Adventitious Roots and Secondary Metabolism

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Abstract: Plants are a rich source of valuable secondary metabolites and in the recent years plant cell, tissue and organ cultures have been developed as an important alternative sources for the production of these compounds. Adventitious roots have been successfully induced in many plant species and cultured for the production of high value secondary metabolites of pharmaceutical, nutraceutical and industrial importance. Adoption of elicitation methods have shown improved synthesis of secondary metabolites in adventitious root cultures. Development of large-scale culture methods using bioreactors has opened up feasibilities of production of secondary metabolites at the industrial levels. In the present review we summarize the progress made in recent past in the area of adventitious root cultures for the production of secondary metabolites.

Keywords: adventitious roots, bioreactor cultures, secondary metabolites

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, pigments, bio-pesticides and food additives[14,26]. Procurement of valuable secondary metabolites by plants under cultivation or from the plants grown in nature is not always satisfactory. It is often restricted to species or genus and might be activated only during a particular growth and developmental stage, or under specific seasonal, stress or nutrient availability. For these reasons in the past several decades a lot of effort has been put into plant cell cultures as a possible production method for plant secondary metabolites[27,31,32]. However, for many of the secondary metabolites of interest the production is too low in the cultured cells, despite extensive studies on the optimization of growth and production media and cell line selection for high production strains. This is usually due to the fact that metabolism is controlled in a tissue-specific manner, tissue de differentiation resulting thus in loss of production capacity. Therefore, root, embryo and shoot cultures have been focused as alternatives for the production of secondary metabolites[31].

Adventitious roots induced by in vitro methods showed high rate of proliferation and active secondary metabolism[19,41]. Adventitious roots are natural, grow vigorously in phytohormone supplemented medium and have shown tremendous potentialities of accumulation of valuable secondary metabolites. In this review we present advancement in the recent past with adventitious root cultures for the production of important secondary metabolites.

I Adventitious root cultures and production of secondary metabolites

Production of secondary metabolites from adventitious root cultures involves four discrete stages, namely, successful induction of adventitious roots from the desirable explants (callus mediated or direct induction, stage one; Figs. 1A, 1B); culturing of adventitious roots in liquid medium in flask-scale or bioreactor cultures and establishing growth kinetics (developing suitable medium components and cultural environment for the biomass and metabolite accumulation, stage two; Figs. 1C, 1D), developing strategies for higher accumulation of metabolites (elicitation strategy, medium or precursor feeding, stage three), culturing of adventitious roots in large scale using bioreactors (developing suitable methodology for large scale cultivation, stage four; Figs. 1E-1G). Downstream processing would be the last stage for the recovery of metabolites. Adventitious roots have been induced for many plant species and roots were cultured in flasks and bioreactors with the objective of production of secondary metabolites. A list of plants in which adventitious

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roots have been induced and cultured successfully for the production of secondary metabolites is given in Table 1. Some are reports of accumulation of secondary metabolites in the adventitious roots, whereas various other researchers have cultured adventitious roots in flasks or bioreactors, established the growth kinetics of adventitious roots and accumulation of secondary metabolites in the cultures. Adventitious roots were successfully induced in mountain ginseng \((Panax ginseng)\)\(^{[10,19]}\) from root derived callus on woody plant medium (WPM) containing 14.8 \(\mu\)mol/L indole butyric acid (IBA) and were cultured in 5 L capacity bioreactors containing WPM medium supplemented with 24.6 \(\mu\)mol/L IBA and 30 g/L sucrose. Yu\(^{[39]}\) grew the adventitious roots in small scale bioreactors using Murashige and Skoog (MS) medium and worked out effects of salt strengths of the medium and osmotic agents on the growth, the formation of biomass and production of ginsenosides from adventitious roots. Jeong \(et\ al\)\(^{[17]}\) worked out gaseous composition (enhanced oxygen, carbon dioxide and ethylene) on biomass growth and accumulation of ginsenosides and compared with atmospheric air composition (\(N_2\) 78%, \(O_2\) 20.8%, \(Ar\) 0.9%, \(CO_2\) 0.03%, \(Ne\) He). They showed that \(CO_2\) and \(C_2H_4\) enhanced the biomass; however these gaseous components were responsible for decreased ginsenoside accumulation.

On the other hand, increased oxygen concentration (40%) was found optimal for the production of adventitious root mass and ginsenoside accumulation. Accumulation of 12.4 mg/L dry weight ginsenosides has been under appropriate cultural conditions. Min \(et\ al\)\(^{[23]}\) have induced adventitious roots from the rhizome of \(Scopolia parviflora\) and maintained in Gamborg’s B5 medium supplemented with 0.1 mg/L IBA and 50 mg/L sucrose in flasks/bioreactors and established growth kinetics. They have analyzed various parameters (inoculum density, culture period, aeration) for cultivation of adventitious roots in bubble column bioreactors. With ideal cultural conditions adventitious roots were accumulating an optimum of 1.8 mg/g dry weight scopolamine and 3.3 mg/g dry weight hyoscymine contents. In \(Raphanus sativus\) (cv. Peking Koushin), Betsui \(et\ al\)\(^{[5]}\) induced adventitious roots from root segments in half strength MS medium supplemented with 0.5 mg/L IBA. The adventitious roots cultured in half strength MS medium supplemented with 0.5 mg/L IBA produced anthocyanin in dark. Adventitious roots were induced in \(Echinacea angustifolia\)\(^{[35]}\) on half strength MS medium 2 mg/g IBA and were cultured in flasks containing MS medium supplemented with 2 mg/L IBA and 50 g/L sucrose. The appropriate conditions for the accumulation of phenolics and flavonoids were: half strength MS medium supplemented

![Fig. 1 Cultivation of adventitious roots of \(Echinacea purpurea\) in flask-scale and large-scale bioreactor cultures](https://example.com/fig1.jpg)

A: induction of callus from leaf explants; B: induction of adventitious roots from callus mosses; C: flask scale cultures; D: 20 L capacity airlift bioreactor cultures; E and F: 500 L (balloon type airlift bioreactor) and 1000 L (horizontal drum type airlift bioreactor) capacity airlift bioreactor cultures; G: adventitious roots harvested from bioreactor cultures
Table 1  List of plants in which adventitious roots have been induced and cultured successfully for the production of secondary metabolites

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Metabolite</th>
<th>Importance</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthemis nobilis</td>
<td>Geranyl isovalerte</td>
<td>Essential oil, fragrance, anti-inflammatory</td>
<td>Omoto et al[24]</td>
</tr>
<tr>
<td>Cornus capitata</td>
<td>Tanins</td>
<td>Anti-oxidants</td>
<td>Tanaka et al[29]</td>
</tr>
<tr>
<td>Dubosis myoporoides-D. leichhardtii hybrid</td>
<td>Scopolamine, hyoscyamine</td>
<td>Spasmolytic, hydriatic agents</td>
<td>Yoshimatsu et al[37]</td>
</tr>
<tr>
<td>Echinacea purpurea E. angustifolia</td>
<td>Caffeic acid derivatives</td>
<td>Immunostimulant, Anti-inflammatory, anti-oxidant</td>
<td>Wu et al[33-35]</td>
</tr>
<tr>
<td>Iris germanica</td>
<td>Irgenin, Iristectorigenin A (Flavonoids)</td>
<td>–</td>
<td>Akashi et al[1]</td>
</tr>
<tr>
<td>Scopolia parviflora</td>
<td>Hyacynine (Alkaloid)</td>
<td>Anticholinergic activity</td>
<td>Kang et al[18], Min et al[23]</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>Ginsenosides (Saponins)</td>
<td>Immunostimulant, Anti-inflammatory, anti-oxidant, anti-cancer, anti-fatigue</td>
<td>Choi et al[19], Kim[19]; Kim et al[20, 21], Jeong et al[17], Son et al[28], Gao et al[3]</td>
</tr>
<tr>
<td>Panax notoginseng</td>
<td>Saponins</td>
<td>Immunistimulant, anti-cancer</td>
<td>Betsui et al[9]</td>
</tr>
<tr>
<td>Rapanus stivsus L. cv. Peking Koushin</td>
<td>Anthocyanin</td>
<td>Food coloring</td>
<td></td>
</tr>
<tr>
<td>Scopolia parviflora</td>
<td>Galloylglycoses, riccionidin A (Polyphenols)</td>
<td>Anti-oxidants</td>
<td>Taniguchi et al[20]</td>
</tr>
</tbody>
</table>

Table 2  Enhancement of secondary metabolites in the adventitious root cultures by elicitation

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Elicitors used</th>
<th>Metabolites</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupleurum kaoi</td>
<td>Methyl jasmonate</td>
<td>Saikosaponin</td>
<td>Chen et al[9]</td>
</tr>
<tr>
<td>Hyoscyamus muticus</td>
<td>Methyl jasmonate</td>
<td>Hyoscyamine, scopolamine</td>
<td>Biondi et al[6, 7]</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>Jasmonic acid Methyl jasmonate Organic germanium Ethephon and methyl jasmonate Methyl jasmonate along with auxin</td>
<td>Ginsenosides (Saponins)</td>
<td>Bae et al[3], Kim[19], Kim et al[20, 21], Yu[39], Yu et al[40, 41]</td>
</tr>
<tr>
<td>Scopolia parviflora</td>
<td>Methyl jasmonate and salicylic acid</td>
<td>Scopolamine</td>
<td>Kang et al[18, 11]</td>
</tr>
</tbody>
</table>

with 2 mg/L IBA, 50 g/L sucrose, 5:25 (mmol/L) ammonium/nitrate ratio, pH 6.0 and inoculum size of 10 g/L (fresh weight). Recently, Wu et al[34] worked out the effects of temperature and light irradiation (photoperiod) on growth of production of caffeic acid derivatives with the adventitious root cultures of E. purpurea. They showed biomass accumulation and production of caffeic acid derivatives was optimal under incubation temperature of 20°C among different incubation temperatures tested (10, 15, 20, 25 and 30°C). Biomass of adventitious roots was highest in cultures grown under dark while accumulation of caffeic acid derivatives was optimal in the cultures grown under 3/12 h light and dark cultural regimes.

2 Enhancement of secondary metabolite production by elicitation

Elicitation and precursor feeding are two strategies followed for enhancing the metabolites in the adventitious root cultures of Bupleurum kaoi, Hyoscyamus muticus, Panax ginseng, Scopolia parviflora (Table 2).

Since the biosynthesis of secondary metabolites in plants is tightly controlled during development and the metabolites are accumulated by plants in response to stress and microbial attack, stress signaling molecules like methyl jasmonate (MeJA) or salicylic acid (SA) are frequently used in elicitation experiments with adventitious roots. Jasmonic acid and MeJA have retarded the growth of adventitious roots; however they have enhanced the accumulation of ginsenosides in the adventitious roots of ginseng. Therefore, a two step strategy was followed for the cultivation of adventitious roots of ginseng: cultivation of adventitious roots without elicitor for the biomass accumulation (for 3 weeks) and elicitation with 100 μmol/L MeJA in the second stage (last two weeks) enhanced the accumulation of ginsenosides significantly[19,21]. Supplementation of ethephon (50 μmol/L) a precursor of ethylene during the initial stage of culture and MeJA in the second stage (last two weeks) enhanced the accumulation of ginsenosides significantly[19,21]. Supplementation of ethephon (50 μmol/L) a precursor of ethylene during the initial stage of culture and MeJA (100 μmol/L) in the second stage of culture have enhanced the adventitious root growth as well as ginsenoside productivity[3]. Similarly use of indole-3-butyric acid (25 μmol/L) and MeJA (100 μmol/L) synergistically also boosted the ginsenoside production in adventitious roots[20]. Yu et al[40] demonstrated usefulness of organic germanium as an elicitor and with the addition of 60 mg/L organic germanium to ginseng adventitious root cultures enhanced both biomass and ginsenosides accumulation.

Increased arginine decarboxylase, ornithine decarboxylase
and diamine oxidase and putrescine N-methyltransferase activities has been demonstrated in root cultures of *Hyoscyamus muticus* upon MeJA treatment, which are responsible for biosynthesis of putrescine and the higher polyamines (sperrmidine and spermine) and accountable for tropane alkaloid production in culture systems. Kang *et al.* studied effect of MeJA and SA on the production of tropane alkaloids (scopolamine and hyoscyamine) and showed expression of putrescine N-methyltransferase (PMT) and hyoscyamine 6-hydroxylase (H6H) genes in adventitious root cultures of *Scopolia carviflora* with MeJA and SA elicitation. MeJA treatments increased the amounts of both scopolamine and hyoscyamine, with growth inhibition of the roots, while SA increased the amounts of scopolamine without negative effects on growth. An elegant work on MeJA-induced transcriptional change in adventitious roots of *Bupleurum koi* has been carried out by Chen *et al.* They have performed real time PCR to verify changes in expression of 36 ESTs (unique expressed sequence tags). Based on their results they showed that genes upregulated by MeJA interacts with other signaling pathways, i.e. auxin homeostasis and ethylene signaling pathways leading to transcriptional reprogramming in *B. koi* adventitious roots.

### 3 Scaled up production of adventitious root biomass and secondary metabolites

Only few studies have been carried out for the production of adventitious roots in large scale at the industrial level. The first successful attempt (scale-up process) was by Choi *et al.* who have successfully achieved 150-fold growth increases when ginseng adventitious roots were grown in 500 L balloon type bubble bioreactors (air-lift bioreactors) for 7 weeks. This biomass increase is tremendous and is higher than the cell, callus and hairy root suspension cultures reported in earlier. The adventitious roots grown in bioreactors contained 1% of dry root weight, which corresponds half of the content for the field grown plants. By adopting MeJA elicitation technique the content of ginsenosides (saponins) could be elevated by up to 2.5%.[21] Based on such results CBN Biotech Company, South Korea (http://www.cbnbiotech.com) is involved in production of ginseng adventitious root biomass on a commercial scale. Second successful example of scale-up process was by Wu *et al.* who have cultivated adventitious roots of *Echinacea purpurea* in 1000 L air lift bioreactors. They were able to achieve 5.1 kg dry biomass of adventitious roots and these roots were possessing higher amounts of chichoric acid (22 mg/g dry mass), clorogenic acid (5 mg/g dry mass) and caftaric acid (4 mg/g dry mass).

### 4 Conclusions

Current advances in plant biotechnology allowed us to culture the plant cells and organs for production of useful secondary metabolites. Plant cell cultures using bioreactors were successful in producing alkaloids, quinines, and pigments.[30] Nevertheless, the large-scale culture of plant cell at the aim for commercial-scale production of useful metabolites has been known to be very difficult due to poor productivity and instability of plant cell culture.[22,31] Some compounds are not synthesized if the cells remain undifferentiated[4,27]. Therefore, undifferentiated cell cultures often lose, partially or totally, their biosynthetic ability to accumulate secondary products.[8] In this respect, the differentiated organ culture seemed to more promising than undifferentiated cell cultures for production of useful secondary metabolites. Hairy roots, the result of genetic transformation by *Agrobacterium rhizogenes*, have attractive properties for metabolite cultures.[11] However, in such studies selectable marker genes are used to identify genetic transformation. The most widely used selectable marker genes include neomycin phosphotransferase II (nptII) encoding resistance to the antibiotic kanamycin, and resistance to herbicides such as glyphosate.[16] Use of selectable markers has raised questions of human health concerns when the target material is a functional food (ginseng for example). With respect to this point of view, improvement of adventitious root culture system through the use of bioreactor seems to be reliable way for the production of pharmaceutically and nutraceutically important metabolites.

### REFERENCES


