Research and Reviews: Journal of Pharmacognosy and Phytochemistry
e-ISSN: 2321-6182
p-ISSN: 2347-2332

Production of Oleanolic Acid by Plant Tissue Culture – A Review

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Review Article

Received: 23/06/2016
Accepted: 13/07/2016
Published: 20/07/2016

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Keywords: Oleanolic acid, Plant tissue culture

ABSTRACT

This review is focused on production of oleanolic acid (a triterpenoid) from different plants using tissue culture. Oleanolic acid has pharmacological actions, such as anti- inflammatory, anti-hyperlipidemic, hepatoprotective, antiviral and anti-tumor. Owing to its pharmacological importance much research is focused on the production and extraction of oleanolic acid. Conventional method of isolation of oleanolic acid from plant material has disadvantages such as extract variability and instability. The production of oleanolic acid by tissue culture offers the opportunity to optimize the yield of the compound and to obtain a uniform, high quality product. Most often plant cell culture technologies not lead to enhanced secondary metabolite production. Therefore, for enhanced oleanolic acid production, conditions such as medium optimization, light, temperature, nutrients, carbon source, pH, elicitors and permeabilisation are to be considered. This review may help in further research on factors effecting the production of high yield of oleanolic acid by tissue culture.

INTRODUCTION

Saponins can be chemically categorized in to two classes based on aglycone moiety. One contains a steroidal aglycone, and the other contains a triterpenoid aglycone. Triterpenes are a versatile group of biologically active ingredients present in different plants. They often occur as glycoconjugates with a distinct bioactivity from their aglycone counterparts. Pentacyclic triterpenoids are the focus of drug research for anti-cancer, hepatoprotective, anti-inflammatory and antimicrobial activities. From a biological perspective, important triterpenoid structures are the oleanane, ursane, lupane, and dammarane-euphane carbon skeletons. Oleanolic acid (3β-hydroxy-olean-12-en-28-oic acid) and its isomer, ursolic acid (3β-hydroxy-olean-12-en-28-oic acid) are pentacyclic triterpenoid compounds with 30 carbon atoms. They exist widely in plants in the form of free acid or aglycones of triterpenoid saponins. In Asia, oleanolic acid has long been known to be anti- inflammatory, anti-hyperlipidemic and hepatoprotective in traditional medicine. It has also been found to have antiviral and anti-tumor actions. Oleanolic acid can be found in varieties of plants (e.g., rosemary, melissa peppermint, sage and ginseng) and exhibit variety of pharmacologically interesting activities. *Ex vivo* production of oleanolic acid offers the opportunity to obtain a uniform, high quality product. Thus, in this review, we provided an overview of the processes for the production of oleanolic acid by tissue culture.

Plant tissue culture techniques may provide continuous and reliable source of natural product and an alternative to intact plant tissues for the production of high valuable secondary metabolites such as OA. These days, biosynthesis of secondary metabolites from plant tissue cultures is very important considering its medicinal values. Thus, due to the importance of OA, production of these triterpenes using cell suspension cultures is of great practical value. One of the most effective strategies to improve the production of secondary metabolites is using elicitation. In tissue culture factors effecting in cultural conditions to production of oleanolic acid such as light, pH, media, agitation speed, plant growth regulators, Nutrients, carbon source and elicitors. Some of the following investigations are reported the cultural conditions for enhancing the production of oleanolic acid.

MEDIA

Components of the basic medium exert a substantial influence on suspension and callus cultures. Generally, medium conditions which most frequently support active secondary metabolism are those which limit rapid cell division. The MS media is mostly favourable for enhancing the oleanolic acid content compared to another media. Plant growth regulators are effective triggers of secondary metabolism. Production of secondary metabolites in plant cell cultures is a function of both cell multiplication and division; growth regulators have a major role in determining the potential productivity of a given culture. The following reports are showed that plant growth regulators are play a significant role for enhance the OA.

Wang et al. [1] cells in suspension culture grown with Murashige & Skoog medium supplemented with 0.2 mg.l^{-1} 2,4-D and 0.5 mg.l^{-1} 6-benzyladenine. After 12 days the cell line produced 16 mg.l^{-1} oleanolic acid (OA).

Szakiel et al. [2] investigation of synthesis of oleanolic acid glucosides in *Calendula officinalis* cell suspension cultures grown with 0.1 mg/L 2,4-D and 0.5 mg/L 2iP (6-(γ,γ-Dimethylallylamino)purine) have been found to synthesize and secrete more oleanolic acid glucosides.

Suspension culture of *Lantana camara* on MS medium with 6-BAP (5 µM), 2,4-D (1 µM), NAA (1 µM) occumulation of more OA. Zhongping et al. [3] *Cyclocarya paliurus* cell suspension cultures grown in liquid basal MS medium with 2,4-D (0.5 mg/L), NAA (0.3 mg/L) and cytokinin (1.0 mg/L) fit for cell growth and oleanolic acid accumulation. Investigation of *Glossogyne tenuifolia* grown with Half-strength MS (Murashige and Skoog) basal medium supplemented with 0.1 mg/L 6-benzyladenine (BA) and 0.1 mg/L α-naphthaleneacetic acid (NAA) induced oleanolic acid content found to be significantly higher (16.89 mg/g) in 3-month old tissue culture raised plants in greenhouse compared to commercially available crude drug (6.51 mg/g, respectively).

Light

The characteristics of radiation which influence plant development in vitro are also those which affect plant tissues and cells *in vitro*. The behaviour of cultures is influenced by photoperiodicity, light quality and light intensity. Several reports indicate that light can have inhibitory effect. Higher levels of accumulation of secondary metabolites under dark rather than light conditions. In Szakiel et al. [4] study *Calendula officinalis* cell suspension cultures interestingly light promoted oleanolic acid glucosides. Suspension culture of *Lantana camara* incubation in the presence of dark are the favourable for production of maximum oleanolic acid content reported *Gentiana straminea* on exposed to extremes of cold and strong UV-B radiation resulted in accumulation of the triterpenoids oleanolic acid.

Nutrients

Generally increased levels of nitrates, potassium, ammonium and phosphate tend to support rapid cell growth while depletion of some of these nutrients is associated with growth limitation and concomitant secondary metabolism. *Cyclocarya paliurus* cell suspension cultures grown in liquid basal MS medium 60 mm total nitrogen, 1.25 mm KH_2PO_4, 2 mm $CaCl_2$, and 2 mm $MgSO_4$ were fit for cell growth and oleanolic acid accumulation in a cell suspension culture of *Cyclocarya paliurus*.

pH

Optimal growth in plant cell cultures usually occurs in media with initial pH values in the ranges 5-6. Media containing undefined organic components, yeast extract are usually well buffered so that the pH changes relatively little during the course of culture development. However, in media without these substances, shifts in pH during culture can be dramatic. The growth of the suspension culture for production of OA in pH of the media at 5.8.

Carbon source

Carbon may be supplied either CO_2 in a photoautotrophic culture or as carbohydrates in a heterotropic cultures. In general, raising the initial sucrose level leads to an increase in the secondary metabolite yields of cultures. Feria-Romero et al. [5] investigated increasing sucrose from 20 g l^{-1} to 50 g l^{-1} in *Uncaria tomentosa* cell suspension cultures enhanced oleanolic acid production from 129 µg g^{-1} ± 61 µg g^{-1} to 553 µg g^{-1} ± 193 µg g^{-1} cell dry weight 3% sucrose level increases the oleanolic acid content in suspension culture of *Lantana camara*.

Rotation speed

In the rotary shake flask cultures, the rotation speed of a shaker (normally 90 rpm to 120 rpm) can have an important effect on growth and metabolic accumulation. Agitation speed 120 rpm can effect on accumulation of OA in suspension culture of *Lantana camara*, Agitation speed 120 rpm, incubation in the presence of dark are the favourable cultural conditions for production of maximum oleanolic acid content **(Table 1).**

Table 1. OA production from different plants through different cultures.

Plant	Culture type	Substance
Camptotheca acuminata	C+S	OA
Actinidia arguta	C	OA
Actinidia chinensis	C	OA
Actinidia polygama	C	OA
Perilla frutescens	S	OA
Taraxacum officinale	C	OA

Hairy root culture

The high-value secondary metabolites in trees are generally synthesized during the later stage of the life cycle and are produced in very less amount, making the development of stable in vitro source mandatory for commercial production of their metabolites. Hairy root systems have not been developed for many important tree species. Since the discovery of *Agrobacterium*

rhizogenes as pathogenic bacteria causing hairy root disease, tremendous development towards establishment of hairy root system as biochemical factory has taken place. Diverse strategies can be developed to improve the yield so as to produce desired metabolites at large-scale and in eco-friendly conditions.

Kuźma [6] reported oleanolic acid was isolated from hairy root culture of *Salvia sclarea* infected with *Agrobacterium rhizogenes* LBA 9402 and culture was grown in growth regulator-free half-strength B5 Gamborg medium with 30 $g.l^{-1}$ sucrose.

Elicitation

Stimulation of particular facets of plant metabolism is achieved by treatment with biotic (microbes especially fungi and their extracts) and abiotic (certain chemicals like methyl jasmonate, etc.) compounds to enhance or increase the yield of desired secondary metabolite.

Yeast elicitor

The following researches reports are with yeast elicitor enhance the secretion of oleanolic acid. Several other authors [7-11] studied cell suspension cultures from *Scutellaria baicalensis* were treated with a yeast elicitor (up to 9 mg/L) more oleanolic acid secreted into the culture medium. Wang et al. [1] study *Perilla frutescens* cells in suspension culture grown with Murashige & Skoog medium supplemented with 0.2 $mg.l^{-1}$ 2,4-D and 0.5 $mg.l^{-1}$ 6-benzyladenine. After 12 days the cell line produced 16 $mg.l^{-1}$ oleanolic acid (OA) [1].The maximum production of oleanolic acid cultures treated with 2% (v/v) yeast elicitor was 19 $mg.l^{-1}$, 46% increase over the control.

Jasmonic acid

Feria-Romero et al. [5] investigated increasing sucrose from 20 g l^{-1} to 50 g l^{-1} in *Uncaria tomentosa* cell suspension cultures enhanced oleanolic acid production from 129 μg^{-1} ± 61 μg^{-1} to 553 μg^{-1} ± 193 μg^{-1} cell dry weight. The maximal concentration of OA (168 $\mu g\ g^{-1}$ ± 39 $\mu g\ g^{-1}$ cell dry weight) was 8 days after elicitation by jasmonic acid compared with yeast extract or citrus pectin. Wiktorowska et al. [9] reported suspension cultures of *Calendula officinalis* was elicitation with jasmonic acid. After 72 h of treatment with 100 µM JA, the intracellular content of OA reached its maximum value (0.84 mg g^{-1} DW), which was 9.4-fold greater than that recorded in an untreated control cultures [12-14]. *Calendula officinalis* suspension cultures elicited with yeast extract released high amounts of oleanolic acid into the medium (0.43 mg/L after 48 h treatment with 200 mg/L yeast extract), whereas jasmonic acid (100 M) induced the highest increases in intracellular levels of OA (to 0.84 mg/g DW, 72 h after treatment; 9.4-fold higher than in controls).

Methyl jasmonate

Depends on concentration of methyl jasmonate enhance the secretion of OA such as. cell suspension of *Hedyotis corymbosa* cultures were successfully induced using 2,4-D plant hormone and then were elicited with methyl jasmonate at different concentrations. It was found that cell suspension biomass decreased 52% in the presence of 10 µM methyl jasmonate concentration after 12 days of culture. The highest OA (17 mg) yields were obtained at 100 µm methyl jasmonate, respectively, on the third weeks of culture. Zhao et al. [8] reported *Gentiana straminea* is exposed to the application of methyl jasmonate (MeJA), significantly enhanced the accumulation of oleanolic acid.

Elicitation with fungi reported results with *Tabernaemontana catharinensis* suspension culture inoculated on MS solid medium supplemented with two combinations of 2,4-D and kinetin (1:1 and 1:0.1) and elicited with *Saccharomyces cerevisiae* showed better results 7 $mg.g^{-1}$ dw, of oleanolic acid respectively. Suspension culture of *Lantana camara* was elicitation with endophytic fungus, *piriformospora indica* at 2.5% (v/v) increases the concentration of O.A by 5.6 fold in the cell cultures of *Lantana camara*.

CONCLUSION

Plant tissue culture techniques may provide continuous and reliable source of natural products and an alternative to plants for the production of high valuable secondary metabolite such as OA. Biosynthesis of secondary metabolites from plant tissue cultures is important considering their medicinal value. The formation of oleanolic acid from different plants in plant tissue cultures is reviewed. The conditions for the enhanced and manipulation of secondary product formation is possible by varying the nutrient composition of the growth medium, light, temperature and pH, and by the use of elicitors, permeabilization are described along with future prospects for the commercial production of secondary products from different cell cultures.

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