J Ginseng Res xxx (2016) 1–5

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Development of a single-nucleotide-polymorphism marker for specific authentication of Korean ginseng (*Panax ginseng* Meyer) new cultivar "G-1"

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ARTICLE INFO

Article history:
Received 10 March 2015
Received in Revised form
11 December 2015
Accepted 15 December 2015
Available online xxx

Keywords:
G-1 cultivar
multiplex polymerase chain reaction
Panax ginseng
Panax quinquefolius
Single-nucleotide polymorphism

ABSTRACT

Background: Korean ginseng (*Panax ginseng*) is a well-known medicinal plant of Oriental medicine that is still in practice today. Until now, a total of 11 Korean ginseng cultivars with unique features to Korean ginseng have been developed based on the pure-line-selection method. Among them, a new cultivar namely G-1 with different agricultural traits related to yield and content of ginsenosides, was developed in 2012.

Methods: The aim of this study was to distinguish the new ginseng cultivar G-1 by identifying the unique single-nucleotide polymorphism (SNP) at its 45S ribosomal DNA and *Panax quinquefolius* region than other Korean ginseng cultivars using multiplex amplification-refractory mutation system–polymerase chain reaction (ARMS-PCR).

Results: A SNP at position of 45S ribosomal DNA region between G-1, *P. quinquefolius*, and the other Korean ginseng cultivars was identified. By designing modified allele-specific primers based on this site, we could specifically identified G-1 and *P. quinquefolius* via multiplex PCR. The unique primer for the SNP yielded an amplicon of size 449 bp in G-1 cultivar and *P. quinquefolius*. This study presents an effective method for the genetic identification of the G-1 cultivar and *P. quinquefolius*.

Conclusion: The results from our study shows that this SNP-based approach to identify the G-1 cultivar will be a good way to distinguish accurately the G-1 cultivar and *P. quinquefolius* from other Korean ginseng cultivars using a SNP at 45S ribosomal DNA region.

1. Introduction

Panax ginseng Meyer is a deciduous perennial herb plant that belongs to the family Araliaceae. They are native to East Asia, while two species of them are found in North America. Ginseng has been used as a medicinal plant for over 2,000 years in Korea, China, and Japan as an immunostimulant, and acts as an agent to foster resistance to fatigue and stress [1–3]. The usage of ginsenoside-based medicinal products is increasing worldwide. Among the different species of ginseng, *P. ginseng* and *Panax quinquefolius* are the most popular for consumption, as well as for medicinal purposes.

Most of the commercial cultivation of *P. ginseng* has been centralized to South Korea and the northeastern part of China and Japan, whereas *P. quinquefolius* has been cultivated in China, Canada, and the United States. In South Korea, the ideal climatic conditions to grow ginseng plants in all four seasons favor the cultivation of many species of *Panax* for commercial purposes, such

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http://dx.doi.org/10.1016/j.jgr.2015.12.007

as *P. ginseng*, *Panax notoginseng* (Chinese ginseng), *Panax japonicus* (Japanese ginseng), and *P. quinquefolius* L. [4]. Recently, there are a total of nine cultivars namely, Yunpoong, Gopoong, Sunpoong, Gumpoong, Chunpoong, Sunun, Sunone, Sunhyang, and Chungsun with features unique to Korean ginseng, which have been selected from three basic (varieties) lines (Jakyung, Chungkyung, and Hwangsook) using the pure-line-selection method [5]. A similar method was followed to develop the K-1, a new cultivar G-1 and registered with the Korea Seed & Variety Service (http://www.seed.go.kr).

Each Korean ginseng cultivar has unique features in relation to the improved agronomical properties, such as root yield, root shape, and disease resistance. Yunpoong has the highest root yield [6] and Chunpoong, Gopoong, Gumpoong have good root shapes. While considering the quality of red ginseng (steamed ginseng roots) in these cultivars, Chunpoong has the highest root yield of Chun Sam and it is considered the first-grade ginseng followed by Gumpoong, Gopoong, Yunpoong, and Sunpoong. Pertaining to the ginsenoside unit content and total content of ginsenoside in 6-year-old ginseng roots, the ginsenoside content is higher in the order of Gopoong, Yunpoong, Chunpoong, Gumpoong, and Sunpoong [7]. The Korean ginseng cultivar G-1 was developed in 2012 and the morphological characteristics of G-1 are short flower stalk, violet color on stem stronger than Chunpoong (green stem with light violet), lighter than K-1, budding later than Sunpoong and red berry color (Table 1). In addition, the G-1 root appearance, ginsenoside analysis and disease resistance were also analyzed (data not shown).

These cultivars are grown in mixed ginseng fields, and are also sold mixed with other *Panax* species in the market. Therefore, the development of a valid authentication method is necessary for the preservation of these varieties, and to protect the rights of farmers and consumers. Although the medicinal components and efficacy of *P. ginseng* have been widely explored [8–11], there is little information available on the genome of *P. ginseng*, making the molecular identification of different cultivars difficult. However, with the development of robust molecular markers, such as polymerase chain reaction (PCR)–restriction fragment length polymorphism [12], single-strand conformation polymorphism [13], randomly amplified polymorphic DNA [14], sequence-characterized amplified region [15], intersimple sequence repeat-derived sequence-characterized amplified region [16], amplification-refractory mutation system (ARMS) [17], amplified fragment length polymorphism, and directed amplification of minisatellite region DNA [18] for the Korean ginseng cultivars, this difficulty is prevailed.

Traditional methods based on phenotypic observations have been used to identify the G-1 cultivar from the rest of the Korean ginseng cultivars, but morphological characteristics are often affected by environmental and developmental factors. Due to very similar phenotypical characteristics of these cultivars, the identification and authentication of G-1 becomes difficult especially during the seed-development and seedling stages. Thus, it is advantageous to use molecular methods to differentiate the ginseng cultivars.

In this study, we investigated the possibility of using a single-nucleotide polymorphism (SNP) in 45S ribosomal DNA (rDNA) to differentiate ginseng cultivars. The nucleolar organizing regions (NORs) are cytologically observed as a secondary constriction containing many tandem repeats of 45S ribosomal ribonucleic acid genes [19]. The 45S rDNA sites were observed to be restricted to the NORs, although in some species, smaller or less active sites have also been detected outside the NORs [20]. Based on the SNP sites found for G-1, other Korean ginseng cultivars, and American ginseng, specific primers were designed and multiplex ARMS–PCR was conducted to authenticate these plants. This method based on DNA analysis is widely accepted as a means of identifying medicinal plants, because it is not affected by the growth stage and environmental conditions.

Table 2
Ginseng plant samples used in this study

Ginseng sample	Voucher	Location	GenBank accession number of 45S
Chunpoong	GB001	Kochang, Korea	KF727964
Yunpoong	GB002	Kochang, Korea	KF727965
Gopoong	GB003	Kochang, Korea	KF727966
Sunpoong	GB004	Kochang, Korea	KF727967
Gumpoong	GB005	Kochang, Korea	KF727968
Sunun	GBD048	Daejeon, Korea	KF727969
Chungsun	GBD073	Daejeon, Korea	KF727970
Sunone	GBD043	Daejeon, Korea	KF727971
Sunhyang	GBD058	Daejeon, Korea	KF727972
K-1	GBD201	Kochang, Korea	KF727973
G-1	GBD101	Kochang, Korea	KF727974
G-1	GBD102	Kochang, Korea	
G-1	GBD103	Kochang, Korea	
Panax quinquefolius	GBD099	USA	KF727975
P. quinquefolius	GBD100	USA	
P. quinquefolius	GBD101	USA	

2. Materials and methods

2.1. Plant materials

Eleven ginseng samples (Table 2) were provided by the Ginseng Resource Bank. All voucher specimens were morphologically identified by Professor Woo-Saeng Kwon (Department of Oriental Medicinal Biotechnology, College of Life Sciences, Kyung Hee University).

Table 1
Main characteristics of aerial parts of 4-year-old ginseng cultivars

Line	Cultivars	Color of stem	Color of berry	Leaf type	Registered date
Jakyung	Yunpoong	Light violet	Red	Having stipule	1998
	Gopoong	Violet	Red	Long oval	2000
	Sunpoong	Violet	Red	Long oval	2000
	Sunun	Violet	Red	Long oval	2004
	Sunone	Violet	Red	Long oval	2004
	Sunhyang	Violet	Red	Long oval, occurrence of stipule	2007
	K-1	Violet	Red	Long oval, tipple	2012
	G-1	Violet	Red	Occurrence of stipule	2013
Chungkyung	Chunpoong	Green and violet spot in green	Orange yellow	Narrow elliptical	1998
	Chungsun	Green	Red	Long oval	2005
Hwangsook	Gumpoong	Green	Yellow	Long oval	2000

Please cite this article in press as: Yang D-U, et al., Development of a single-nucleotide-polymorphism marker for specific authentication of Korean ginseng (*Panax ginseng* Meyer) new cultivar “G-1”, Journal of Ginseng Research (2016), http://dx.doi.org/10.1016/j.jgr.2015.12.007