Response of Different *Agrobacterium Rhizogenes* Strains for *in vitro* Hairy Root Induction and Accumulation of Rosmarinic Acid Production in *Agastache Rugosa*

1Woo Tae Park, 1Thanislas Bastin Baskar, 1Sun Kyung Yeo, 2Nam Il Park, 3Jong Seok Park and 1Sangun Park

1Department of Crop Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon, 34134, Korea  
2Department of Plant Science, Gangneung-Wonju National University, Gangneung-si, Gangwon-do 25457, Korea  
3Department of Horticultural Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Korea

Abstract: In this study a total of seven different *Agrobacterium rhizogenes* strains were evaluated for their ability to transform the plant *Agastache rugosa* and to produce the secondary metabolite rosmarinic acid. All the strains of *A. rhizogenes* i.e., 13333, 15834, A4, LBA9402, R1000, R1200 and R1601 strains tested here in this study, were able to induce hairy root formation in leaf tissue explants. The strain A4 had the highest rate of infection (94.1±8.3%) and the strain R1000 had the lowest rate (88.6±6.9%). The highest frequency of hairy roots per explant (13.6±1.4) was found for strain R1601 and the tallest root length (20.4±1.7 mm) was found for strain 13333. We also evaluated dry weight and level of rosmarinic acid in the hairy roots and found that the highest growth (310.1±14.6 mg/flask) was occurred after infection with strain R1200, while the highest production of rosmarinic acid (68.2±3.8 mg/g dry weight) was noted using strain 13333. Our study showed that *A. rhizogenes* strain 13333 was the most effective of the 7 tested strains for production of transformed root cultures as well as rosmarinic acid in the hairy roots.

Keywords: *Agastache Rugosa*, Hairy Root, Rosmarinic Acid, Strain of *Agrobacterium Rhizogenes*

Introduction

The ornamental plant *Agastache rugosa* (family Lamiaceae), also known as Korean mint, is mainly found in Eastern Asia and is widespread in Korea, Japan and China as reported by (Kang et al., 2013; Zielinska and Matkowski, 2014). In recent years, *A. rugosa* has become one of the species of choice for medical research due to its interesting physiological and pharmacological properties (Wilson et al., 1992; Song et al., 2001; Shin and Kang, 2003; Jun et al., 2010) and also have anti-inflammatory and anti-a rogenic properties (Min et al., 1999). *A. rugosa* possesses properties of potentially great importance for development of drugs against diseases such as cholera and for other medical conditions such as vomiting and for the treatment of disorders in intestine (Kim et al., 2001; Chae et al., 2005; Lee et al., 2008; Li et al., 2013).

*A. rugosa* also has use in food industries as it can act as a flavoring agent and add a spicy flavoring to food (Hong et al., 2001). Presently, there is considerable interest in Korea on exploiting native plant species like as persimmon, hardy rubber and wild mulberry used as efficient foods (Han, 2010; Park et al., 2012). *A. rugosa* leaves may possibly be of use in high-standard useful foods especially for tea, cakes and so on. The leaves can also be used to extract a fragrance reagent for perfumes. Thus, *A. rugosa* exhibits a variety of valuable properties with economic and social benefits.

*Agrobacterium rhizogenes* (family Rhizobiaceae) is a well-known gram-negative bacterium, can induce hairy root at the place of contamination in plants. By using hairy Root Inducing (Ri)-plasmids, *A. rhizogenes* transmitted T-DNA into plant cells to bring into being hairy root diseases (Hamill et al., 1987). Now a day *A. rhizogenes* has been used to induce hairy roots...
to a vast number of plant species (Guillon et al., 2006). There is a considerable interest in hairy root cultures for its quick growth, genetic and biochemical stability as well for their ability to produce higher amount of secondary metabolites (Christey and Braun, 2005; Georgiev et al., 2007; Srivastava and Srivastava, 2007).

There is a continuing demand for production of rosmarinic acid from different plant sources because of their various pharmacological uses (Petersen and Simmonds, 2003; Gao et al., 2005; Swarup et al., 2007). Rosmarinic acid has a wide range of health benefitted biological properties i.e., anti-inflammatory, antioxidiant and so on those were reported by (Domitriović et al., 2013; Rocha et al., 2015).

Hairy root cultures from A. rugosa can be used to achieve production of rosmarinic acid. Elicitors upon contact with the cells of higher plants trigger increased production of secondary metabolites in different plant species (Uddin et al., 2010; Kim et al., 2013; Bong et al., 2015). Optimization of secondary metabolite production could be achieved not only by elicitors or phytohormones but also could be done by using bioinformatics methods (Tambunan et al., 2014).

Here we investigated both the induction of hairy roots and the levels of rosmarinic acid accumulation from A. rugosa using different A. rhizogenes strains and compare the outcome with the previously reported work on another strain of A. rhizogenes (Li et al., 2010).

**Materials and Methods**

**Procedure of Sterilization and Germination of Seeds**

Before placing seed germination, seeds of A. rugosa were sterilized with 70% (v/v) ethanol for 1 min and then 4% (v/v) sodium hypochlorite solution was used for 10 min and finally seeds were rinsed three times with sterilized water.

Six seeds were kept on a 25 mL−1 of agar-solidified culture medium having the size of ×15 mm petri dishes for germination. The basal MS medium contained salts and was solidified by using 0.8% (w/v) agar following the techniques described by (Murashige and Skoog, 1962.) The pH of the medium was attuned to 5.8 before adding agar and there after the medium was sterilized through autoclaving maintaining temperature of 121°C for a period of 20 min. For germination the seeds were kept in a growth chamber following a temperature of 25°C, a flux rate of 35 µmol s−1 m−2 and a 16-h photoperiod under standard cool white fluorescent tubes.

**Growth of Agrobacterium Rhizogenes**

Cultures of A. rhizogenes strains used in this study i.e., 13333, 15834, A4, LBA9402, R1000, R1200 and R1601 were instigated from glycerol stocks. They were allowed to grow in a liquid Luria-Bertani medium, to mid-log phase (OD600 = 0.5) overnight maintaining the temperature of 28°C with shaking at 180 rpm. A. rhizogenes were cultured for a period of 10 min through centrifugation at 224×g and then suspended again in the MS medium containing 30 g L−1 sucrose. The cell density of A. rhizogenes was maintained at 600 nm of 1.0 for inoculation through a spectrophotometer absorption unit.

**Establishment of Hairy Root Cultures**

Leaves of A. rugosa were sampled for establishing hairy roots, from the plants those were grown in vitro condition and were cut at the ends with a size of 7×7 mm. The cut pieces of leaves were put into the culture of A. rhizogenes strains 13333, 15834, A4, LBA9402, R1000, R1200 or R1601 in liquid inoculation medium for 10 min and then these samples were dry on sterile filter paper and finally incubated in dark condition on MS medium containing agar-solidified at 25°C. After 2 days of co-cultivation the explants were moved to a hormone-free medium, where medium contained a combination of MS salts and vitamins, 30 g L−1 sucrose, 500 mg L−1 cefotaxime and 8 g L−1 agar. Within 2 weeks many hairy roots were initiated from the injured sites of the explants. The hairy roots those initiated from explants were separated and then they were cultured again in the dark condition keeping them on agar-solidified MS medium at 25°C. Immediately after repeated transfers to fresh medium rapidly growing hairy root cultures were found. An amount of 0.5 g [DW/l] of isolated roots were moved to a 30 mL−1 MS liquid medium (30 g L−1 sucrose), in 100 mL−1 flask. Root cultures keeping in 100 mL−1 flasks were moved in a growth chamber putting them in a gyratory shaker (100 rev/min) at 25°C with a flux rate of 35 µmol s−1m−2 and a 16-h photoperiod under standard cool white fluorescent tubes. Hairy roots were allowed to grow for 21 days and then they were harvested and finally from that samples dry weights as well as level of rosmarinic acid contents were measured. Three flasks were used for each culture and experiments were repeated thrice.

**HPLC Analysis of Rosmarinic Acid**

For rosmarinic acid analysis in HPLC, 1 g of collected hairy roots were frozen in liquid N2 and then were ground using a mortar and pestle to a fine powder and finally extracted using 10 mL−1 methanol for two times for a period of 24 h at 25°C. After drying under vacuum the crude extracts were achieved and before placing in HPLC analysis it was finally dissolved in methanol. Rosmarinic acid was quantified using a
System Gold 126 HPLC and 128 photodiode array detector manufactured by Beckman-Coulter, Mississauga, Canada under a C18 reverse phase column having 4.6 mm internal diameter, 250 mm length; Ultra sphere, Beckman-Coulter at room temperature. A solvent gradient was used as in the mobile phase by mixing of 70% solvent A (3% acetic acid in water) to 30% solvent B (methanol); where the solvent gradient reached 100% solvent B after 50 min. The solvent flow rate was maintained in a constant at 1.0 ml/min. Samples were detected at 280 nm wavelength.

Data Analysis

The data are reported as means±standard deviation.

Results

*A. rugosa* leaf explants showed susceptible for infection by all the strains of *A. rhizogenes* used in this study. No significant differences were observed in the morphologies for the hairy roots production in either of the strains used in this study. The rates of hairy root formation produced by the different *A. rhizogenes* strains were: A4 (94.1±8.3%), 13333 (93.2±8.4%), R1200 (92.7±7.4%), LBA9402 (91.6±9.1%), 15834 (91.5±7.8%), R1601 (89.3±8.2%) and R1000 (88.6±6.9%). These results were shown in Fig. 1.

The numbers of hairy roots per explant at 30 days after inoculation were as follows: 13.6±1.4 for strain R1601; 13.0±1.7 for 13333; 12.3±0.8 for R1601; 11.7±0.8 for A4; 10.6±1.3 for LBA9402; 9.7±1.3 for 15834; and 9.6±1.3 for R1000. The hairy root lengths in well-developed roots for each strain were as follows: 20.4±1.7 mm for strain 13333; 19.4±1.4 mm for R1200; 18.2±1.3 mm for LBA9402; 17.4±1.1 mm for R1601; 16.5±1.4 mm for A4; 16.2±1.1 mm for 15834; and 15.8±0.8 for R1000, results were shown in Table 1.

Hairy root cultures from each of the seven strains were subculture in a fresh medium for a period of two to three months and there after transferred into liquid medium. In the liquid culture, MS medium was used and then kept them to grow for 21 days. At this level dry weight of hairy roots and level of rosmarinic acid s were evaluated in response to different strains (Fig. 2). The highest growth was found in R1200 strain with 310.1±14.6 mg/flask. For the other strains, we found: 306.8±16.4 mg/flask for strain 13333; 296.3±12.1 mg/flask for A4; 292.5±18.8 mg/flask for LBA9402; 291.3±12.0 mg/flask for R1000; 283.2±21.9 mg/flask for 15834; and 278.8±23.8 mg/flask for R1601. The levels of rosmarinic acid were (Fig. 3) 68.2±3.8 mg/g DW for strain 13333; 67.7±4.4 mg/g DW for R1200; 60.8±4.4 mg/g DW for R1000; 56.4±4.4 mg/g DW for R1601; 55.6±4.1 mg/g DW for LBA9402; 52.0±4.6 mg/g DW for 15834; and 51.8±5.3 mg/g DW for A4.

![Fig. 1. Effect of strains of *A. rhizogenes* on the infection frequency of *A. rugosa* hairy root cultures (The values represent the means±SD of three independent measurements)](image-url)
Fig. 2. Effect of strains of *A. rhizogenes* on the growth of *A. rugosa* hairy root cultures (The values represent the mean±SD of three independent measurements)

Fig. 3. Effect of *A. rhizogenes* on rosmarinic acid production in *A. rugosa* hairy root cultures (The values represent the mean±SD of three independent measurements)
Table 1. Influence of strains of *A. rhizogenes* on the growth of *A. rugosa* hairy root cultures

| Agrobacterium strains | Number of hairy roots | Root length (mm) |
|-----------------------|-----------------------|------------------|
| 13333                 | 13.0±1.7              | 20.4±1.7         |
| 15834                 | 9.7±1.3               | 16.2±1.1         |
| LBA9402               | 11.7±0.8              | 16.5±1.4         |
| R1000                 | 10.6±1.3              | 18.2±1.3         |
| 13333                 | 9.6±1.3               | 15.8±0.8         |
| R1200                 | 13.6±1.4              | 19.4±1.4         |
| R1601                 | 12.3±0.8              | 17.4±1.1         |

The values represent the mean±SD from three independent measurements.

**Discussion**

It is well reported that *A. rhizogenes* strains showed positive response for hairy root development and also responded highly showing vigorous growth behavior and finally a wide range variation of accumulation of secondary metabolites. From a previous study which was reported a long time before where different *A. rhizogenes* strains were used to examine rooting ability, accumulation of saponin and amount of astragal sides in *Astragalus on gholicus* (Ionkova et al., 1997). The hairy roots in *Capsicum* species was initiated through *A. rhizogenes* strains has also been reported (Setamam et al., 2014). From the research findings of (VanHALA et al., 1995; Mateus et al., 2000) where they reported that *Agrobacterium* infection was shown to increase root growth and higher accumulation of alkaloids and tropane content in *Hyoscyamus niger* transgenic root cultures. *Gentiana macrophylla* hairy root cultures were evaluated after infection with four *A. rhizogenes* strains, where different responses were observed in each hairy root line for their root growth and also for accumulation of secoiridoid glucoside, gentiopicroside (Tiwari et al., 2007). The selection of an effective *Agrobacterium* strain is highly responsible for the production of transformed root cultures on types of plant species and it could be determined empirically.

The comparative abilities of the *A. rhizogenes* strains like 13332, 15834, R1000, R1200 and R1601 were studied in *Rubia akane* to check the ability of hairy root formation and level of anthraquinones production (Lee et al., 2010). It is reported that different *A. rhizogenes* strains (i.e., A1, 15834, K599, LBA 9402, 9365 and 9340) helped to induce transformed hairy roots in shoot tip meristem explants of *Artemisia annua* Giri et al. (2001).

Rosmarinic acid is found in a variety of plant species as an ester of caffeic acid. Previous studies reported that hairy root cultures *A. rugosa* produces rosmarinic acid by inducing *A. rhizogenes* strain R1000 from the hairy root of the mint family of many species like *Ocimum basilicum* (Tada et al., 1996), *Salvia miltiorrhiza* (Chen et al., 2001), *Coleus forskohlii* (Li et al., 2005) and *Salvia of ficinalis* (Grzegorczyk et al., 2006), rosmarinic acid was also found. The technique of hairy roots induction could be a best planting material for the accumulation of secondary metabolites. Here in this study, we compared the abilities of seven different *A. rhizogenes* strains to induce hairy roots and identified different responses in terms of infection frequency, number of roots, root lengths, dry weights and rosmarinic acid production in *A. rugosa*. We found that *Agrobacterium rhizogenes* strain 13333 was best for accumulation of rosmarinic acid in the hairy roots of *A. rugosa*.

**Conclusion**

In conclusion, this report describes a quick and effective protocol of *A. rhizogenes* mediated transformation for the enhancement of hairy root cultures and accumulation of a vital secondary metabolite like rosmarinic acid in *A. rugosa* which can easily compare the effectiveness of *A. rhizogenes* strains 13333, 15834, A5, LBA9402, R1000, R1200 and R1601. Strain 13333 showed the best characteristics to initiate hairy root and product of rosmarinic acid. All seven strains induced hairy root formation and production of rosmarinic acid but at different levels. From our observations, we conclude that *A. rugosa* is a promising and suitable candidate for accumulation of rosmarinic acid in the in vitro root cultures.

**Funding Information**

This study was supported by a Grant (Project no. 116068-03-2-HD020) from Korean Institute of Planning and Evaluation for Technology of Food, Agriculture, Forestry and Fisheries (IPET).

**Author’s Contributions**

Woong Tae Park, Thanislas Bastin Baskar, Sun Kyung Yeo and Jong Seok Park: Conduction of experiments and analyzed the data

Nam Il Park: Planning the idea of the study and preparation of manuscript

Sangun Park: Planning, coordination and editing the manuscript.

**Ethics**

This article is original and contains unpublished material. The corresponding author confirms that all of
the other authors have read and approved the manuscript and no ethical issues involved.

References

Bong, S.J., M.R. Uddin, S.J. Kim, J.S. Park and S.U. Park, 2015. Influence of auxins and wounding on glucosinolate biosynthesis in hairy root cultures of Chinese cabbage (Brassica rapa ssp. pekinensis). Biosci. Biotechnol. Res. Asia. 12: 1041-1046.

Chae, Y.A., H.C. Ohk and J.S. Song, 2005. Variability of the volatile composition of Agastache rugosa in South Korea. Proceedings of the Bioprospecting and Ethno Pharmacology, (BEPS' 05).

Chen, H., F. Chena, F.C. Chiu and C.M. Lo, 2001. The effect of yeast elicitor on the growth and secondary metabolism of hairy root cultures of Salvia miltiorrhiza. Enzyme Microb. Technol., 28: 100-105.

Christey, M.C. and R.H. Braun, 2005. Production of hairy root cultures and transgenic plants by Agrobacterium rhizogenes-mediated transformation. Methods Mol. Biol., 286: 47-60.

Dominov, R., M. Skoda, V.V. Marchesi, O. Cvijanovic and E.P. Pugel et al., 2013. Rosmarinic acid ameliorates acute liver damage and fibrogenesis in carbon tetrachloride-intoxicated mice. Food Chem. Toxicol., 51: 370-378.

Gao, L.P., H.L. Wei, H.S. Zhao, S.Y. Xiao and R.L. Zheng, 2005. Antiapoptotic and antioxidant effects of rosmarinic acid in astrocytes. Pharmazie, 60: 62-65.

Georgiev, M.I., A.I. Pavlov and T. Bley, 2007. Hairy root type plant in vitro systems as sources of bioactive substances. Applied Microbiol. Biotechnol., 74: 1175-1185.

Giri, A., S.T. Ravindra, V. Dhingra and M.L. Narasu, 2001. Influence of different strains of Agrobacterium rhizogenes on induction of hairy roots and artemisinin production in Artemisia annua. Curr. Sci., 81: 378-382.

Grzegorczyk, I., A. Krolka and V. Wysokinska, 2006. Establishment of salvia of ficinalis L. hairy root cultures for the production of rosmaragic acid. Z. Naturforsch, 61: 351-356.

Guillon, S., J. Tremouillaux-Guiller, P.K. Pati, M. Rideau and P. Gantet, 2006. Harnessing the potential of hairy roots: Dawn of a new era. Trends Biotechnol., 24: 403-409.

Hamill, J.D., A.J. Parr, M.J.C. Rhodes, R.J. Robins and N.J. Walton, 1987. New routes to plant secondary products. Biotechnol, 5: 800-804.

Han, S.H., 2010. Effects of eucommia ulmoides oliveri tea extract on aluminum accumulation rate and tissue function in aluminum-administered rats. Korean J. Food Culture, 25: 839-846.

Hong, J.J., J.H. Choi, S.R. Oh, H.K. Lee and J.H. Park et al., 2001. Inhibition of cytokine-induced vascular cell adhesion molecule-1 expression; possible mechanism for anti-atherogenic effect of Agastache rugosa. FEBS Lett., 495: 142-147.

Ionkova, I., T. Kartnig and W. Alfermann, 1997. Cycloartane saponin production in hairy root cultures of Astragalus mongholicus. Phytochem, 45: 1597-1600.

Jun, H.J., M.J. Chung, K. Dawson, R.L. Rodriguez and S.J. Houng et al., 2010. Nutrigenomic analysis of hypolipidemic effects of Agastache rugosa essential oils in HepG2 cells and C57BL/6 mice. Food Sci. Biotechnol., 19: 219-227.

Kang, M.J., S. Sundan, G.A. Lee, H.C. Ko and J.W. Chung et al., 2013. Genetic diversity and population structure of Korean mint Agastache rugosa Kuntze (Lamiaceae) using ISSR markers. Korean J. Plant Res., 26: 362-369.

Kim, S.J., W.T. Park, M.R. Uddin, Y.B. Kim and S.Y. Nam et al., 2013. Glucosinolate biosynthesis in hairy root cultures of broccoli (Brassica oleracea var. italica). Natural Product Commun., 8: 217-220.

Kim, T.H., J.H. Shin, H.H. Baek and H.J. Lee, 2001. Volatile flavour compounds in suspension culture of Agastache rugosa Kuntze (Koreanmint). J. Sci. Food Agric., 81: 569-575.

Lee, S.Y., H. Xu, Y.K. Kim and S.U. Park, 2008. Rosmarinic acid production in hairy root cultures of Agastache rugosa Kuntze. World J Microb. Biot., 24: 969-972.

Lee, S.Y., S.G. Kim, W.S. Song, Y.K. Kim and N.I. Park et al., 2010. Influence of different strains of Agrobacterium rhizogenes on hairy rootinduction and production of alizarin and purpurin in Rubia akane Nakai. Rom. Biotechnol. Lett., 15: 5405-5409.

Li, G.S., W.L. Jiang, J.W. Tian, G.W. Qu and H.B. Zhu et al., 2010. In-vitro and in-vivo antifibrotic effects of rosmarinic acid on experimental liver fibrosis. Phytoemed, 17: 282-288.

Li, H.Q., Q.Z. Liu, Z.L. Liu, S.S. Du and Z.W. Deng, 2013. Chemical composition and nematocidal activity of essential oil of Agastache rugosa against Meloidogyne incognita. Molecules, 18: 4170-4180.

Li, W., K. Koike, Y. Asada, T. Yoshikawa and T. Nikaido, 2005. Rosmarinic acid production by Coleus forskohlii hairy root cultures. Plant Cell Tissue Organ Cult., 80: 151-155.

Mateus, L., S. Cherkaooui, P. Christen and K.M. Oksman-Caldentey, 2000. Simultaneous determination of scopoline, hyoscyamine and litorine in plants and different hairy root clones of Hyoscyamus muticus by micellar electrokinetic chromatography. Phytochem, 54: 517-523.

Min, B.S., M. Hattori, H.K. Lee and Y.H. Kim, 1999. Inhibitory constituents against HIV-1 protease from Agastache rugosa. Arch. Pharm. Res., 22: 75-77.
Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant, 15: 473-497.

Park, S., W.Y. Jeong, J.H. Lee, Y.H. Kim and S.W. Jeong et al., 2012. Determination of polyphenol levels variation in Capsicum annum L. CV. Chelsea (yellow bell pepper) infected by anthracnose (Colletotrichum gloeosporioides) using liquid chromatography–tandem mass spectrometry. Food Chem., 130: 981-985.

Petersen, M. and M.S.J. Simmonds, 2003. Rosmarinic acid. Phytochem, 62: 121-125.

Rocha, J., M. Eduardo-Figueira, A. Barateiro, A. Fernandes and D. Brites et al., 2015. Anti-inflammatory effect of rosmarinic acid and an extract of Rosmarinus officinalis in rat models of local and systemic inflammation. Basic Clin. Pharmacol. Toxicol., 116: 398-413.

Setamam, N.M., N.J. Sidik, Z.A. Rahman and C.R.C.M. Zain, 2014. Induction of hairy roots by various strains of Agrobacterium rhizogenes in different types of Capsicum species explants. BMC Res. Notes, 7: 414-414.

Shin, S. and C.A. Kang, 2003. Antifungal activity of the essential oil of Agastache rugosa Kuntze and its synergism with ketoconazole. Lett. Applied Microbiol., 36: 111-115.

Song, J.H., M.J. Kim, H.D. Kwon and I.H. Park, 2001. Antimicrobial activity and components of extracts from Agastache rugosa during growth period. J. Food. Sci. Nutri., 6: 10-15.

Srivastava, S. and A.K. Srivastava, 2007. Hairy Root culture for mass-production of high-value secondary metabolites. Crit. Rev. Biotechnol., 27: 29-43.

Swarup, V., J. Ghosh, S. Ghosh, A. Saxena and A. Basu, 2007. Antiviral and anti-inflammatory effects of rosmarinic acid in an experimental murine model of Japanese encephalitis. Antimicrob. Agents Chemother., 51: 3367-3370.

Tada, H., Y. Murakami, T. Omoto, K. Shimomura and K. Ishimaru, 1996. Rosmarinic acid and related phenolics in hairy root cultures of Ocimum basilicum. Phytochem, 42: 431-434.

Tambunan, U.S.F., A. Randy and A.A. Parikesit, 2014. Design of Candida antarctica Lipase B thermost ability improvement by introducing extra disulfide bond into the enzyme. Online J. Biol. Sci., 14: 108-118.

Tiwari, R.K., M. Trivedi, Z.C. Guang, G.Q. Guo and G. Zheng, 2007. Genetic transformation of Gentiana macrophylla with Agrobacterium rhizogenes: Growth and production of secoiridoid glucoside gentiopicrosidein transformed hairy root cultures. Plant Cell Rep., 26: 199-210.

Uddin, M.R., K.W. Park, Y.K. Kim, S.U. Park and J.Y. Pyon, 2010. Enhancing sorgoleone levels in grain sorghum root exudates. J. Chem. Ecol., 36: 914-922.

VanHala, L., R. Hiltunen and K.M. Oksman-Calendentey, 1995. Virulence of different Agrobacterium strains on hairy root formation of Hyoscyamus muticus. Plant Cell Rep., 14: 236-240.

Wilson, L.A., N.P. Senechal and M.P. Widrlechner, 1992. Headspace analysis of the volatile oils of Agastache. J. Agirc. Food. Chem., 40: 1362-1366.

Zielińska, S. and A. Matkowski, 2014. Phytochemistry and bioactivity of aromatic and medicinal plants from the genus Agastache (Lamiaceae). Phytochem Rev., 13: 391-416.