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Review

Biotechnological applications for rosmarinic acid production in plant

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Rosmarinic acid, an important phenolic compound, is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae. However, it is also found in species of other higher plant families and in some fern and hornwort species. Rosmarinic acid has a number of interesting biological activities, e.g. antiviral, antibacterial, anti-inflammatory, and antioxidant. The presence of rosmarinic acid in medicinal plants, herbs and spices has beneficial and health promoting effects. In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defence compound. The biosynthesis of rosmarinic acid starts with the amino acids phenylalanine and tyrosine. Plant cell cultures, e.g. from *Coleus blumei* or *Salvia officinalis*, accumulate rosmarinic acid in amounts much higher than in the plant itself (up to 36% of the cell dry weight). Similarly some other biotechnological researches for production of rosmarinic acid were done in the past i.e. from shoot culture, producing hairy root, using bioreactor, and the treatment of elicitors. As a review paper the aim of this study is to gather all the possible biotechnological ways to produce rosmarinic acid, thus will help the scientists to take action for future study in this discipline.

Key words: Biotechnology, *in vitro* culture, plant, rosmarinic acid.

INTRODUCTION

Rosmarinic acid (Figure 1) derived from caffeic acid and (*R*)-(+)-3-(3, 4-dihydroxyphenyl) lactic acid represents one of the most common caffeic esters in plant material and is accumulated constitutively (Ellis and Towers, 1970). It is a well-known natural product from rosemary (*Rosmarinus officinalis*), lemon balm (*Melissa officinalis*), and other *Lamiaceae* as well as other plant families, e.g. the medicinal plants like thyme, oregano, savory, peppermint, sage (Lu and Foo, 1999; Kochan et al., 1999; Zheng and Wang, 2001). Rosmarinic acid (RA) exhibits various pharmacological activities including prevention of oxidation of low density lipoprotein, inhibition of murine cell proliferative activity and of cyclooxygenase, and anti-allergic action. The biological activity of RA is described as antibacterial, antiviral, and antioxidative (Szabo et al., 1999; Hras et al., 2000). Its activity especially against rheumatic and inflammatory conditions makes it a sought-after substance for use in phytotherapy (Pabsch et al., 1991). More recently, rosmarinic acid was reported to have anti-HIV activities (Chen et al., 1999).

Secondary compounds from plant have been incorporated into a wide range of both commercial and industrial applications, and in many cases, rigorously controlled plant *in vitro* culture can generate the same valuable natural products. Plant as well as *in vitro* plant cell or tissue culture have served as resources for preservatives, natural pigments, flavors, enzymes, biobased fuels and plastics, cosmetics, and bioactive compounds (Mary, 2005). A series of distinct advantages such as production can be more reliable, simple, and more predictable; isolation of the phytochemical can be rapid and efficient; interfering compounds that occur in field-grown plant can be avoided in vitro cultures; cell cultures can yield a source of standard phytochemicals in

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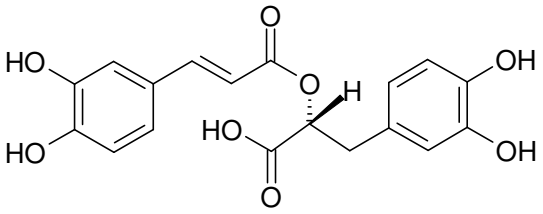

Figure 1. Chemical structure of rosmarinic acid.

large volumes; secondary products through *in vitro* plant culture and some other ways can be generated on a continuous year-round basis without seasonal constraints; production is independent of ambient weather producing a valuable secondary product *in vitro* plant cultures, rather than in the whole crop plant.

There are several *in vitro* culture systems (callus, suspension cell, shoot, and hairy root cultures) for production of plant secondary metabolites. This paper presents a modest contribution of rosmarinic acid to use as a large scale for the well being of human being and others collecting the knowledge of biotechnologically derived rosmarinic acid for further study.

Source of rosmarinic acid in plant

Rosmarinic acid occurs throughout the Boraginaceae, whereas within the Lamiaceae it is restricted to the sub-family Nepetoideae (Litvinenko et al., 1975). For example, it occurs in ferns of the family Blechnaceae (Häusler et al., 1992), lower plants such as the hornworts (Takeda et al., 1990) and in monocotyledonous plants like the sea grass family Zosteraceae (Ravn et al., 1994), the related Potamogetonaceae as well as the Cannaceae.

Properties of rosmarinic acid

Rosmarinic acid has antioxidant, anti-inflammatory and antimicrobial activities. The antioxidant activity of rosmarinic acid is stronger than that of vitamin E. Rosmarinic acid helps to prevent cell damage caused by free radicals, thereby reducing the risk for cancer and atherosclerosis. Rosmarinic acid has anti-inflammatory properties. Perilla, rich in rosmarinic acid, is used for its anti-allergic activity. A study by Sanbongi et al. (2004) has shown that the oral administration of rosmarinic acid is an effective intervention for allergic asthma. Another study by Youn et al. (2003) demonstrated that rosmarinic acid suppressed synovitis in mice and that it may be beneficial for the treatment of rheumatoid arthritis. Unlike antihistamines, rosmarinic acid prevents the activation of immune responder cells, which cause swelling and fluid formation.

Rosmarinic acid is also used for food preservation. In Japan the perilla extracts, rich in rosmarinic acid, is used to garnish and improve the shelf life of fresh seafood. Rosmarinic acid is used to treat peptic ulcers, arthritis, cataract, cancer, rheumatoid arthritis and bronchial asthma.

DIFFERENT BIOTECHNOLOGICAL APPROACHES FOR ROSMARINIC ACID PRODUCTION

Plant cell culture

Production of secondary metabolites through plant cell culture has been carried out in many plant species, especially in medicinal plant. Screening, selection, elicitation and media optimization are the methods applied for improving production of secondary metabolites in cell cultures. Most attempts to produce secondary metabolites *in vitro* have failed because the cells have not produced the compound in sufficient quantity and yields have been unpredictably variable. These two factors have usually been considered to be associated with the use of undifferentiated cells (Verpoote et al., 1998; Mary, 2005). Up till now there are so many researchers that have tried to produce rosmarinic acid using cell culture of plant.

The pool sizes of free L-phenylalanine and L-tyrosine, the precursors of rosmarinic acid in *Anchusa officinalis* L. cell suspension cultures, fluctuated during the culture cycle (Eknamkul and Ellis, 1989). The major increase in pool sizes was preceded by a peak of prephenate aminotransferase activity, while the subsequent decrease coincided with the presence of high activities of phenylalanine ammonia-lyase and tyrosine aminotransferase, the two entry point enzymes of the rosmarinic acid biosynthesis pathway. Callus and suspension cultures of *Coleus blumei* Benth. were cryopreserved and stored for various periods of time in liquid nitrogen. The best results were obtained using cells from the early growth period. The duration of storage was 3, 6, 12 and 15 months, respectively. The growth characteristics and the accumulation of rosmarinic acid, a compound of pharmaceutical value, were found to be in the range of untreated controls (Reuff et al., 1988).

Rosmarinic acid is a natural antioxidant produced by cell suspension cultures of sage (*Salvia officinalis* L.). The growth and production of RA by these cells can be modified by the type of culture medium. From a study, Hippolyte et al. (1992) stated that production can be increased 10-fold to attain 6.4 g/l under optimal conditions. Suspension cultures of *C. blumei* accumulate very high amounts of rosmarinic acid, an ester of caffeic acid and 3, 4-dihydroxyphenyllactate, in medium with elevated sucrose concentrations (Petersen et al., 1994). Since the synthesis of this high level of rosmarinic acid occurs in only five days of the culture period, the activities of the enzymes involved in the biosynthesis are very high. High cell density and rosmarinic acid (RA) productivity have been achieved by applying periodic culture perfusion

to the *A. officinalis* cell suspension. Experimental results from Su and Lei (1993) showed that RA productivity increased with the inoculum size, up to 4 g dry weight/l. Further increases in the inoculums size did not yield a higher RA productivity regardless of culture perfusion.

Salvia chamelaeagnea is an attractive and aromatic South African plant widely used for medicinal purposes. Explants can be easily induced to callus on Murashige and Skoog (MS) medium containing 2,4-D (1-2 mg/l) and induce shoots on the same medium containing 1 mg/l BA. Transfer of the shoots on to MS medium containing NAA (0.5-2 mg/l) resulted in the formation of fast-growing roots. Chromatographic techniques indicated that extracts from both callus and leaves produced rosmarinic acid, with higher levels in the callus (Huang and Van, 2002). The influence of sucrose concentration in the nutrient medium on cell growth and accumulation of rosmarinic acid by *Lavandula vera* MM cell culture was investigated. The results showed that 7% sucrose in the nutrient medium ensured a steady growth of the cell suspension and increased the yield of rosmarinic acid (Llieva and Pavlov, 1997). The accumulation of rosmarinic acid (RA) in *Salvia fruticosa* callus, cell suspension, and root cultures was studied by Karam et al. (2003). For callus induction, leaves excised from micro shoots were cultured on MS medium containing thidiazuron (TDZ) (0, 2.3, 4.6, 6.9, 9.2, or 11.5 µM) and indole-3-acetic acid (IAA) (0 or 3 µM). Culture duration of 5 weeks resulted in maximum callus growth and RA yield (2.12 mg/100 mg dry weight). Recently callus and cell suspension cultures of *Agastache rugosa* were established for the production of rosmarinic acid *in vitro.* In cell suspension culture of *A. rugosa*, the maximum growth (7.7 g/l) and the highest rosmarinic acid production (11.5 mg/g) were attained in the liquid B5 medium supplemented with 2 mg/l 2, 4-D and 0.1 mg/l BAP at 10 days after culture. The present results demonstrate that cell culture of *A. rugosa* might be an alternative approach for the production of rosmarinic acid (Xu et al., 2008).

In vitro shoot culture

Researches were done to synthesis rosmarinic acid using shoot portion of plant, some of them are presented below. Phenolic metabolites from oregano and related species in the family Lamiaceae are important sources of antimicrobials and antioxidants. Clonal lines were generated from multiple shoots induced by 1 mg/l benzylaminopurine in standard Murashige and Skoog (MS) medium with 3% sucrose. Under these optimum conditions 7 - 10 shoots per explants were generated for further clonal propagation or regeneration of plants.

Following 30 days of growth on hormone-free MS medium indicate that *Pseudomonas* sp. mediated stimulation of phenolics and rosmarinic acid in various oregano clonal lines were directly correlated to tolerance to *Pseudomonas* sp. (Eguchi et al., 1996). A biosynthesis in shoot cultures was also compared due to its relevance to greenhouse production and organ culture. Callus maintained in light grows more rapidly when compared to shoot grown in light and callus grown in dark. The ratio of biomass accumulated by callus (Light) : callus (Dark) : shoot was 4:1:1 based on fresh weight and 4:1:2 based on dry weight. The ratio of peak RA levels (expressed mg/g DW) was 1:1:10 (Komali and Shetty, 1998).

Rosmarinic acid and total phenolics were assayed in all treated clonal lines and compared to uninoculated and untreated shoot explants of corresponding line. The *Pseudomonas* and A2C (azetidine-2-carboxylate) treatment strategy allowed the rapid tissue culture-based screening of potentially high phenolic antioxidant-producing clonal lines of spearmint for future field and greenhouse evaluation. Targeted elite lines had combinations of *Pseudomonas* tolerance with no loss in biomass in response to the bacterium and enhanced levels of total phenolics and rosmarinic acid in response to A2C (Hussein et al., 1999). Oregano clones, like other plants, carry out an elicitor-mediated defense response by inducing the phenylpropanoid pathway. Free phenolics and rosmarinic acid are produced via this pathway. Free proline, rosmarinic acid, and free phenolics in oregano shoots were measured. It was found that the induced higher levels of rosmarinic acid in some *Pseudomonas* inoculated clones and at several stages is often correlated to higher levels of proline in those same clones. From this study clonal line O-4 appeared to be the best line to investigate the role of proline-linked pentose phosphate pathway in regulating rosmarinic acid synthesis (Perry and Shetty, 1999).

Lavender is a good source of essential oils and phenolic metabolites for food, medicine, and cosmetic applications. Due to cross-pollination, lavender has substantial plant to plant variation and therefore a high degree of genetic inconsistency in the level of phytochemicals produced for diverse applications. Tissue culture methods, using benzyladenine-induced shoot organogenesis, were used to isolate clonal lines originating from individual heterozygous seeds among a heterogeneous seed population to exploit the genetic heterogeneity. On the basis of tolerance to *Pseudomonas* and proline analogue treatments, multiple shoot forming ability, biomass, rosmarinic acid, total phenolics, and total chlorophyll, 20 separate clonal lines were screened and isolated for further vegetative propagation and evaluation (Hussein et al., 1999). Rosmarinic acid synthesized by multiple shoot cultures of *Mentha arvensis* was evaluated in four different basal media, at various sucrose concentrations, at altered KH_2PO_4 levels, and in the presence of agents like phenylalanine or peptone. Shoots grown in liquid MS medium supplemented with 3% sucrose and phenylalanine (30 mg/l) produced highest amount of rosmarinic acid (0.21 mg/g) on fresh weight basis. This is the first report of synthesis of rosmarinic acid in the cultured shoots of *M. arvensis* (Phatak and Heble, 2002).

Hairy root culture

Agrobacterium rhizogenes infects wounds of many plant species and the infections are characterized by production of adventitious roots with numerous root hairs. Hairy root cultures established by transformation with *A. rhizogenes* are attractive for the production of secondary metabolites as such cultures are genetically and biochemically stable, show rapid growth rates, and have the ability to synthesize useful natural compounds at levels comparable to those produced by wild type roots. Hairy root cultures may thus be useful in studies on the production of important natural products (Hamill et al., 1987; Signs and Flores, 1990; Giri and Narasu, 2000; Guillon et al., 2006). Rosmarinic acid was also synthesized from hairy root, some of the important research findings regarding hairy root culture are given below.

Hyssopus officinalis transformed roots were induced by infection with *A. rhizogenes.* The transformed roots grew well in hormone-free Woody Plant liquid medium producing high levels of phenolic compounds such as rosmarinic acid (maximum: 8.03% of dry weight) and lithospermic acid B (maximum: 3.89% of dry weight) (Murakami et al., 1998). Hairy root cultures of *Salvia miltiorrhiza* were established by infecting sterile plantlets with *A. rhizogenes* ATCC 15834, and the transformation was proved by direct detection of the inserted T-DNA by the polymerase chain reaction. As determined by HPLC, these hairy root cultures had the ability to produce lithospermic acid B, rosmarinic acid and other related phenolic compounds, the water-soluble active components of the plant (Chen et al., 1999). Hairy roots of *H. officinalis* L. were induced by infection of petioles with *A. rhizogenes* LBA 9402 and studied for production of phenolic acids, especially rosmarinic acid (RA). The highest content of rosmarinic acid (about 6% of dry weight) was obtained in hairy roots grown in Gamborg's B5 liquid medium containing 10% (w/v) sucrose. The level was at least 60% higher than those found in callus, cell suspension culture and roots of one-year-old field grown plants (Kochan et al., 1999).

Five clones of *Ocimum basilicum* hairy roots, A-1 and A-2 (included by *A. rhizogenes* ATCC 15834), and J-1, J-2 and J-3 (induced by *A. rhizogenes* MAFF 03-01724), grew well in hormone-free Murashige Skoog, Gamborg B5 and Woody plant liquid media. In these cultures, a large amount of rosmarinic acid was produced (maximum: 14.1% dry wt, by J-1 in MS medium) together with small amounts of the related phenolics, lithospermic acid (ca 1.70% dry wt) and lithospermic acid B (ca 0.17% dry wt) (Tada et al., 1996). Hairy root cultures of *O. basilicum* transformed with *A. rhizogenes* (ATCC-15834) showed three-fold increases in growth and rosmarinic acid production compared to the untransformed normal roots. Upon elicitation with fungal cell wall elicitors (CWE) from *Phytophthora cinnamoni*, the production of RA was enhanced 2.67-fold compared with the untreated control. Roots were induced to exude RA by fungal in situ challenge with *Pythium ultimum*, to our knowledge an undocumented observation (Bais et al., 2002). Recently, several studies have reported the establishment of hairy root cultures of *Coleus forskohlii* (Li et al., 2005), *S. officinalis* (Grzegorczyk et al., 2006), and *Agastache rugosa* Kuntze (Lee et al., 2008). All these studies addressed rosmarinic acid production in the mint family (*Lamiaceae*).

Bioreactor

Bioreactor is a vessel in which a chemical process which involves organisms or biochemically active substances derived from such organisms is carried out. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel. The advanced bioreactor system is a key step towards commercial production of secondary metabolites by plant cell and tissue cultures. Rosmarinic acid could be synthesized using bioreactor, some of the important research findings while using different bioreactor to synthesis rosmarinic acid are presented below. A perfusion fermentation of *A. officinalis* was carried out in a stirred tank bioreactor integrated with an internal cross-flow filter. Bubble-free aeration via micro porous membrane fibers was used to provide oxygen. A two-stage culture was successfully conducted in this reactor without filter fouling. In 17 day fermentation, a cell density of 26 g dry weight/l and a rosmarinic acid productivity of 94 mg/l-day were achieved. This productivity is three times that obtained in a batch culture (Su and Humphrey, 1991). *C. blumei* cells were immobilized in a column reactor packed with *Luffa cylindrica* pieces. Medium was fed from the top of the column using a spray system and cells maintained high viability for 52 days. Cell growth was slower but rosmarinic acid production was better compared to immobilized cells in the shake flasks (Martinez and Park, 1994). Permeabilized *C. blumei* cells were cultivated in an immobilized state to study the effect of dimethyl sulfoxide (DMSO) concentrations and growth regulators on cell growth and rosmarinic acid (RA) production characteristics. Cell growth rate and RA production were approximately half that obtained in cell suspension cultures. Cell yield was similar to that of cell suspension cultures. The absence of growth regulators did not promote an increase of RA production but did decrease the cell mass. The second step preconditioning with 0.5% DMSO did not improve the cell's adaptability to higher DMSO concentrations and the cell mass did not increase with 2.5% DMSO (Park and Martinez, 1994).

In the bioreactor culture, rosmarinic acid production was found very sensitive to agitation and aeration conditions as well as dissolved oxygen concentration. A maximum cell density of 35 g dry weight/l and a rosmarinic acid concentration of 3.7 g/l were obtained by maintaining the dissolved oxygen concentration above

30% air saturation, gradually raising the impeller tip speed from 34 to 72 cm/s, and keeping the aeration rate at 0.44 vvm while increasing the O_2: air ratio in the gas feed stream to 4:1 (Su et al., 1995).

The treatment of elicitor

Elicitation is the induced or enhanced biosynthesis of metabolites due to addition of trace amounts of elicitors. Elicitor may be defined as a substance which, when introduced in small concentrations to a living cell system, initiates or improves the biosynthesis of specific compounds. Elicitors can be classified on the basis of their 'nature' like abiotic elicitors or biotic elicitors, or on the basis their 'origin' like exogenous elicitors and endogenous elicitors (Namdeo, 2007). Recently, some scientists did some works to synthesis rosmarinic acid using elicitor and most of the cases they were successful.

Batch suspension cultures of *C. blumei* cells preconditioned with 0.1% dimethyl sulfoxide (DMSO) maintained viability at above 85% during a 14-day culturing period for initial sucrose concentrations of 30-70 g/l. At 60 g/l sucrose, cells grew rapidly with a doubling time of 10.7 h, and dry cell mass reached a maximum of 15.6 g/l. Broad optima of cell yield (0.46-0.48 g of cell/g of sucrose), rosmarinic acid (RA) yield (3.1-3.5 g of RA/100 g of sucrose), and rosmarinic acid production (1.0-1.1 g of RA/l) were observed at 40-60 g/l sucrose concentration. Even at sucrose concentrations of 40-70 g/l, sucrose consumption over 14 days was limited to 33 g/l (Martinez and Park, 1993). Continuous permeabilization of preconditioned *C. blumei* cells with dimethyl sulfoxide (DMSO) is shown to be an effective strategy for the enhanced release of rosmarinic acid while preserving cell viability. When non-preconditioned cells were permeabilized with DMSO, they lost their viability at DMSO concentrations higher than a critical value located between 0.1 and 0.5% DMSO. Product release reached a maximum of 2.85 g RA/100 g DCW (dry cell weight) at 0.5% DMSO, which was 66.4% of the total rosmarinic acid produced (Park and Martinez, 1992). Suspension cultures of *C. blumei* (Lamiaceae) treated with either an elicitor preparation from the culture medium of the phytopathogenic oomycete *Pythium aphanidermatum* or with methyl jasmonate enhanced accumulation of rosmarinic acid approximately threefold. The specific activities of phenylalanine ammonia lyase and rosmarinic acid synthase were also enhanced after addition of the fungal elicitor (Szabo and Petersen, 1999).

A transient increase in rosmarinic acid (RA) content in cultured cells of *Lithiospermum erythrohizon* was observed after addition of yeast extract (YE) to the suspension cultures, reaching a maximum at 24 h. The highest increase of the RA content (2.5-fold) was obtained when 6-day-old cells in the exponential growth phase were treated with YE (Mizukami et al., 1992). Cell suspension cultures of *Orthosiphon aristatus* were shown to accumulate rosmarinic acid (RA) at concentrations of 1.0-2.0 mM/g fresh weight. Addition of yeast extract (4-6 g/l) to the liquid growth media resulted in a large increase of RA accumulation in treated cells independent of the growth stage. The highest concentration of RA observed in treated cells (carbohydrate polymer 10 µmol/g fresh weight) was usually reached 72-96 h after addition of yeast extract (Sumaryono et al., 1991).

The stimulation of RA biosynthesis in oregano clonal line O-1 in response to proline, proline precursors (ornithine and arginine), and proline analogue (azetidine-2-carboxylate, A2C) was reported by Yang and Shetty (1998). Following exogenous treatment with proline and proline precursors in the presence or absence of proline analogue A2C, significantly enhanced RA content and concurrently higher levels of endogenous proline were observed compared to control. Callus and suspension cultures were established from the leaves of *A. rugosa*. The suspension cell growth was maximum at 15 days after inoculation. The cellular content of rosmarinic acid increased slowly and reached maximum (0.42 mg g/l dry wt.) during the stationary phase of culture, after 18 days of inoculation. The addition of yeast extract preparation (MW < 10,000) at 50 ug/ml elevated the rosmarinic acid content up to 5.7-fold of that found in non-elicited suspension cells (Kim et al., 2001).

CONCLUSION

It is well known to all that the current era of globalization is directly linked with biotechnology which is in a phase of evolution with endeavors closely linked to development, product and services. This manuscript has gathered different biotechnological ways of producing rosmarinic acid and development efforts to improve on current knowledge of the subject and expertise for the benefit of present and future generations. These issues will help to do basic research and development discoveries shared can be translated into technology that will impact positively on pharmaceuticals, diagnostic tools and modern health management technologies and thus benefit researchers and related industries in the areas of improved research and development networking, teaching and learning as this rosmarinic acid has a number of interesting biological activities, e.g. antiviral, antibacterial, anti-inflammatory and antioxidant. The presence of rosmarinic acid in medicinal plants, herbs and spices has beneficial and health promoting effects. In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defense compound.

REFERENCES

Bais HP, Walker TS, Schweizer HP, Vivanco JM (2002). Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum*. Plant Physiol. Biochem. 40: 983-995.
Chen H, Chen F, Zhang YL, Song JY (1999). Production of lithospermic

acid B and rosmarinic acid in hairy root cultures of *Salvia miltiorrhiza*. J. Ind. Microbiol. Biotechnol. 22: 133-138.
Eguchi Y, Curtis OF, Shetty K (1996). Interaction of hyperhydricity-preventing Pseudomonas sp. with oregano (*Origanum vulgare*) and selection of high phenolics and rosmarinic acid-producing clonal lines. Food Biotechnol. 10: 191-202.
Eknamkul W, Ellis BE (1989). Behavior of free aromatic amino acid pools in rosmarinic acid-producing cell cultures of *Anchusa officinalis* I. Plant Physiol. 89: 429-433.
Ellis BE, Towers GHN (1970). Biogenesis of rosmarinic acid in *Mentha*. Biochem. J. 118: 291-297.
Giri A, Narasu MJ (2000). Transgenic hairy roots: recent trends and applications. Biotechnol. Adv. 18: 1-22.
Grzegorczyk I, Krolicka A, Wysokinska H (2006). Establishment of *Salvia officinalis* L. hairy root cultures for the production of rosmarinic acid. Z Naturforsch. [C] 61: 351-356.
Guillon S, Tremouillaux-Guiller J, Pati PK, Rideau M, Gantet P (2006). Harnessing the potential of hairy roots: dawn of a new era. Trends Biotechnol. 24: 403-409.
Hamill JD, Parr AJ, Rhodes MJC, Robins RJ, Walton NJ (1987). New routes to plant secondary products. Biotechnology. 5: 800-804.
Häusler E, Petersen M, Alfermann AW (1992). Rosmarinsäure in *Blechnum*-Spezies. In: H.P. Haschke and C. Schnarrenberger, Editors, *Botanikertagung*, Berlin. Akademie Verlag, Berlin (1992), p. 507.
Hippolyte I, Marin B, Baccou JC, Jonard R (1992). Growth and rosmarinic acid production in cell suspension cultures of *Salvia officinalis* L. Plant Cell Rep. 11: 109-112.
Hraš AR, Hadolin M, Knez Z, Bauman D (2000). Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem. 71: 229-233.
Huang LD, Van SJ (2002). *Salvia chamelaeagnea* can be micropropagated and its callus induced to produce rosmarinic acid. S. Afr. J. Bot. 68: 177-180.
Hussein AA, Mansour BMM, Toaima N, Korus RA, Shetty K (1999). Tissue culture based screening for selection of high biomass and phenolic producing clonal lines of lavender using *Pseudomonas* and azetidine-2-carboxylate. J. Agric. Food Chem. 47: 2937-2943.
Llieva M, Pavlov A (1997). Rosmarinic acid production by *Lavandula vera* MM cell-suspension culture. Appl. Microbiol. Biotechnol. 47: 683-688.
Karam NS, Jawad FM, Arikat NA, Shibli RA (2003). Growth and rosmarinic acid accumulation in callus, cell suspension, and root cultures of wild *Salvia fruticosa*. Plant Cell Tissue Organ Cult. 73: 117-121.
Kim HK, Oh SR, Lee HK, Huh H (2001). Benzothiadiazole enhances the elicitation of rosmarinic acid production in a suspension culture of *Agastache rugosa* O. Kuntze. Biotechnol. Lett. 23: 55-60.
Kochan E, Wysokinska H, Chmiel A, Grabias B (1999). Rosmarinic acid and other phenolic acids in hairy roots of *Hyssopus officinalis*. Z. NaturforschI. 54c: 11-16.
Komali AS, Shetty K (1998). Comparison of the growth pattern and rosmarinic acid production in rosemary (*Romarinus officinalis*) shoots and genetically transformed callus cultures. Food Biotechnol. 12: 27-41.
Lee SY, Xu H, Kim YK, Park SU (2008). Rosmarinic acid production in hairy root cultures of *Agastache rugosa* Kuntze. World J. Microbiol. Biotechnol. 24: 969-972.
Li W, Koike K, Asada Y, Yoshikawa T, Nikaido T (2005). Rosmarinic acid production by *Coleus forskohlii* hairy root cultures. Plant Cell Tissue Organ Cult. 80:151-155.
Litvinenko VI, Popova TP, Simonjan IG, Sokolov VS (1975). "Gerbstoffe" und Oxyzimtsäureabkömmlinge in *Labiaten*. Planta Med. 27: 372-380.
Lu YR, Foo LY (1999). Rosmarinic acid derivatives from *Salvia officinalis*. Phytochemistry. 51: 91-94.
Martinez BC, Park CH (1994). Immobilization of *Coleus blumei* cells in a column reactor using a spray feeding system. Biotechnol. Techniq. 8: 301-306.
Martinez BC, Park CH (1993). Characteristics of batch suspension cultures of preconditioned *Coleus blumei* cells: Sucrose effect. Biotechnol. Prog. 9: 97-100.
Mary AL (2005). Valuable secondary products from *in vitro* culture. CRC Press LLC. 285-288
Mizukami H, Ogawa T, Ellis BE (1992). Induction of rosmarinic acid biosynthesis in *Lithospermum-erythrorhizon* cell suspension cultures by yeast extract. Plant Cell Rep. 11(9): 480-483.
Murakami Y, Omoto T, Asai I, Shimomura K, Yoshihira K, Ishimaru K (1998). Rosmarinic acid and related phenolics in transformed root cultures of *Hyssopus officinalis*. Plant Cell Tissue Organ Cult. 53: 75-78.
Namdeo AG (2007). Plant Cell Elicitation for Production of Secondary Metabolites**:** A Review. Phcog. Rev. 1: 69-79.
Pabsch K, Petersen M, Rao NN, Alfermann AW, Wandrey C (1991). Chemo- enzymatic synthesis of rosmarinic acid. Recl. Trav. Chim. Pays-Bas. 110: 199-205.
Park CH, Martinez B (1994). Growth and production characteristics of permeabilized *Coleus blumei* cells in immobilized fed-batch culture. Plant Cell Rep. 13: 459-463.
Park, CH, Martinez BC (1992). Enhanced release of rosmarinic acid from *Coleus blumei* permeabilized by dimethyl sulfoxide dmso while preserving cell viability and growth. Biotechnol. Bioeng. 40: 459-464.
Perry PL, Shetty K (1999). A model for involvement of proline during Pseudomonas-mediated stimulation of rosmarinic acid levels in oregano shoot clones. Food Biotechnol. 13: 137-154.
Petersen M, Haeusler E, Meinhard J, Karwatzki B, Gerlowski C (1994). The biosynthesis of rosmarinic acid in suspension cultures of *Coleus blumei*. Plant Cell Tissue Organ Cult. 38: 171-179.
Phatak SV, Heble MR (2002). Rosmarinic acid synthesis in shoot cultures of *Mentha arvensis* Linn. Ind. J. Biotechnol. 1: 381-385.
Ravn H, Pedersen MF, Andray J, Borum C, Anthoni U, Christophersen C, Nielsen PH (1994). Seasonal variation and distribution of two phenolic compounds, rosmarinic acid and caffeic acid, in leaves and roots-rhizomes of eelgrass (*Zostera marina* L.). Ophelia. 40: 51-61.
Reuff I, Seitz U, Ulbrich B, Reinhard E (1988). Cryopreservation of *Coleus-blumei* suspension and callus cultures. J. Plant Physiol. 133: 414-418.
Sanbongi C, Takano H, Osakabe N, Sasa N, Natsume M, Yanagisawa R, Inoue KI, Sadakane K, Ichinose T, Yoshikawa T (2004). Rosmarinic acid in perilla extract inhibits allergic inflammation induced by mite allergen, in a mouse model. Clin. Exp. Allergy. 34(6): 971-977.
Signs M, Flores H (1990). The biosynthetic potential of plant roots. Bioessays. 12: 7-13.
Su WW, Humphrey AE (1991). Production of rosmarinic acid from perfusion culture of *Anchusa officinalis* in a membrane-aerated bioreactor. Biotechnol. Lett. 13: 889-892.
Su WW, Lei F (1993). Rosmarinic acid production in perfused *Anchusa officinalis* culture: Effect of inoculum size. Biotechnol. Lett. 15: 1035-1038.
Su WW, Lei F, Kao NP (1995). High density cultivation of *Anchusa officinalis* in a stirred-tank bioreactor with in situ filtration. Appl. Microbiol. Biotechnol. 44: 293-299.
Sumaryono W, Proksch P, Hartmann T, Nimtz M, Wray V (1991).Induction of rosmarinic acid accumulation in cell suspension cultures of *Orthosiphon aristatus* after treatment with yeast extract. Phytochemistry. 30: 3267-3272.
Szabo E, Thelen A, Petersen M (1999). Fungal elicitor preparations and methyl Jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei*. Plant Cell Rep. 18: 485-489.
Tada H, Murakami Y, Omoto T, Shimomura K, Ishimaru K (1996). Rosmarinic acid and related phenolics in hairy root cultures of *Ocimum basilicum*. Phytochemistry. 42: 431-434.
Takeda R, Hasegawa J, Sinozaki M (1990). The first isolation of lignans, megacerotonic acid and anthocerotonic acid, from non-vascular plants, *Anthocerotae* (hornworts). Tetrahedron Lett. 31: 4159-4162.
Verpoorte R, van der Heijden R, ten Hoopen HJG, Memelink J (1998). Metabolic engineering for the improvement of plant secondary metabolite production. Plant Tissue Culture Biotechnol. 4: 3-20.
Xu H, Kim YK, Jin XJ, Lee SY, Park SU (2008). Rosmarinic Acid Biosynthesis in Callus and Cell Cultures of *Agastache rugosa* Kuntze. J. Med. Plants Res. 2: 237-241.
Yang R, Shetty K (1998). Stimulation of rosmarinic acid in shoot cul-

tures of oregano (*Origanum vulgare*) clonal line in response to proline, proline analogue, and proline precursors. J. Agric. Food Chem. 46: 2888-2893.

Youn J, Lee KH, Won J, Huh SJ, Yun HS, Cho WG, Paik DJ (2003). Beneficial Effects of Rosmarinic Acid on Suppression of Collagen Induced Arthritis. J. Rheumatol. 30: 1203-1207.

Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49: 5165-5170.