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Review

Eleutherococcus senticosus as a crude medicine: Review of biotechnological effects

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Eleutherococcus senticosus **(Rupr. et Maxim.) Harms (=*Acanthopanax senticosus*, Araliaceae), also called Siberian ginseng, Ciwujia in Chinese and Gasiogalpi in Korean, is a woody medicinal plant, distributed in the cold regions of Northeast Asia. *E. senticosus* (ES, thereafter) has been found to possess efficacies on strengthening spleen and nourishing kidney in the theory of Traditional Chinese Medicine. As its great medicinal and economic significance, ES is popularly considered as an "adaptogen" like *Panax ginseng*. In recent decades, a great number of biotechnological, chemical, pharmacological, and clinical studies on ES have been carried out worldwide. The goal of present review is to up-to-date and comprehensively analyze the biotechnological trials on traditional propagation, embryogenesis, and molecular classification of ES. Due to the poor and/or even failed seed setting and over-exploitation, ES has been prescribed as an endangered plant by the Environmental Ministry in Korea. Despite conventional propagations including seed and stem cutting propagation, have achieved a great process, their productivity could not come up to that through *in vitro* micro- and mass-propagation of ES. And the *in vitro* regenerated plantlets have been used as materials for production of secondary metabolites and other biotechnological applications. Molecular classification by randomly amplified polymorphic DNA (RAPD) analysis could help further understanding of relationships among populations, growth conditions, and quality as medicinal materials to improve farm cultivation of ES.**

Key words: *Eleutherococcus senticosus, Acanthopanax senticosus,* propagation, biotechnology, medicinal plant, adaptogen.

INTRODUCTION

Eleutherococcus senticosus (Rupr. et Maxim) Harms (=*Acanthopanax senticosus*, Araliaceae, ES, thereafter), also called Siberian ginseng, Ciwujia in Chinese and Gasiogalpi in Korean, is a woody medicinal plant, distributed in southeast Russia, northeast China, Korea, and Japan (Lee, 1979; Hahn et al., 1985). The cortical root and stem tissues of this species have long been used for medicinal properties (Umeyama et al., 1992; Davydov and Krikorian, 2000). Main active compounds such as triterpene saponins isolated from ES possess important pharmacological activities, including inhibiting histamine release, improving immune system, fighting cancer and aging, and improving adrenal function (Umeyama et al., 1992; Gaffney et al., 2001). However, the poor and/or even failed seed setting, seed dormancy and over-exploitation always puzzle this species (Yu et al., 2003). Thus, improving its propagation efficiency on enhancing yield and quality to achieve efficient farm

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Abbreviation: ES, *Eleutherococcus senticosus*; **RAPD,** randomly amplified polymorphic DNA; **GA_3,** gibberellic acid; **MS,** Murashige and Skoog; **2,4-D,** 2,4-Dichlorophenoxyacetic acid; **LEDs,** light emitting diodes; **JA,** jasmonic acid; **ROS,** reactive oxygen species; **hLf,** human lactoferrin; ***PgSS1*;** squalene synthass-encoding gene derived from *Panax ginseng*; **ISSR,** inter-simple sequence repeat.

cultivation and considerable economic benefits has become an important issue. To achieve this goal, many investigations have been reported, including conventional propagations, habitat conditions, molecular classification, and mass production through *in vitro* tissue cultures.

Conventional propagations of this species have two means: seed propagation and stem cutting propagation. However, until now, two propagations are still considered difficult because of long-term stratification prior to the maturation of the zygotic embryos in mature seeds or difficultly rooting induction from stem cuts (Isoda and Shoji, 1994). Based on this situation, plant cell culture techniques have been applied as a new means for propagation of this species (Choi et al., 1999a, b). To date, a high frequency of mass production system of ES plantlets has also been established (Choi et al., 2002; Jung et al., 2004), and the produced plant cells or/and tissues, and plantlets through various *in vitro* culture techniques, have been directly applied as a source of secondary products and medicinal raw materials (Fowler, 1983; Jung et al., 2004), just like *Panax ginseng* (Furuya et al., 1983).

Due to no comprehensive review of the biotechnological effects on ES to date, we here, summarize the currently available scientific information on ES, aiming to provide the basis for further understanding of this species.

PROPAGATION IN KOREA

Due to the over-exploitation of ES, combined with poor seed setting and/or failure to set seed (Yu et al., 2003), this plant has become an endangered species in several countries, and even classified as rare, protected plants by the Environmental Ministry in Korea (Jung et al., 2004). To develop the farm cultivation of ES, many investigations are involved in the natural growth conditions of habitats. As known that the region, Hokkaida in Japan is the location adapting to the natural growth and seed production of ES, Park et al. (1995) therefore compared the natural condition factors such as local temperature and sunshine duration of Hokkaida in Japan with several locations in Korea to select a proper seed production site in Korea. This investigation suggested that Daegwanryeong in Korea is the most suitable for ES cultivation from seed propagation, for that its climate characteristics are mostly similar to those of Hokkaido. In the further investigation, Park et al. (1996) mentioned Mountain Deokyu situated at 127º45'E, 35º52'N, is one of main habitats of ES in Korea. To understand more habitat information to instruct farm cultivation of ES in Korea, Park et al. (1996) surveyed the local climate, soil components, and symbiotic plant species as information inferences. To optimize the cultivation conditions of ES, Han et al. (2001) investigated the effect of shading treatments on the growth of ES, and suggested that 50% shading net treatment was most effective for yield. Not only apparent quantum yield but carboxylation efficiency and re-phosphorylation could be increased by shadding treatments (Kim et al., 2003).

Since long-term stratification during afterripening period is required to induce maturation of the zygotic embryos in mature seeds (Isoda and Shoji, 1994), Park et al. (1997) studied the characteristics of embryo elongation after stratification and the dehiscence rate during afterripening period, that helped improve seed propagation of this species. In addition, seed dormancy also entangles germination and propagation of this species (You et al., 2005). To date, several studies have been attempted to break seed dormancy in order to promote the seed germination. For example, Li et al. (2003a) investigated a method for breaking the physiological dormancy of dehisced ES seeds, and suggested that storage at 5°C for 85 d could most effectively increase germination rates up to more than 90%. In the report by Li et al. (2003b), they performed cold stratification before sowing combined with gibberellic acid (GA_3) soak. This result showed 10 d-cold stratification at 4°C following after ripening and soaking in 500 ppm GA_3 for 3 d could also effectively promote germination. As the positive affect of GA_3 soak on germination, Lim et al. (2008) also applied this method as pretreatment of ES seeds, however, due to the different experimental materials and specific sensitivity to GA_3 soak, they elucidated that the optimal GA_3 concentration was 300 mg/l for promoting the seed afterripening. Toros sterilization was also synchronously performed in ES seeds, showing positive effect on reducing dehiscent rates and suppressing fungi actions.

Except of seed propagation, stem cutting propagation is widely used for ES propagation, however, difficult rooting is a major problem to resolve. Park et al. (1994) suggested that rooting could be successfully induced from cut of stems after 3 ~ 12 d-culture, and the season for cutting propagation is also important, the late September being the best cutting season in Korea. Han et al. (2001) indicated that up-ground 30 cm length cutting was the most effective for branching stem length, plant height and yield.

Despite a great process has been achieved on conventional propagation, this propagation pattern is known limited by some disadvantages, such as requiring enormous time and labor, and particularly long-term stratification for ES (Choi and Jeong, 2002). Thus, the establishment of more efficient propagation methods is urgently needed.

IN VITRO PLANT REGENERATION

Based on the plasticity and totipotency of plants, tissue culture technology has now become a remarkably useful tool in experimental studies, such as rapidly achievement

of plant breeding and mass propagation. Through one hundred years' investigation, plant tissue culture technologies have achieved a great progress in many aspects including the effects of plant growth regulators, auxins, and cytokinins, genotype-dependence, and callus type-dependence. For ES, *in vitro* tissue culture and plant regeneration has been firstly reported through direct secondary somatic embryogenesis from immature zygotic embryos (Gui et al., 1991). Later, Somatic embryos were produced directly from the surface of zygotic embryos of this species without forming an intervening callus (Choi and Soh, 1993). In this report, two kinds of somatic embryos were induced from various explants, including hypocotyls, cotyledon, radicle: one was single embryos with closed radicle mainly formed on cotyledon and radicle, the other was polyembryos mainly formed on hypocotyls.

To improve the *in vitro* tissue culture conditions, Yu et al. (1997a, b) attempted to induce embryogenic callus from immature embryos, and obtained high callus formation of 83% on modified SH medium and 100% on B5 medium with 2,4-D addition. Plant regeneration capability of embryogenic callus was different depending on the mature degree of the explants, immature embryos. Choi et al. (1999a) established a high frequency of plant production *via* somatic embryogenesis from callus with cultured on Murashige and Skoog (MS, Murashige and Skoog, 1962) medium with 4.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D) for somatic embryo induction and then MS medium lacking 2,4-D before plant regeneration. In the following report by Choi et al. (1999b), various explants such as cotyledon, hypocotyl and root were investigated in plant regeneration *via* direct somatic embryogenesis, of which hypocotyls segments showed the highest somatic embryo formation frequency (75%). This report obtained the highest germination rate of 93% from somatic embryos, and thus established an efficient means for mass propagation though somatic embryogenesis of ES. As known that the somatic embryogenesis and plant regeneration in plants were genotype-specific and explants-specific (Liu et al., 2004; Sun and Hong, 2010), Li and Yu (2002) investigated somatic embryogenesis from various explants including young leaf, stem, node, petiole, peduncle, flower and root using three different genotypes of ES Korean, Russian and Japanese accessions. In this report, the highest callus formation frequency was obtained from flower explants, and normal plantlets were produced from somatic embryos when transferred to 1/4 MS medium.

To achieve *in vitro* mass propagation of ES, cell suspension cultures using hypocotyls-derived callus have been conducted by Choi et al. (1999a). However, the somatic embryo formation capacity of suspension cultured cells was significantly lower compared to that from callus cultures. Later, improved cell suspension cultures were observed that 35 g dotyledonary embryos (about 12,000) were converted to 567 g fresh mass of plantlets with initially culture in 500-ml flask, followed by culture in 10L plastic tank, and then low-strength MS medium (Choi et al., 2002). This report established an efficient protocol for the mass production of ES plantlet from tank culture of somatic embryos. In the year 2003, the *in vitro* mass propagation conditions were further improved by shortening the maturation time from immature zygotic embryos to somatic embryos within one month (Han and Choi, 2003). Based on the above results, it indicated that *in vitro* mass propagation could be practically applicable for systematic procedure of plant production of ES, and the *in vitro* plantlets could be satisfied as a source of medicinal raw materials.

In addition, Choi and Jeong (2002) overcame the disadvantages of conventional propagation, and reported firstly encapsulated somatic embryos as ES artificial seeds to achieve all development status from artificial seeds to whole plants. As known that seed dehydration accompanied by the maturation of zygotic embryos results in the dormancy of zygotic embryos (Gray et al., 1987), thus, the desiccation of somatic embryos not detrimental to survival is very efficient in the long-term conservation of somatic embryos. In light of these theories, Choi and Jeong (2002) investigated the dormancy characteristics of ES somatic embryos and induced encapsulated somatic embryos maintain in the dormancy status by a high sucrose treatment. Moreover, maintaining ES somatic embryos from cell suspension cultures under low temperature (4℃) was also considered to be able to achieve long-term animatingly conservation (Li et al., 2004). These treatments help a long-term conservation of artificial seeds and an enhanced resistance to dehydration of somatic embryos. However, the artificial seeds of encapsulated somatic embryos have one disadvantage of low soil survival (Redenbaugh et al., 1986). To resolve this problem, Jung et al. (2004) determinate the roles of addition of carbon sources to the encapsulation matric, and suggested that this treatment could enhance post-germinative growth of embryos, when adding 2% sucrose and 1% starch powder having the highest efficiency. You et al. (2005) carried out that removal of endosperm from seeds could markedly stimulate the growth of rudimentary zygotic embryos to induce more rapid germination of rudimentary zygotic embryos by *in vitro* culture of excised seeds and in their later investigation (You et al., 2006), the roles of plasmolyzing pretreatment for zygotic embryos were evaluated on the induction of somatic embryos, suggesting that this pretreatment could result in sharply increased callose concentration in ES zygotic embryos, and callose accumulation could then stimulate the reprogramming of epidermal cells into embryogenically competent cells and finally induce somatic-embryo development from single cells. The further and detailed mechanism of enhanced somatic embryo formation through plasmolysis treatment was revealed that the expression level of callose synthase gene increased with

response to 2,4-D, sucrose, and mannitol, and the callose played an important role in separating cell in epidermis from neighboring cells and consequently developing into embryogenic potential cells (Xilin et al., 2010).

APPLICATION FOR BIOCHEMICAL EVENTS

Somatic embryogenesis has been studied as a model system for understanding the physiological, biochemical, and molecular biological events occurring during plant embryo development (Zimmerman, 1993). Among them, production of secondary metabolites through cell culture, particularly in medical plant, has long been used for commercial purposes (Roberts, 2007). To improve the culture conditions and then increase the production efficiency, many scientists have been investigated many factors affecting growth of culture materials and *in vivo* accumulation of active compounds. Ahn et al. (2003) investigated the effect of inorganic nitrogen sources such as KNO_3 and NH_4NO_3 on cell growth and production of chlorogenic acid and eleutheroside E derivative. In another investigation by Ahn et al. (2007), the effect of NO_3^- and NH_4^+ on the adventitious root growth of ES and production of eleutheroside derivatives were investigated, and eleutheroside B (249 µg/g), E (788 µg/g), and E1 (43 µg/g) were increased at the highest levels by 40, 120, and 40 mM total nitrogen source, respectively. These results suggested that production of secondary metabolites through *in vitro* cultured cells could be manipulated by controlling the total concentration of nitrogen sources and the concentration ratio of NO_3^-/NH_4^+ in the culture medium.

Except of nitrogen sources, light is another important factor affecting growth and organogenesis, but a factor stressing plants to consequently regulate the secretion mechanism of secondary products (Shohael et al., 2006a). Higher H_2O_2 content, malondialdehyde content and lipoxygenase activities were observed in cultured embryos under red light compared dark grown embryos, as well as activities of some antioxidant enzymes such as catalase, superoxide dismutase, glutathione *S* transferase, and ascorbate peroxidase were also stimulated in red light irradiated embryos. Of course, the contents of eleutheroside E and E1 were synchronously accumulated 51 and 21% higher than control under red light irradiation. Jeong et al. (2009) compared the effects of red, blue, and far-red light by irradiaton of light emitting diodes (LEDs) with white fluorescent lamp, on growth, morphogenesis and eleutheroside contents of *in vitro* cultured ES. The results indicated that *in vitro* cultured plantlets under the red/blue LEDs were taller than control, and those under blue LED showed greater leaf area, root length, and fresh weight than other light sources. Contents of eleuthroside B and E in plantlets were higher under blue LED, while content of eleuthroside E1 was the highest under fluorescent lamps.

Ahn et al. (2007, 2010) investigated the impacts of jasmonic acid (JA) on adventitious root culture of ES and eleutherosides accumulation, suggesting that JA inhibited the root growth but increased eleutherosides accumulation, as well as total phenolic contents and antioxidant activity. The highest levels of accumulation of eleutheroside B (359.9 µg/g), E (798.1 µg/g), and E1 (197 µg/g) were found under 40, 10, and 10 µM of methyl jasmonate addition, respectively.

Effects of temperature on secondary metabolite production such as eleutheroside B, E, E1, total phenolics, flavonoids, and chlorogenic acid and antioxidant enzyme activities were investigated by Shohael et al. (2006b), suggesting that culture at 24°C caused the highest production efficiency of secondary metabolites, and either lower or higher temperature could cause severe oxidative stress to form a cellular damage.

Based on above results, the production of secondary metabolites, one side, was considered as the consequent result of cultured cell metabolism, the other side, as the outcome stimulated by some stress treatments. Therefore, to control the balance between reactive oxygen species (ROS) formation derived by stress treatments and consumption correlated with an array of antioxidant enzymes and redox metabolites becomes required and important. Shohael et al. (2007) further examined the ascorbate-glutathione cycle enzymes and other enzymes metabolism during somatic embryogenesis of ES, and suggested that the alterations of the glutathione redox systems play a significant role in somatic embryo development.

APPLICATION FOR MOLECULAR BIOLOGICAL EVENTS

To authors' knowledge, only two transformation events through Agrobacterium-mediated transformation occurred in ES. Jo et al. (2005) transformed the human lactoferrin (*hLf*) gene into ES cells, and these transgenic ES cultured cells could produce *hLf* protein as cell growth increasing proportionally.

As lactoferrin is an iron-binding glycoprotein with many biological roles, including the protection against microbial and virus infection and stimulation of the immune system, *hLf* transgenic ES plants could be used as a medicinal raw material for production of secondary metabolites. Another successful transformation event of ES was obtained in the report by Seo et al. (2005) that a squalene synthass-encoding gene derived from *Panax ginseng* (*PgSS1*) was successfully introduced into ES plants through Agrobacterium-mediated transformation. The transgenic plants showed up to 3-fold of squalene synthase enzyme activity higher than that of wild-type plants. Moreover, the introduced *PgSS1* gene in transgenic plants enhanced the metabolisms of phytosterol and triterpenoides, with 2 ~ 2.5-fold increments of their levels. These results indicated that transgenic ES

cultured cells would be biotechnologically useful for the commercial production of medicinal plant cell cultures.

CLASSIFICATION

To identify the *Eleutherococcus* species and further understand this genus, Kim et al. (1997) studied morphological and anatomical characteristics of five *Eleutherococcus* species. Among them, ES showed the highest identification with *E. chiisanensis* based on their morphological data, however, both species could be distinguished by anatomical characteristics such as cork thickness, secondary phloem and cork cell layer number. It indicated that the leaf and spine shapes were the main factors to identify the *Eleutherococcus* species, and the numbers and length of spine on the stem were prominent characters to identify ES. To describe the infraspecific genetic structure of ES in Korea, Hong et al. (2000) investigated eight Korean accessions, one Russia accession and one China accession using inter-simple sequence repeat (ISSR) markers. There were relatively high genetic variations among all accessions as 62.8%, but relatively low variation within eight Korean accessions. Later, randomly amplified polymorphic DNA (RAPD) analysis was performed to analyze genetic structure among 10 different populations from Deokyu, Bukhaedo and Odae using 20 primers. These populations were divided into two major groups at the similarity coefficient value of 0.65, of which Deokyu 5 showed the highest genetic variation with other populations (Lim et al., 2000). In further investigations (Yu et al., 2003), RAPD analysis was conducted using 10 primers among 10 *Eleutherococcus* collections differed from geographical regions. The 10 collections were classified into two groups at a similarity coefficient of 0.50. One group included ES from Bukhaedo, Japan, *E. sessliliflorus* from Youngwal, Korea, *E. seoulense* and *E. chiisanesis*, while the other group included several internal and Russian collections.

OTHER INVESTIGATION ON ES

In markets, the dried cortical tissues of the roots and shoots are used for various medicinal purposes (Brekhman, 1960; Choi et al., 2002), thus, the processing and drying condition received attentions and became important: one side, it did not affect material quality such as material color; the other side, it must keep the least loss and change of active compounds. To satisfy these demands, Jeong et al. (2010) examined cortex color, dry weight ratio change, and effective compound contents including Eleutheroside B and E, phlophenolic and flavonoid compounds, acid Insoluble ash, and water extract under different steaming times and drying treatments, and suggested that less than 20 min-steaming for peeling bark could maintain high quality of ES cortex, drying at 50°C was considered as the most optimal for drying and keeping effective compound contents. Keon and Park (2008) described pharmacognostical characteristics of 9 *Eleutherococcus* species, including ES as well as *E. chiisanensis*, *E. divaricatus*, and other *Eleutherococcus* species.

Choi et al. (2007) investigated and identified three diseases commonly coming on ES, including black ring spot caused by *Phoma* sp., gray mold caused by *Botrytis cinerea* and leaf blights caused by *Rhizoctonia solani*, and three insect pests including aphids, stinkbugs and *Bothrogonia japonica*. This result would help understand prevention and cure of diseases and insect pests in this species.

To meet commercial needs of ES, Jeong et al. (2008) selected two new cultivars ‘Cheonsu’ and ’Misu’ from 896 wild-type ES populations collected from 35 different regions, and the selected two cultivars showed high biomass yield, high fruiting capacity, and strong disease tolerance.

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