNL3	702	MZ149951	<i>Panax vietnamensis</i>	99.72
20	SNL4	705	MZ149952	<i>Panax vietnamensis</i>	99.86
21	SVD1	698	MZ149953	<i>Panax stipuleanatus</i>	100
22	SVD2	703	MZ149954	<i>Panax stipuleanatus</i>	99.72
23	SVD3	642	MZ149955	<i>Panax stipuleanatus</i>	99.53

## RESULTS AND DISCUSSION

**PCR and DNA sequence:** Although there are few studies reported the difficulty in amplifying and sequencing ITS region (Gonzalez et al., 2009; Wang et al., 2016), all PCR and sequencing reactions in this study were performed successfully. The sequences length is 693 bp on average, varying from 631 bp to 707 bp. The similarity of obtained sequences to homologous sequences in NCBI Genbank ranges from 99.52 to 100%. The Latin names of collected samples were identified and presented in Table 2.

After identification, 23 samples were found belonging to four species consisting of *Panax notoginseng*, *Panax stipuleanatus*, *Panax japonicus* var. *bipinnatifidus*, *Panax vietnamensis* var. *fuscidiscus*, and *Panax vietnamensis*

Thus, two different plant groups in Vietnam local names consisting of “Tam that hoang” including TTH1 and TTH2 samples and “sam vu diep” including SVD1, SVD2 and SVD3 samples were identified as *Panax stipuleanatus*. This confirms the low accuracy in traditional method for *Panax* classification due to several uncontrollable factors such as changes of environmental conditions and developmental stages (Ali et al., 2012).

**Sequence alignment and Phylogenetic analysis:** Together with 23 obtained sequences from this study, additional 10 ITS sequences from different species in *Panax* genus were downloaded from NCBI Genbank and combined for sequence alignment and the alignment summary is presented in Table 3. Totally, up to three 33 variation sites were detected within analyzed sequences.

Table 3. Variable sites in 33 ITS sequences from different *Panax* species.

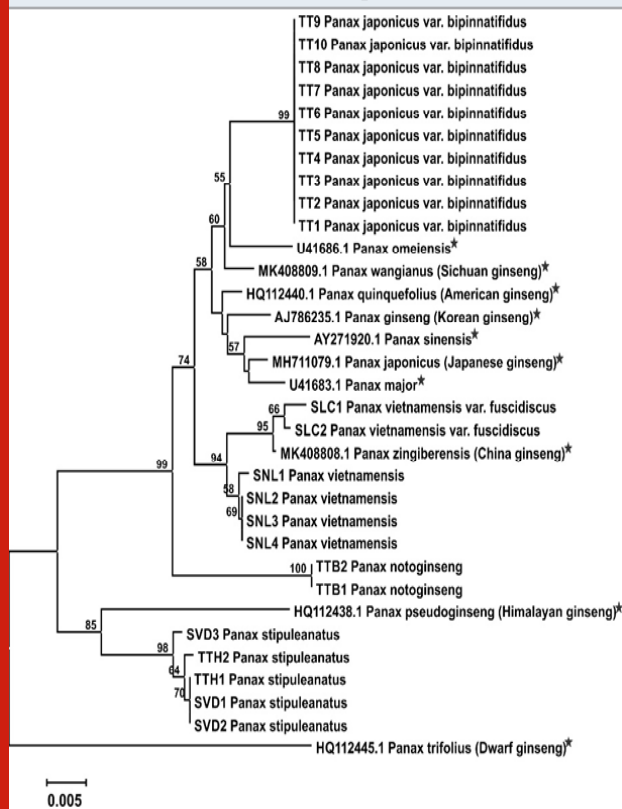
Consensus	A	C	A	T	G	G	A	C	G	T	G	C	C	A	C	A	A	C	A	T	C	G	C	C	C	A	G	C	G	C	C			
TTB2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
TTB1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
TTH1	.	.	.	C	A	A	.	T	A	.	T	T	.	T	G	.	.	C	G	.	T	.	.	.	G	.	T	A	.	.	.	.		
TTH2	T	.	.	C	A	A	.	T	A	.	T	T	.	T	G	.	.	C	G	.	T	.	.	.	G	.	T	A	.	.	.	.		
TT1	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT2	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT3	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT4	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT5	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT6	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT7	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT8	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT9	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
TT10	.	T	C	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	
SLC1	C	T	.	.	.	.	.	.	.	.	T	G	.	.	G	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	
SLC2	T	T	.	.	.	.	.	.	.	.	T	G	.	.	G	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	
SNL1	C	T	.	.	.	.	.	.	.	.	T	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	
SNL2	.	T	.	.	.	.	.	.	.	.	T	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	
SNL3	.	T	.	.	.	.	.	.	.	.	T	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	
SNL4	.	T	.	.	.	.	.	.	.	.	T	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	
SVD1	.	.	.	C	A	A	.	T	A	.	T	T	.	T	G	.	.	C	G	.	T	.	.	.	G	.	T	A	.	.	.	.		
SVD2	.	.	.	C	A	A	.	T	A	.	T	T	.	T	G	.	.	C	G	.	T	.	.	.	G	.	T	A	.	.	.	.		
SVD3	C	.	.	C	A	A	.	T	A	.	T	T	.	T	G	.	.	C	G	.	T	.	.	.	G	.	T	A	.	.	.	.		
AJ786235.1*	C	T	.	.	.	.	.	A	.	A	T	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.		
MH711079.1*	T	T	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	T	T
U41683.1*	G	T	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T
U41686.1*	G	T	C	.	.	.	T	A	.	T	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	.	.	
HQ112438.1*	T	.	.	C	A	A	.	.	A	C	T	T	.	.	G	.	.	C	G	.	T	.	.	.	T	.	T	.	.	.	.	.		
HQ112440.1*	T	T	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	
AY271920.1*	G	T	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	
HQ112445.1*	T	.	.	C	A	C	G	.	.	C	.	T	.	.	T	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	
MK408809.1*	T	T	C	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	
MK408808.1*	T	T	.	.	.	.	.	.	.	.	T	G	.	.	G	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	

(\*: sequences were retrieved from NCBI Genbank)

Previously, several research groups have employed the nucleotide variations to develop molecular makers for plant identification such as in *Taxus* (Liu et al., 2011) or onion (Ipek et al., 2014). Thus, these variations are valuable for developing specific markers or diagnosis KIT for discriminating different *Panax* species. The genetic distances among 23 *Panax* samples were calculated based on Kimura-2 parameter (K2P) method of MEGA 6 software. The lowest genetic distance is 0.000 indicating the sequence similarity among samples, while the highest is 0.052 this result showing the close genetic relationship among these accessions when using ITS regions. The phylogenetic trees constructed by either neighbor-joining (NJ) or Maximum Likelihood methods are shown in Figure 2 and Figure 3, respectively.

In general, the results show the high similarity between Neighbor-Joining and Maximum Likelihood methods. *Panax* samples collected from Vietnam in this study is clustered into several groups corresponding to its identified species using NCBI BLAST. Interestingly, Vietnam ginsengs are also separated from different ginseng species collected from different countries. The grouping of *Panax* species in obtained phylogenetic trees of this study is slightly different with previous study of Yang and colleagues in Korea in which they reported the closer clustering of *P. notoginseng* and *P. pseudoginseng* as well as *P. omeiensis* and *P. zingiberensis* (Yang et al., 2001).

Figure 2: Neighbor-Joining tree with 1000 bootstrap replicated based on ITS sequences (Star symbols indicating sequences retrieved from NCBI Genbank and the availability of common names are shown in parentheses).



## CONCLUSION

The obtained data from this study confirms the effectiveness of using ITS marker to classify *Panax* samples in Vietnam. Furthermore, this marker also shows high discrimination power to distinguish *Panax* species from Vietnam and from abroad. Future study should focus on develop specific primer pairs or diagnosis KIT to serve for identification and authentication activities of *Panax* plants.

**Ethics approval and consent to participate:** Not applicable.

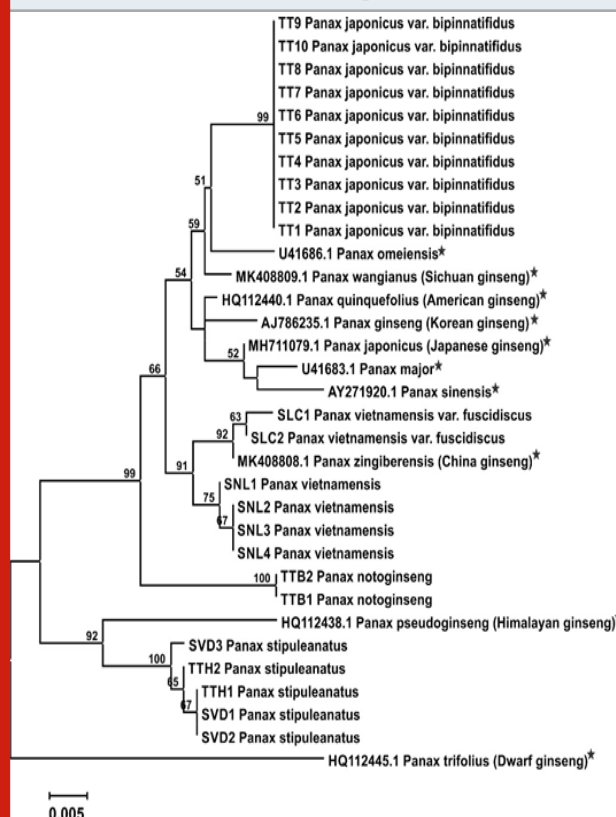
**Conflict of Interests:** The author has no conflict of interest.

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Figure 3: Maximum Likelihood tree with 1000 bootstrap replicated based on ITS sequences (Star symbols indicating sequences retrieved from NCBI Genbank and the availability of common names are shown in parentheses).



## REFERENCES

- Ahmedand THM, Mohamed ZMA (2014). Genetic diversity of mango (*Mangifera indica* L.) cultivars in Shendi Area. *Extensive journal of applied sciences* 3 (6): 219-224.
- Ali MA, Al-Hemaid FM, Lee J et al. (2012). Assessing nrDNA ITS2 sequence based molecular signature of ginseng for potential use in quality control of drug. *African Journal of Pharmacy and Pharmacology* Vol 6 No 39 Pages 2775-2781. DOI: 10.5897/AJPP.12.384
- Bang KH, Kim YC, Lim JY et al (2015). Internal Transcribed Spacer Barcoding DNA Region Coupled with High Resolution Melting Analysis for Authentication of *Panax* Species. *Korean Journal of Medicinal Crop Science* Vol 23 No 6 Pages 439-445. <http://dx.doi.org/10.7783/KJMCS.2015.23.6.439>
- Barcaccia G, Lucchine M, Cassandro M (2016). DNA Barcoding as a Molecular Tool to Track Down Mislabeling and Food Piracy. *Diversity* Vol 8 No 2 doi:10.3390/d8010002.
- CBOL Plant Working Group et al (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* Vol 106: 12794-12797.
- Chase MW, Cowan RS, Hollingsworth PM et al (2007). A proposal for a standardized protocol to barcode all land plants. *Taxon* Vol 56 Pages 295-299.

- Giudicelli GC, Mader G, Freitas LBD (2015). Efficiency of ITS sequences for DNA barcoding in Passiflora (Passifloraceae). *International Journal of Molecular Sciences* Vol 16 Pages 7289–7303.
- Gonzalez MA, Baraloto C, Engel J et al (2009). Identification of Amazonian Trees with DNA Barcodes. *PLoS ONE* Vol 4 No 10: e7483. <https://doi.org/10.1371/journal.pone.0007483>.
- Harkey MR, Henderson GL, Gershwin ME et al (2001). Variability in commercial ginseng products: an analysis of 25 preparations. *The American Journal of Clinical Nutrition* Vol 73 Pages 1101–1106.
- Hollingsworth PM, Graham SW, Little DP (2011). Choosing and using a plant DNA barcode. *PLoS ONE* Vol 6 No 5: e19254 doi:10.1371/journal.pone.0019254
- Hong HD, Cho CW, Kim YC et al (2012). Morphological Characteristics of Korean Dried Ginseng Products. *Journal of Ginseng Research* Vol 36 No 3 Pages 314–321. DOI: 10.5142/jgr.2012.36.3.314.
- Hu SJ, Hu HY, Gao H et al. (2019). DNA barcode and rapid identification of the precious herb *Herba Anoectochili*. *Chinese journal of Natural medicines* 17(10): 0738–0745.
- Hyeonah, Shim, Nomar Espinosa Waminal, Hyun Hee Kim & Tae-Jin Yang (2021) Dynamic evolution of *Panax* species *Genes & Genomics* volume 43, pages 209–215
- Ipek M, Ipek A, Simon WP (2014). Testing the utility of matK and ITS DNA regions for discrimination of *Allium* species. *Turkish Journal of Botany* Vol 28 Pages 203–212.
- Kim JH, Kim MK, Wang H et al (2016). Discrimination of Korean ginseng (*Panax ginseng* Meyer) cultivar Chunpoong and American ginseng (*Panax quinquefolius*) using the auxin repressed protein gene. *Journal of Ginseng Research* Vol 40 No 4 Pages 395–399. <https://doi.org/10.1016/j.jgr.2015.12.002>.
- Kress WJ, Wurdack KJ, Zimmer EA et al (2002) Use of DNA barcodes to identify flowering plants, *Proceedings of the National Academy of Sciences of the United States of America* Vol 102 No 23 Pages 8369–8374.
- Lee HS, Kim SW, Lee SW et al (1995). Agrobacterium mediated transformation of ginseng (*P. ginseng*) and mitotic stability of the inserted beta-glucuronidase gene in regenerates from isolated protoplasts. *Plant Cell Reports* Vol 14 No 9 Pages 545–549. doi: 10.1007/BF00231935
- Lee SM, Bae BS, Park HW et al (2015) Characterization of Korean Red Ginseng (*Panax ginseng* Meyer): History, preparation method, and chemical composition. *Journal of Ginseng Research* Vol 39 No 4 Pages 384–391. <https://doi.org/10.1016/j.jgr.2015.04.009>
- Li J, Wang S, Jing Y et al (2013). A modified CTAB protocol for plant DNA extraction. *Chinese Bull Bot* Vol 48 Pages 1–7.
- Liu J, Moller M, Ga LM et al (2011). DNA barcoding for the discrimination of Eurasian Yews (*Taxus* L., Taxaceae) and the discovery of cryptic species. *Molecular Ecology Resources* Vol 11 Pages 89–100.
- Liu J, Liu Y, Zhao L et al (2016). Profiling of ginsenosides in the two medicinal *Panax* herbs based on ultra-performance liquid chromatography-electrospray ionization–mass spectrometry. *Springer Plus* Vol 5, 1770. <https://doi.org/10.1186/s40064-016-3427-3>
- Muellner AN, Schaefer H, Lahaye R (2011). Evaluation of candidate DNA barcoding loci for economically important timber species of the Mahogany family (Meliaceae). *Molecular Ecology Resources* Vol 11 Pages 450–60.
- Panday AK, Ali MA (2011). Intraspecific variation in *Panax assamicus* Ban. Populations based on internal transcribed spacer (ITS) sequences of nrDNA. *Indian Journal of Biotechnology* Vol 11 Pages 30–38.
- Peng D, Wang H, Qu C et al (2012). Ginsenoside Re: Its chemistry, metabolism and pharmacokinetics. *Chinese Medicine* Vol 7 No 2. <https://doi.org/10.1186/1749-8546-7-2>
- Pham QT, Nguyen MD, Khuong TB et al (2018). Genetic diversity of several ginseng plants collected in Lai Chau. *Vietnam Journal of Science, Technology and Engineering* Vol 60 No 2 Pages 27–31.
- Proctor JTA, Sullivan AJ, Rupasinghe HPV et al (2011). Morphological and Ginsenoside Differences among North American Ginseng Leaves. *Journal of Ginseng Research* Vol 35 No 2 Pages 155–161. DOI: 10.5142/jgr.2011.35.2.161
- Serrone PD, Attorri L, Gallinella B et al (2006). Molecular Identification of *Panax* ginseng C.A. Meyer in Ginseng Commercial Products. *Natural Product Communications* Vol 1 No 12 Pages 1137–1140. DOI: 10.1177/1934578X0600101213
- Shim YH, Choi JH, Park CD et al (2003). Molecular differentiation of *Panax* species by RAPD analysis. *Archives of Pharmacal Research* Vol 26 No 8 Pages 601–605.
- Wang A, Gopurenko D, Wu H et al (2016). Evaluation of six candidate DNA barcode loci for identification of five important invasive grasses in eastern Australia. *PLoS One* Vol 12 No 4: e0175338
- Wen J, Zimmer EA (1996). Phylogeny and Biogeography of *Panax* L. (the Ginseng genus, Araliaceae): Inferences from ITS sequences of Nuclear Ribosome DNA. *Molecular Phylogenetics and Evolution* Vol 6 No 2 Pages 167–177.
- Yang DC, Yang KJ, Yoon ES (2001). Comparison of ITS (Internal Transcribed Spacer) and 5.8S rDNA sequences among varieties and cultivars in *Panax ginseng*. *Journal of Photoscience* Vol 8 No 2 Pages 55–60.
- Yang JB, Wang YP, Möller M et al (2012). Applying plant DNA barcodes to identify species of *Parnassia* (Parnassiaceae). *Molecular Ecology Resources* Vol 12 Pages 267–75.
- Zhang D, Jiang B, Duan L et al (2016). Internal transcribed spacer (ITS), an ideal DNA barcode for species discrimination in *Crawfordia* wall (Gentianaceae). *African Journal of Traditional, Complementary and Alternative Medicines* Vol 13 No 6 Pages 1010–1106.
- Zhang H, Abid S, Ahn JC et al (2020). Characteristics of *Panax ginseng* cultivars in Korea and China. *Molecules* Vol 25 No 11 2635. doi.org/10.3390/molecules25112635