

# Advances in Ginseng Tissue Culture Research

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**Abstract.** *Panax ginseng* C.A. Meyer is a traditional and precious Chinese medicinal herb that is widely used in the fields of food, medicine, and cosmetics. This article provides a comprehensive overview of the research progress on *Panax ginseng* C.A. Meyer tissue culture from four aspects: the current utilization of *Panax ginseng* C.A. Meyer resources, *Panax ginseng* C.A. Meyer adventitious root culture, hairy root culture, and elicitor. The aim is to provide a reference for the rapid acquisition of secondary metabolites of *Panax ginseng* C.A. Meyer and promote the development and sustainable utilization of *Panax ginseng* C.A. Meyer resources.

**Keywords:** plant tissue culture; *Panax ginseng* C.A. Meyer; adventitious root; hairy root.

## 1. Introduction

*Panax ginseng* is the dried root and rhizome of *Panax ginseng* C.A. Meyer, a perennial herb in the family Panaxaceae, which translates to "cure all diseases" in Greek, emphasizing the high status of *Panax ginseng* C.A. Meyer in herbal medicine [1, 2]. Modern studies have shown that *Panax ginseng* C.A. Meyer based on ginsenosides, *Panax ginseng* C.A. Meyer polysaccharides, peptides, volatile oils, proteins, amino acids, vitamins, organic acids, and flavonoids have a wide range of pharmacological activities[3-5], including antitumor[6], antioxidant[7],anti-inflammatory[8], antiviral[9],antidiabetic[10],anti-osteoporosis[11],immunomodulatory[12],neuroprotective[13], anti-aging[14]and anti-atherosclerotic[15]. In China, wild ginseng mainly grows in the long and narrow area of Changbai City, Jilin Province, and its supply is currently very limited [16, 17]. Furthermore, the artificial cultivation of ginseng has a long growth cycle and is greatly affected by climate, environment, and cultivation conditions, as well as pests and diseases such as red skin disease, root rot, and rust disease [18, 19]. Field cultivation often involves the heavy use of pesticides, leading to serious issues of pesticide residues and heavy metal residues [20]. The tissue culture of medicinal plants is an effective way to address these problems. To meet the increasing clinical demand for active components of ginsenosides, tissue culture technology is used to obtain ginseng tissue cultures and produce ginsenosides, thus resolving the supply-demand conflict of ginseng medicinal materials.

## 2. Ginseng Tissue Culture Technology

### 2.1. A Brief History of Plant Tissue Culture Technology

Plants, due to their high regeneration capacity, typically exhibit significant developmental plasticity. Under suitable conditions, plant cells are totipotent, with a single cell able to develop autonomously into a complete plant through embryogenesis [21]. Based on this theoretical foundation, plant tissue culture technology came into being. Plant tissue culture refers to the aseptic cultivation of cells, tissues, organs, and their components under specified physical and chemical conditions outside the plant, to obtain cells, tissues, or whole organisms [22]. It is an indispensable and valuable technology for studying morphogenesis, embryogenesis, clonal propagation, crop improvement, production of pathogen-free plants, gene transfer and expression, as well as secondary metabolite production. Plant tissue culture is a fundamental and applied aspect of plant biology.

The era of plant tissue culture technology began in the early 20th century, and the concept of tissue culture was first proposed by the Austrian botanist Gottlieb Haberlandt (1854-1945) in a lecture delivered to the German Academy of Sciences in 1902, regarding experiments on single-cell

culture[23, 24]. He believes that it is possible to cultivate individual nutrient cells of higher plants in a simple nutrient solution to successfully grow artificial embryos. Through multiple experiments, he attempted to cultivate differentiated epidermal cells of *Ornithogalum* and other species in an artificial culture medium but was unsuccessful in each attempt[25]. Based on his 1902 lecture and his pioneering experiments before and after, Haberlandt is recognized as the father of plant tissue culture[22].

In 1922, Robbins and Kotte successfully cultured root tips of peas and corn in different synthetic media, achieving the earliest successful organ cultures[26, 27]. Under sterile conditions, White (1934) established isolated tomato root tip cultures in a simple liquid medium of known composition. Excised tomato root tips could grow indefinitely in the liquid medium, marking the first successful experiment in sustaining growth and cell division in plant tissue culture[28]. Ball (1946) obtained complete plants from cultured shoot meristems. This heralded the advent of *in vitro* asexual propagation methods, with Ball being regarded as the father of what's known as micropropagation [29, 30]. Subsequently, the observation of substances related to plant growth and differentiation—plant hormones auxin and cytokinin—and their specific ratios regulating shoot and root regeneration, propelled the development of plant tissue culture techniques[30-32]. By the late 1950s, Reinert and Steward became the first to confirm Haberlandt's prediction that embryos can be regenerated from cultured differentiated somatic cells[33-35]. The plant regeneration pathway later came to be known as "somatic embryogenesis," with initiation restricted to single cells[36].

Based on the aforementioned research, the scientific community can now employ a new set of procedures and techniques to control the regeneration and morphogenesis of plants in culture. Plant cell, tissue culture, and regeneration systems have been successfully applied to hundreds of plant species and various explants for plant propagation, resulting in the production of plants with desirable traits.

With the increasing demand for food, medicine, spices, cosmetics, and renewable fuels, plants have become an attractive natural source for high-value products such as secondary metabolites. However, secondary metabolites from plant sources typically have long production cycles, stringent growth conditions, and low abundance, making large-scale isolation and purification unfeasible. The use of plant tissue culture technology can overcome these difficulties [37]. Plant tissue culture technology is not affected by natural environmental factors such as seasonality, climate, and geography. Controlling and optimizing various production parameters, allows for the artificial regulation of the production of secondary metabolites, making it a high-quality product that can be cultivated under controlled, large-scale, and standardized conditions.

The study of cell culture from plant tissues and organs has a history of more than 100 years. Benefiting from breakthroughs in the intrinsic physiological mechanisms of plants and the rapid development in fields such as information technology, engineering, instrumental analysis methods, and materials science, innovative breakthroughs have been made in plant tissue culture technology. The combination of traditional tissue culture techniques with artificial intelligence modeling methods can be effectively used for the prediction and optimization of parameter conditions in plant tissue culture [38, 39]. Nanotechnology has also shown positive effects in eliminating microbial contaminants, improving seed germination rates, inducing callus tissue responses, somatic embryogenesis, organogenesis, enhancing plant growth and yield, secondary metabolite production, somatic cell cloning variations, protection or management of plant diseases, plant genetic engineering, and plant genetic modification [40]. The integration of new materials and methods with plant tissue culture technology has pushed the development of plant tissue culture technology to a new level.

So far, plant cell and tissue culture technology has become increasingly mature. Not only has it achieved the regeneration of whole plants through single-cell culture, but it has also promoted the production of natural secondary metabolites using cell and organ cultures. Through plant tissue culture technology, the industrial-scale and large-scale production of valuable secondary metabolites

not only helps meet the needs of human health and life but also contributes to the protection of the natural ecological environment and the promotion of the sustainable development of human society.

## **2.2. Ginseng adventitious roots**

Adventitious root culture is employed to harvest secondary metabolites from plants with significant medicinal value, which are then used in the production of pharmaceuticals, food products, dietary supplements, and cosmetics, providing an alternative strategy for the propagation of medicinal plants and the production of natural active compounds [41, 42].

The application of different ginsenosides affects the biomass of adventitious roots. When cultivated ginseng adventitious roots were treated with different concentrations of Rb1 and Re for 45 days, it was found that cell proliferation and adventitious root branching decreased in the presence of ginsenoside Rb1 and high concentrations of ginsenoside Re but increased when treated with low concentrations of Re[43].

The growth and differentiation ability of ginseng adventitious roots may be influenced under different incubation temperatures (15, 20, 25, and 30°C for 35 days). Research indicates that low-temperature stimulation can promote the accumulation of ginsenosides, with the total content of ginsenosides increasing by 2.53 times under conditions of 10°C for 7 days followed by 25°C for 28 days, compared to the temperature of 25°C[44].

Ginseng adventitious root extract (GARE) significantly reduces blood glucose levels in mice, while also lowering the levels of total cholesterol (TC), triglycerides (TG), and glycosylated hemoglobin (GHb). This indicates that GARE may have potential therapeutic effects on type 1 diabetes (T1DM) and provides a theoretical basis for the development and utilization of ginseng adventitious roots (GAR) resources[45].

## **2.3. Ginseng hairy roots**

The hairy root culture technique is a novel plant tissue culture technology that developed in the late 1980s. It involves the integration of T-DNA fragments from the Ri plasmid carried by *Agrobacterium rhizogenes* into the nuclear genome of plant cells, inducing the production of abundant byproducts, namely hairy roots, thus establishing a hairy root culture system[46, 47].

Using the plasmid construct containing the *rolA*, *rolB*, and *rolC* genes isolated earlier from the TL-DNA of *Agrobacterium rhizogenes* for the transformation of ginseng cell cultures (strain 1c). The levels of ginsenosides in the resulting transgenic tissue were measured to assess the potential role of the *rol* genes in ginsenoside formation, and it was found that the plant oncogene *rolC* is responsible for the increased ginsenoside formation in ginseng hairy root cultures[48].

The transformation efficiency and biomass production of five distinct ginseng genotypes were evaluated. Of them, the ‘Yunpoong’ cultivar, and a local landrace ‘Ganghwa’ had the highest transformation efficiencies of 66.11% and 65.00%, respectively. The biomass production of transgenic hairy roots was 1.5–2.1 times faster than that of non-transgenic adventitious roots without hormone supplementation[49].

The allelopathic autotoxicity of ginsenosides is an important factor contributing to the continuous cropping obstacle in ginseng cultivation. Ginseng hairy roots exhibit more severe cell apoptosis under the stress of low-polarity ginsenoside component (ZG70). ZG70 exerts allelopathic autotoxicity by regulating key enzyme genes in the biosynthetic pathways of *cis*-zeatin (*cZ*), indole-3-acetic acid (IAA), and jasmonic acid (JAs) signaling transduction pathways[50].

## 2.4. Ginseng inducer

The term "elicitor" originated in the 1970s, when scientists referred to small molecular polysaccharides and proteins produced by plant pathogens as "elicitors."

From the perspective of plant pathology, an elicitor is a chemical substance or biological factor that can induce plants to produce antitoxins (phytotoxins) and hypersensitive reactions to protect themselves by triggering the plant's disease resistance response[51]. From the perspective of plant tissue culture, an elicitor is a chemical substance or biological factor that can promote the production of target metabolites in plant cells and cause physiological changes in a certain tissue. Therefore, elicitors are often used to increase the content of secondary metabolites in medicinal plants and the study of metabolic regulation mechanisms.

A biotic elicitor is an important biotechnological strategy to promote the production of metabolites in adventitious root culture. Co-cultivation of ginseng adventitious roots with immobilized spores of *Aspergillus niger* (ISAN) enhances the accumulation of ginsenosides. Under the conditions of 102 spores/mL ISAN concentration, 4% volume ratio, and a co-cultivation time of 24 hours, the maximum content of ginsenosides ( $26.43 \pm 0.49$  mg/g) was achieved, which was 3.09 times higher than that of ginsenosides without the addition of ISAN[52].

The impact of endophytic bacterial elicitor on biomass and ginsenoside production in ginseng adventitious root cultures. The endophytic bacterium LB 5-3, as an elicitor, can increase the biomass and ginsenoside accumulation in ginseng adventitious root cultures. After induction with 10.0 mL of the LB 5-3 strain for 6 days, the total content of ginsenosides reached 2.026 mg g<sup>-1</sup>, which was four times higher than that in unchallenged roots[53].

In the future, *Alternaria panax* mycelia can serve as an economical elicitor for commercial production of ginsenosides in ginseng adventitious root cultures. The concentration and duration of mycelia elicitor significantly influence the accumulation of ginsenosides, with the highest content achieved when 30-day-old adventitious roots were treated with 200 mg/L mycelia elicitor for 8 days. In comparative experiments, the ginsenoside accumulation induced by the mycelia elicitor was similar to that induced by mycelia extract, with ginsenoside content reaching approximately 25 mg/g DW, significantly higher ( $p < 0.05$ ) than methyl jasmonate[54].

Abscisic acid (ABA) was involved in the cold-induced accumulation of ginsenosides Rg1 and Re in *P. ginseng* adventitious roots (PGARs), and exogenous application of ABA efficiently promoted the accumulation of ginsenosides Rg1 and Re in PGARs[55].

## 3. Summary

As a traditional and valuable Chinese medicinal herb, ginseng has extremely high application value. Through research on ginseng tissue culture, not only can rapid reproduction of ginseng be achieved, but also the content of its main medicinal components can be increased, which is of great significance for the long-term development of traditional Chinese medicine and environmental protection.

In addition, plant metabolic processes are complex, and analytical techniques are relatively underdeveloped. The metabolic pathways of most active substances are still unclear, and due to technical difficulties, the yield of metabolites in plant tissue cultures is relatively low. With the emergence and application of new technologies and analytical instruments in the future, researchers will gradually gain a clearer understanding of the metabolic networks of plants. Plant tissue culture technology will gradually become more precise and scientific, meeting the requirements for large-scale production and industrialization of more precious active substances.

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