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In vitro cultivation of *Panax ginseng* C.A. Meyer

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ABSTRACT

Ginseng is a perennial plant that grows slowly and has a long production cycle (4–6 years). It belongs to genus *Panax* that comprises of 11 different species and valued for their immense potential to cure all diseases, especially against fatigue. Korean ginseng is known for its homeostasis, anti-Alzheimer, anti-amnesia and anticancer properties. Since the approval of its medicinal properties through modern scientific technology and increased dependence of the western world on herbal remedies, a surge for Korean ginseng in the international market was seen during last decades. Around the world Korean ginseng have been found to be superior in the content of ginsenosides, non-saponin, phenol and polysaccharide compounds than American and Sanchi ginseng. World ginseng production needs alternative approaches to meet the modern-day market demand for standardized plant products of uniform quality and free of toxic agrochemicals. For the purpose in-vitro tissue culture approaches have extensively been studied and adopted for uniform and continuous supply of the products. This review elaborates on the traditional medicinal uses and cultivation practices of *Panax ginseng* C.A. Meyer and explains modern day tissue culture approaches for the enhanced production of important medicinal compounds.

1. Introduction

Korean *Panax ginseng* C.A. Meyer a perennial plant belonging to genus *Panax* and member of Araliaceae family is an important medicinal plant of long history. There are 13 Known species of this genus, distributed in Korea, China, Japan, USA, Canada, and eastern Himalaya. It was domesticated back in 11 B.C. by transplantation of wild ginseng followed by an attempt in 1122 AD. to propagate ginseng from seeds during King In Jong of the Koryo Dynasty (Yun, 2001). However, its proper cultivation by Korean people was started during 1900s century when the demand for ginseng was higher than its supply from wild (Ang-Lee et al., 2001; Kee, 1999).

Historically, ginseng roots were used to increase vigour, mind booster, anti-aging and believed to strengthen the soul. The word ginseng is derived from Chinese word, Ren-Shen named after its roots that look like a little man and was believed that God sent these miraculous roots from mountains to give strength and save weak people. The detail information about its history has inclusively been reviewed in Kim et al. (2016), Baeg and So (2013), Park (2002), and Yun (2001) reviews. Currently, Korea is the second largest producer (27,480 ton) and exporter of ginseng roots after China (Baeg and So, 2013). Since the increased perception of safe and healthy food, the demand for ginseng among people has enormously increased and believed to be expanding rapidly. The long cultivation period, replant disease, little arable land, and laborious cultivation practices have hindered the farmers to fulfil the market demand. Also, the use of expensive pesticides and variation in environmental conditions due to global warming have forced the academicians and plant scientist to look for alternative approaches to fulfil the global demands.

The demanding alternative approach in this modern era is believed to be plant tissue culture, that is a collective term used for not only tissues (meristem, leaf tissues, etc.) but also to organ cultures (adventitious roots, hairy roots, etc.) and cell culture. This promising alternative approach offers the potential benefits of being in a controlled environment and its plausible manipulation to increase the content of targeted medicinal metabolites. It is believed that this approach is safe, continuous, regarded as good manufacturing practice and easier to extract the desired compounds (Davies and Deroles, 2014). The history of plant tissue culture dates back to the theory of cell, proposed by Schleiden and Schwann (1838–1839) and later proved by Haberlandt (1914) experiments of culturing mesophyll cells and epidermal tissue. That was further succeeded after the design of medium by Murashige and Skoog (1962) that is known as MS medium and used to date as such or with minor modifications. Since than the discovery of nutrient

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medium and plant growth regulators, the plant tissue culture research turned into a business of numerous opportunities and profits. The plant tissue culture approach helped in swiftness of plant transformation, breeding, clonal propagation, and conservation of medicinal, horticulture and crops plants.

It was Butenko who found the method for sterilization of explant and expressed her desire to establish *in vitro* culture of ginseng (Butenko, 1967). In 1967 with her co-workers she pioneered the *in vitro* tissue cultures of *P. japonicas*, *P. quinquefolius*, and *P. pseudoginseng* (Butenko et al., 1968; Slepyan et al., 1967). The work was further extended by Slepyan to report callus culture from ginseng root explant and noticed an increase in endurance activity of callus extract in mice (Slepyan, 1968). Further, in 1969 Kitra and Sungi defined nutrient medium for P. ginseng tissue culture. Succeeding Slepyan work, Furuya et al. (1970) studied the chemical composition of 5 years old ginseng callus culture and successfully isolated panaxatriol. Later on, in 1972 first German patent for saponin production in ginseng tissue cultures was owed by Furuya et al. (1970). Though, Metz and Lang (1966) had already claimed a German patent for saponin production in ginseng root organ culture (Metz and Lang, 1966). From Korea, it was Lee who reported ginseng culture for the first time in 1971 (Lee and Huemer, 1971). Afterward, Kim et al. (1972) and Jhang et al. (1974) established ginseng tissue cultures and compared the contents of saponin in roots, leaves and stems, and cultured tissues of Korean and American ginseng (Jhang et al., 1974; Kim et al., 1972). The continuous efforts in ginseng tissue culture research directed NITTO DENKO CO. to culture ginseng cells in large scale (20,000 and 25,000L) bioreactors and since 1988 it sells ginseng healthy food products in Japan. But this didn‘t stops there and to date, several research articles have been published for the perfection of *in vitro* culture system to enhance the secondary metabolite yield. In this review, we have elaborated the efforts of several tissue culturists, biochemical engineers, Phyto-chemists, and others for the establishment of a promising alternative approach to fulfil the ginseng market demand.

2. Panax: Photochemistry and phytochemistry and pharmacology

The genus name *Panax* is derived from two Greek words ‘pan’ means all and ‘axos’ means cure, this name was accredited to this group because of orient belief. It was 1854 when Garriques isolated panaquinone from *P. quinquefolius* and afterward in 1921 Wong isolated pale-yellow ginseng oil, and sapogenin. Since then Chinese, Japanese, and Korean researchers studied ginseng in detail and reported its active ingredients, ginsenosides. Although its medicinal use is a history of thousand years and originally it was believed to be a plant of miracles to heal all kind of diseases. With the introduction of pure compound isolation from plant extracts, advanced therapeutic potentials of ginseng other than conventional uses were studied e.g., cardioprotective, antidiabetic, antioxidant, anxiolytic, anticancer, and aphrodisiac (Table 1).

The genus *Panax* has medicinally rich phytochemicals, most notably saponin, and also polyacetylenes, phenolic compounds (flavonoids, and phenolic acids), essential oils, polysaccharides, microelements, and vitamins (Fig. 1) (Kim et al., 2010a; Kim, 2016). But most prominent attention has been paid to its saponin fraction and most exclusively to ginsenosides. Ginsenosides are triterpene saponin, composed of 17 carbons in four rings (Leung and Wong, 2010). To date, more than 150 ginsenosides have been identified in plants (Zhao et al., 2015) and are grouped into two main groups i.e., dammarane, and oleanane (Attele et al., 1999). On the bases of sugar moiety position on the dammarane skeleton, it is further divided into three groups: 1) 20 (S)-protopanaxatriol (PPT), 2) 20 (S)-protopanaxadiol (PPD), and 3) ocotillol (Matsuura et al., 1984). These compounds are named as ‘Rx’, where ‘R‘ stands for the root and ‘x‘ describes the chromatographic polarity in an alphabetical order (Shibata et al., 1965), for example Ra is the least polar than Rb. Beside these some rare ginsenosides, such as the ocotillol saponin F11 (24-R-pseudoginsenoside) and pentacyclic oleanane saponin Ro (3, 28-*O*-bisdesmoside) have also been identified (Sanada et al., 1974). The medicinal value of different ginsenosides have been summarized by Li and Gong (Li and Gong, 2015) and Nag et al. (Nag et al., 2012). Moreover, anticancer, cardioprotective, anti-Alzheimer and other medicinal properties of ginsenosides have comprehensively been reviewed (Lee and Kim, 2014; Leung and Wong, 2010; Lim et al., 2015; Sheng et al., 2015).

3. Problems of conventional cultivation

Ginseng cultivation requires a commitment to grow it in the field for 5–10 years under optimal temperature, 80% shade and 20% humidity to thrive in deep rich loamy soil. With the rapidly increasing demand for ginseng, special efforts on ginseng cultivation have been carried out, including the development of culture varieties by selection, field cultivation, reutilization of the wasted ginseng plantation, ginseng cultivation under the forest, as well as prevention and treatment of ginseng diseases, and pests. These efforts have greatly improved the quality and quantity of the ginseng. But, still its stringent environmental requirements and prolonged cultivation period have made this commodity unacceptable to farmers. Ginseng plant requires an optimal temperature between 10 °C and 20 °C during the leafing phase and 21–25 °C during the flowering, and fruiting phases (Mork et al., 1981). An increased temperature beyond 30 °C for a prolonged period causes whole leaf burning or bleaching due to pigment destruction in leaf tissues (Rahman and Punja, 2005).

Formerly, ginseng was a wild plant collected from its natural niches in Korea (between 33°7′N and 43°1′N) and north-eastern China (between 43°N and 47°N). Due to the higher market value of wild grown ginseng, farmers developed wild simulated and wood cultivated methods for obtaining roots with higher similarity to the wild one. These methods have proven to be economical and also save arable lands for cultivation of other food crops. But still, the fear of rodents, pathogen, and thieves attack have made it unappealing to the growers. Whereas growing them in paddy fields under the artificial shades of a wood-lath or polypropylene-cloth and ploughing 15 times in a year makes it more expensive and labour intensive. Its long-term cultivation practice exerts an extra pressure of fungal pathogens attack and compromises the roots quality (Punja, 2011). Furthermore, replant diseases in field grown ginseng hinders its sustainable production as well.

On top of these all, the quality and quantity of ginsenosides in ginseng is affected by several other factors including age, genotype, plant tissue type, soil, temperature, method of cultivation, season, and light (Liu et al., 2017). Previously, Sengupta et al. (2004) reported higher Rg1:Rb1 ginsenoside ratio in Asian ginseng while conversely American ginseng had lower Rg1:Rb1 ratio. Searels et al. (2013) reported regional chemotypes of *P. quinquefolius* L that differed in Rg1 and Re contents. Tissue specific variations in ginsenosides have also been reported and in a study the authors indicated 12 times higher total ginsenosides in leaves than main roots (Kang and Kim, 2016). Xie et al. (2004) reported higher Re in leaf and stem instead of main ginseng roots. Lim et al. (2005) determined the genotypes and environmental factors affecting the ginsenoside profile in eight wild populations of *P. quinquefolius*. Peng et al. (2012) have mentioned in his review that Re content varies with population but not with locations, whereas Rb1, Rc and Rb2 only varies with locations, and Rg1 and Rd varies with both. Ginsenosides levels are decreased, while ginseng growth is increased, at an intensively managed garden location. These all influencing factors have been found appealing to plant biologist to look for alternative ways of ginseng cultivation, that are discussed here as below.

4. In vitro tissue culture approach

In vitro tissue culture approach is an advantageous system then conventional cultivation practices. The necessary conditions that make