

Research Article

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American Ginseng: Research Developments, Opportunities, and Challenges

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American ginseng (*Panax quinquefolius* L.) is grown in some regions of the USA and Canada and marketed for its health promoting attributes. While cultivation of this plant species has taken place in North America for over 100 years, there are many challenges that need to be addressed. In this article, the current production method used by growers is described and the challenges and opportunities for research on this valuable plant are discussed. These include studies on pharmacological activity, genetic diversity within the species, genetic improvement of currently grown plants, molecular characterization of gene expression, and management of diseases affecting plant productivity. The current research developments in these areas are reviewed and areas requiring further work are summarized. Additional research should shed light on the nature of the bioactive compounds and their clinical effects, and the molecular basis of active ingredient biosynthesis, and provide more uniform genetic material as well as improved plant growth, and potentially reduce losses due to pathogens.

Keywords: *Panax quinquefolius*, Genetic variation, Disease, Transgenic research

INTRODUCTION

Ginseng (*Panax* spp.) is a slow-growing perennial herbaceous plant (Order Umbelliferales, Family Araliaceae) that is grown for its highly valued root, which provides a source of vitalizing and stimulating agents [1,2]. There are more than six described *Panax* species, including *Panax quinquefolius* L. (American ginseng) and *Panax ginseng* Meyer (Ginseng), which are the two predominant species grown commercially in North America and Asia, respectively [3]. American ginseng is native to eastern North America, and grows as an understory plant in deciduous and mixed forests in regions of the northeastern states of the United States and the Canadian provinces of Quebec and Ontario [2]. Commercial cultivation occurs in Wisconsin, Michigan, North Carolina, Ohio, Tennessee and several other states in the US, while Ontario and British Columbia represent the major grow-

ing regions in Canada. Cultivation of *P. quinquefolius* in Canada began over 100 yr ago when it became apparent that harvesting of existing wild populations was leading to their extinction. Under commercial cultivation, an average of 3 to 4 yr of growth is required in order for the roots to reach a marketable size. Artificial shade is provided over the crop (Fig. 1A) using a synthetic shade cloth to ensure that only about 30% of the incident sunlight reaches the plants below (Fig. 1B) to mimic the conditions found in the forest habitat [2]. A layer of straw mulch, consisting primarily of wheat or barley, is placed on the soil surface to reduce damage due to cold weather in the winter and to maintain soil moisture in the summer. During the third and fourth years of growth, the plants produce an umbel-shaped inflorescence (Fig. 1C) and the flowers develop into mature red berries over the

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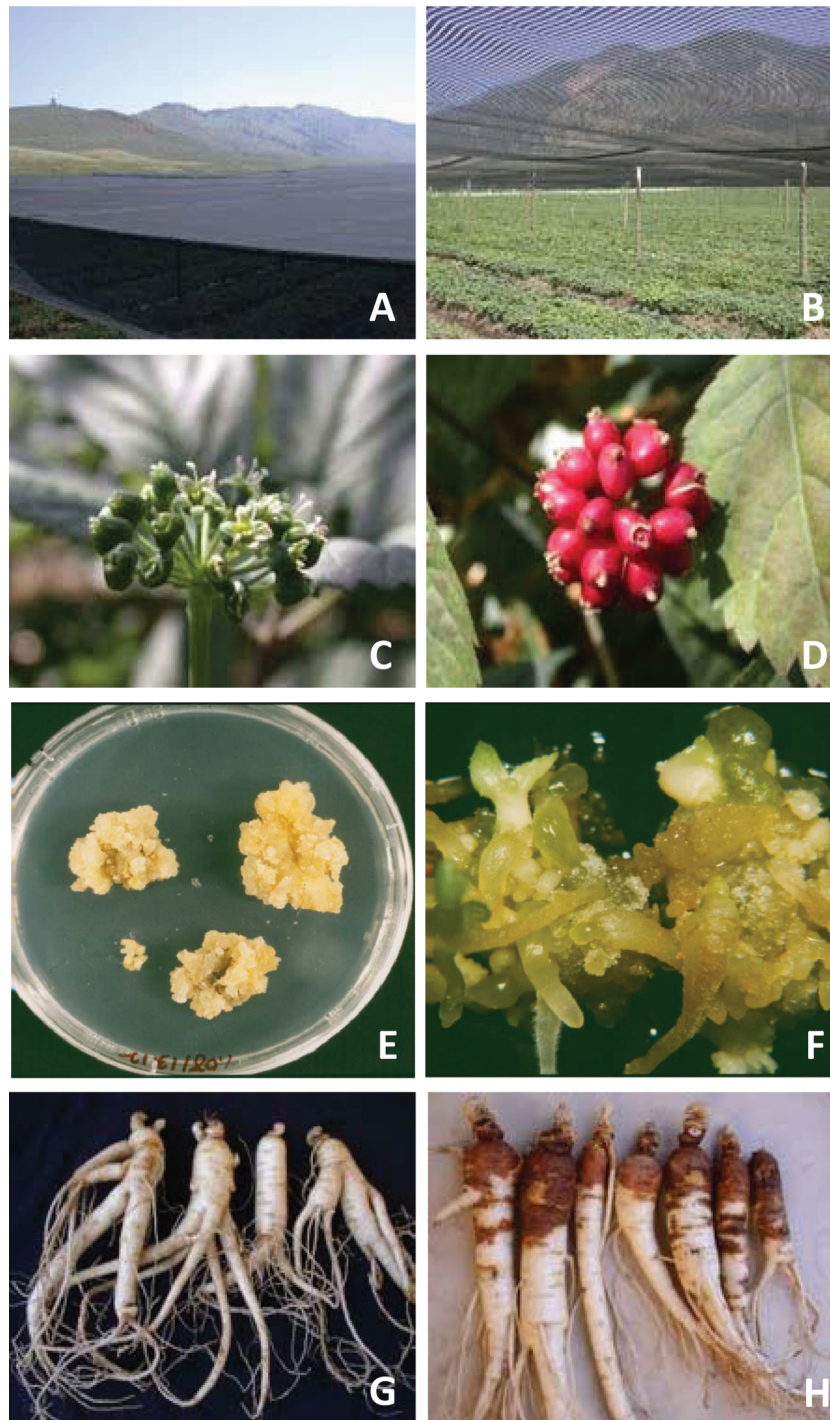


Fig. 1. American ginseng production. (A) Artificial shade cloth used during commercial production. (B) A view of the ginseng plants under the shade cloth. (C) Young developing ginseng inflorescence, showing flowers and green berries. (D) Mature red berries. (E) Tissue culture of ginseng root explant, showing callus development. (F) Somatic embryo and root formation on differentiated ginseng callus. (G) Healthy four-year old American ginseng roots. (H) Roots with extensive reddish-brown discoloration on the crown area due to the rusty root problem.

ensuing 8 to 12 wk period (Fig. 1D). The seeds (ranging from 1 to 3) contained inside the berries are used to propagate the next crop after a period of prolonged seed stratification cycle consisting of exposure to cool-warm-cool periods over 12 to 18 mo [4]. The commercial

production of American ginseng has encountered many challenges but there are also a range of opportunities and developments in research on American ginseng. These are discussed here with an emphasis on improvement of cultivation methods and productivity.

GINSENSIDES AND PHARMACOLOGICAL ACTIVITY

Compared to *P. ginseng*, which has been used as a medicinal plant for centuries and has been studied extensively for its pharmacological activity, the amount of information regarding *P. quinquefolius* is considerably less. However, a recent review by Yuan *et al.* [5] provides an assessment of the current understanding of the analysis and pharmacological effects of saponins found in *P. quinquefolius*. In addition, there are other references on this topic [1,6]. The roots contain valuable pharmaceutically-active components, which include ginsenosides (triterpenoid saponins), polyacetylenes, polyphenolic compounds and acidic polysaccharides. There are differences in the types of ginsenosides present between American and Asian ginseng. At least 30 of the possible 150 known ginsenosides are known to occur in *P. quinquefolius*. The major ginsenosides are Rb₁, Rc, Rd, Re, Rg₁ and Rb₃ (accounting for up to 70% of the total), while Rf is absent in American ginseng but present in Asian ginseng [5,7,8]. Ginsenoside levels are influenced by root age, and vary in different parts of the plant [8-10]. Highest levels are found in the leaves and root hairs, followed by the root and stem [8]. Ginsenosides levels also differ widely within and between plant populations [11,12]. The pharmacological effects, which involve the cardiovascular system, the immune system, the central nervous system as well as anti-cancer and anti-diabetes, are described by Yuan *et al.* [5] and are not discussed further here.

GENETIC DIVERSITY

There are currently no identified cultivars of American ginseng but efforts in selection for improved horticultural characteristics have been initiated. This is in contrast to Korean ginseng, where selection of pure lines over many years has led to improved varieties with enhanced growth, root quality and ginsenoside levels [13]. It is reported that cultivation of American ginseng originated from seed or plants collected from the wild, followed by exchange of materials within and between the major production areas. Therefore, plants grown within a commercial field may exhibit considerable phenotypic variability, such as in leaf size and shape, plant height, number of flowers, and root size [14]. This variation can also have an impact on ginsenoside content and levels in roots and eventually in commercial products of American ginseng. This variability could in turn potentially influence the outcome of pharmacological studies and affect the con-

sistency of results and therefore remains a challenge to be overcome.

To assess the basis of this genetic diversity, a number of molecular studies have been conducted. Using random amplified polymorphic DNA markers (RAPD), researchers have documented the extent of genetic diversity between cultivated and wild populations of American ginseng, between plants cultivated in different regions of Canada and the US, and between plants growing in a commercial field or originating from the same or different seed lots [14-16]. The findings affirm that American ginseng currently represents a heterogeneous mix of genetic material. The observed genetic diversity in cultivated populations in Canada have resulted largely from a mixing of different seeds lots. In addition, there is a level of heterozygosity within plants that can be maintained through cross-pollination [14]. This type of genetic diversity analysis if conducted on Asian ginseng populations would provide an interesting comparison to assess the extent to which genetic material may differ between different growing regions. The variation seen in American ginseng can be beneficial, however, as it provides a broad base from which to identify vigorous desirable plants for further selection and propagation. The broad-based genetic variation also could confer broader adaptive ability of the ginseng populations to varying biotic or abiotic stresses.

GENETIC IMPROVEMENT

The long reproductive cycle and difficulties associated with seed germination, and the lack of defined selection criteria, present difficulties in establishing a breeding program for variety development in American ginseng. Notwithstanding this, efforts have already begun to identify and select superior plants in research conducted in Canadian laboratories [17,18]. As a part of the efforts to initiate genetic improvement of American ginseng, several researchers have previously developed tissue culture methods for regeneration of ginseng plantlets from different explant tissue sources for propagation purposes [19-21]. This avoids the need for the prolonged seed stratification and germination phases required for normal seed development and germination. Starting with segments of leaf, or root zygotic embryos, the tissues are induced to produce callus (undifferentiated tissue) (Fig. 1G) on hormone-containing defined tissue culture media [19]. This is followed by a differentiation phase over a 3 to 6 month period in which either somatic embryos or shoots are produced (Fig. 1H). These somatic embryos can also

be multiplied in large numbers in a liquid suspension culture medium [20]. When rooted, the somatic embryos can give rise to plantlets which then have to undergo an acclimatization phase and be induced to develop a functioning root system [21]. The latter still remains a challenge to the high frequency plantlet recovery that would be required to make tissue culture propagation a practical tool in a breeding program. With further research, the potential still exists for the large-scale clonal propagation of ginseng plants to become a reality. Biochemical and molecular markers can then be used to confirm the genetic identity and stability of the tissue-culture derived plants [20]. Similar tissue culture approaches have also been described for Korean ginseng [22,23].

The ability to regenerate plants *in vitro* using the above tissue culture methods also provides opportunities for genetic transformation and expression of potentially useful novel genes in ginseng. Methods for genetic transformation have been developed and transgenic plants expressing novel genes have been described for American ginseng [24,25]. These have all utilized *Agrobacterium*-mediated gene transfer to achieve the expression of a gene encoding a chitinase protein origination from rice plants, a thaumatin-like protein also from rice, as well as a bacterially-derived gene conferring herbicide resistance [24,25]. This approach is still a long way from being utilized in a practical breeding program and the challenge remains as to whether these transgenic plants with foreign genes expressing unique traits would be acceptable for widespread use. This challenge is not unique to ginseng and is encountered in other crop species [26].

MOLECULAR STUDIES

DNA-based approaches can be used to distinguish between American and Asian ginseng and to establish their relationship to other species of the genus *Panax*. Both nuclear ribosomal DNA and chloroplast DNA have been examined [27,28]. These molecular approaches can also be useful for authentication of products containing fresh or dried roots [29-31]. These techniques can ultimately be used to provide a molecular fingerprint of selected desirable plants or varieties for identification purposes or proprietary protection. DNA markers have not yet been developed which can be used in marker-assisted selection for desired phenotypic traits, such as large root size or high ginsenoside content.

In the context of identifying genes that are expressed in ginseng plants, screening of expressed sequence tags (EST's) from American ginseng root complementary

(cDNA) libraries followed by sequencing of select differentially expressed clones has revealed a large number of genes (>175) with high similarity to those of other plant species found in published databases [32]. These include genes involved in metabolic pathways, cell communication, cellular transport, defense responses, and various other functions [32]. These types of studies are useful to provide a more thorough understanding of the biochemical pathways and gene products present in ginseng which could have a role in its pharmacological activity. Recent studies are identifying through transcript profile analysis putative genes involved in ginsenoside biosynthesis [33,34]. This type of research complements earlier studies conducted on Korean ginseng which revealed EST's associated with ginsenoside biosynthesis [35,36]. Proteomic studies have also been conducted with *P. ginseng* but not *P. quinquefolius*.

DISEASE MANAGEMENT

Undoubtedly the biggest challenge to American ginseng production is the management of diseases which render the ginseng root unmarketable due to infection primarily by fungal pathogens. These micro-organisms infect and damage both the foliage and the roots, causing considerable damage and eventual plant death [37]. The production environment for ginseng, in which thick straw mulch and a shade cloth are used during the summer to simulate a forest canopy environment, provide conditions that are conducive for the survival and multiplication of fungal pathogens. Research has shown that the most prevalent fungal pathogens infecting ginseng include *Cylindrocarpon destructans* (causing root decay) [38,39], *Phytophthora* (causing foliar blight and root rot) [37], *Alternaria alternata* and *Botrytis cinerea* (causing foliar and seed infection) [37], and *Fusarium* species and *Rhexocercosporidium* (causing root infection or rusty root) [40,41]. Many of these fungi have the ability to also colonize the developing berries through release of airborne spores and therefore potentially invade the seed [42]. This presents a challenge as the wide dissemination of ginseng seeds has the potential to also spread seed-borne pathogens from one region to another. In addition, many seedling-infecting fungi such as *Pythium* and *Fusarium* species, and *Rhizoctonia solani*, cause seedling death and reduce plant numbers [43,44]. Molecular techniques have now been developed which allow for the rapid identification of the pathogens infecting ginseng seed [41,42]. Growers currently have available only a few disease management options, which include the use

of fungicides (where registered) or soil amendments to reduce disease severity. Induction of naturally occurring defense responses in the roots through induction of gene expression by elicitors, an approach that has been used in other crops, is an avenue worthy of further investigation in ginseng [45]. Additional approaches to develop environmentally benign methods to ameliorate the impact of fungal pathogens include the use of biological control agents [46] or cover crops/green manure that would promote the establishment of antagonistic soil microflora. However, the perennial nature of ginseng roots presents a challenge with regard to whether these microflora could protect the roots over a 3 to 4 yr period.

A major problem that affects ginseng roots worldwide is the appearance of reddish-brown to orange-brown discolored areas on the root surface, referred to as rusty root or rust spot (Fig. 1H). This significantly reduces the economic value of the roots, in some cases by up to 40% [47]. The affected tissues display significant biochemical changes, including a build-up of phenolic compounds, accumulation of cations such as Fe, Mg, Si and Mg, as well as an increase in defense-related enzymes [47]. It was postulated that infection by fungal pathogens leads to the induction of these responses in the root. Rusty root remains a challenge in most ginseng production areas, and more research is required to understand the epidemiology of the fungi reported to induce these symptoms and their management.

FUTURE AVENUES FOR RESEARCH

There are many opportunities for additional research on American ginseng that can provide a better understanding of the genetic and biochemical basis for its reported health-promoting activities. As a step toward ensuring a more uniform end-product for use by consumers, more consistent levels of ginsenosides and other active metabolites need be achieved through cultivar development. There is sufficient variation currently present to allow for selection to be successful. The availability of specific DNA markers linked to phenotypic traits of interest would be valuable to assist with the selection and improvement of desired attributes. Advancements in molecular breeding and biotechnology which have been developed for many agricultural crops have not yet been utilized for ginseng. Furthermore, research on gene expression and gene regulation can provide useful information on the roles of specific proteins/enzymes that may be involved in the pharmacological activity of American ginseng and how these metabolites could be regulated.

There has been some progress in the utility of transcript profile analysis to elucidate the expression of genes in ginseng tissues and additional work would shed light on the specific genes involved in the complex biochemical pathways likely present in ginseng roots. The availability of transgenic technologies for ginseng would also allow for transgene expression analysis. On the applied side, research on ginseng pathogens and their management should be an on-going priority to avoid losses due to these diseases. Non-chemical approaches have not yet been researched sufficiently to warrant widespread use by growers. This could ultimately provide an opportunity to reduce reliance on fungicides. In addition, there may be opportunities to expand on the current American ginseng products by investigating niche opportunities that may include American 'red' ginseng or organically-grown ginseng in the future. Finally, the pharmacological activities of the American ginseng root components (saponins as well as other compounds) should be actively researched and compared in clinical studies to the Asian ginseng.

CONCLUSION

American ginseng has not been researched as extensively as *P. ginseng* at the present time but there are many opportunities to better understand and utilize this species for its health-promoting properties. Areas of research include, but are not limited to, cultivar development, molecular and genetic studies of bioactive compounds, improved production practices and disease management, and clinical studies on pharmacological effects compared to Asian ginseng.

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