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Research Note

Exogenous auxins and polyamines enhance growth and rosmarinic acid production in hairy root cultures of *Nepeta cataria* L.

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Abstract

To enhance growth and rosmarinic acid production in catnip (*Nepeta cataria* L), hairy root cultures were grown for 15 days in media supplemented with various concentrations of auxins or polyamines. Three auxins (0.1–1 mg·L^{-1} IAA, IBA, and NAA) enhanced the growth of hairy roots and production of rosmarinic acid. IBA was the most effective auxin. Hairy root cultures treated with 0.5 mg·L^{-1} IBA produced the highest levels of growth (13.5 g·L^{-1} culture) and rosmarinic acid (19.2 mg·g^{-1} dry weight). Similarly, putrescine was the most effective polyamine. Hairy root cultures treated with 50 mg·L^{-1} putrescine produced the highest levels of growth (13.3 g·L^{-1} culture) and rosmarinic acid (18.4 mg·g^{-1} dry weight). These findings indicate that exogenous auxins and polyamines can enhance growth and rosmarinic acid production in hairy root culture of *N. cataria.*

Keywords: Auxins; Catnip; hairy root; Polyamines; Rosmarinic acid
Abbreviation: IAA- indole-3-acetic acid; IBA- indole-3-butyric acid; NAA-1-naphthaleneacetic acid; HPLC - high-performance liquid chromatography

Introduction

Nepeta cataria L., commonly known as catnip, is a perennial herb that belongs to the mint family (Labiatae). Traditionally, catnip is believed to have sedative, carminative, and antispasmodic properties. Therefore, it has been used to treat insomnia, flatulence, and upset stomach. It has also been used to treat the common cold, flu, and fevers (Grognet, 1990; Tucker and Tucker, 1988). Several classes of secondary metabolites have been isolated from *N. cataria*, such as flavonoids, phenolic compounds, and essential oils containing monoterpenes, terpenoids, and sterols (Chauhan et al., 2005; Ganzera et al., 2001; Klimek and Modnicki, 2005; Modnicki et al., 2007). Rosmarinic acid (Fig. 1), an ester of caffeic acid, is abundant in several plant species and is an active constituent of *N. cataria*. Rosmarinic acid has many useful biological properties, including astringent, antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and antiviral effects (Ly et al., 2006; Parnham and Kesselring, 1985). Hairy root cultures are *in vitro* plant tissue cultures established by transformation of plant cells with *Agrobacterium rhizogenes*. Hairy root cultures have many advantages, including biochemical and genetic stability, independence from seasonal and geographical conditions, rapid growth, and the ability to produce secondary metabolites at levels comparable to plants grown naturally. As a result, these cultures have been used to produce pharmaceuticals, cosmetics, and food additives from many plant species (Giri and Narasu, 2000; Guillon et al., 2006; Signs and Flores, 1990). Recently, rosmarinic acid production by *N. cataria* was detected using hairy root cultures (Lee *et al.*, 2010). Since factors such as phytohormones influence growth and production of secondary metabolites in hairy root cultures of plants (Hu and Du, 2006), we investigated the effects of several auxins and polyamines on growth and rosmarinic acid biosynthesis in hairy root cultures of *N. cataria*.

Materials and methods

H*airy root cultures*

Hairy root cultures of *N. cataria* were established and maintained as described previously (Lee, et al., 2010). In brief, hairy roots of *N. cataria* were subcultured on fresh agar-solidified MS medium (Murashige and Skoog, 1962), and then transferred to MS liquid culture medium for experiments. Hairy root cultures were maintained in MS liquid medium and subcultured every 15 days.

Culture and treatment conditions

The basal MS medium contained salts, vitamins, and 30 g·L^{-1} sucrose. The pH of the medium was adjusted to 5.8 before adding agar, and then autoclaved for 20 min. Hairy root cultures were maintained at 25 °C on a gyratory shaker (100 rpm) in a growth chamber with a 16-h photoperiod and cool white fluorescent lights (flux rate of 35 mol·s^{-1}·m^{-2}). The hairy root cultures were treated with 6 plant hormones. Specifically, the effects of 3 auxins, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA), were tested at 3 different concentrations (0.1, 0.5, and 1.0 mg·L^{-1}). In addition, 3 polyamines, putrescine, spermidine, and spermine, were tested at 3 different concentrations (10, 50, and 100 mg·L^{-1}). Three flasks were used for each treatment condition, and experiments were performed in duplicate.

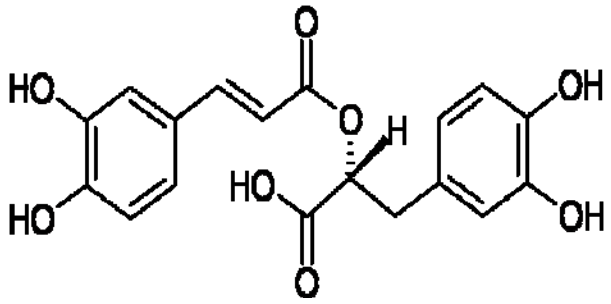

Fig 1. Chemical structure of rosmarinic acid.

Preparation of samples for high-performance liquid chromatography analysis of rosmarinic acid

After 15 days of culture, hairy roots were harvested and freeze-dried at -80 °C for at least 48 h using a FD-5508 freeze dryer (Ilsin Engineering Co., Seoul, Korea). Dried samples (0.5 g) were ground into a fine powder using a mortar and pestle, and then extracted twice with 10 mL methanol for 24 h at 25°C. Extracts were vacuum dried, and then the residue was resuspended in methanol for high-performance liquid chromatography (HPLC) analysis. HPLC was performed with an Ultrasphere C_{18} reversed phase column (4.6 × 250 mm; Beckman-Coulter). Rosmarinic acid was purified by a linear gradient of 30–100% solvent B (methanol) for 50 min at room temperature. Solvent A was 3% acetic acid in water. The flow rate was maintained at 1.0 ml·min^{-1} and the injection volume was 20 μl. Elution was monitored at 280 nm. The amount of rosmarinic acid in samples was determined from a standard curve of rosmarinic acid.

Statistical analysis

Each result shown in the figures was the mean of three replicated treatments. The significant differences between treatments were statistically evaluated by standard deviation.

Results

To induce hairy roots from leaf explants of *N. cataria*, *A. rhizogenes* R1000 was tested for its ability. For removing *A. rhizogenes* explant tissues were transferred to agar-solidified MS medium containing 200 mg/l Timentin, 2 days after co-cultivation with *A. rhizogenes* R1000. Wounded *N. cataria* leaf explants were susceptible to infect by *A. rhizogenes* R1000. It infected more than 70% of the leaf explants 30 days after inoculation. Hairy root initials emerged from wound sites on leaf within 15 days after inoculation. After 30 days, it was shown that hairy roots of *N. cataria* began to grow more rapidly. Rapidly growing hairy roots were excised from the explant tissues and transferred on agar-solidified MS medium containing 200 mg/l Timentin in every two weeks. After repeated transfer to fresh medium for three months, then hairy root clones were replaced to MS liquid culture medium.

Effects of auxins on growth and rosmarinic acid production in hairy root cultures

To study the effects of different auxins on growth and rosmarinic acid production in catnip hairy root cultures, hairy roots were grown for 15 days in media supplemented with various concentrations of different auxins. Our results revealed that all tested auxin treatments increased the growth rates of the hairy roots and rosmarinic acid production in hairy roots (Fig. 2). IBA was the most effective auxin. Hairy root cultures treated with 0.5 mg·L^{-1} IBA produced the highest levels of hairy root growth (13.5 g·L^{-1} culture) and rosmarinic acid (19.2 mg·g^{-1} dry weight (D.W.)). For both IBA and NAA, concentrations up to 0.5 mg·L^{-1} increased the dry weight and rosmarinic acid content; however, higher concentrations decreased these parameters. Here NAA was more sensitive than IBA as at the highest level of NAA (1 mg·L^{-1}) the amount of reduction of dry weight and rosmarinic acid production was much lower than IBA. In contrast, increasing concentrations of IAA monotonically increased the dry weight and rosmarinic acid content.

Effects of polyamines on growth and rosmarinic acid production in hairy roots

To examine the effect of polyamines on hairy root growth and rosmarinic acid production in catnip hairy root cultures, hairy roots were grown for 15 days in media supplemented with various concentrations of different polyamines. All three polyamines (putrescine, spermidine, and spermine) increased growth and rosmarinic acid production in hairy roots (Fig. 3). Putrescine was the most effective polyamine. Hairy root cultures treated with 50 mg·L^{-1} putrescine produced the highest levels of hairy root growth (13.3 g·L^{-1} culture) and rosmarinic acid (18.4 mg·g^{-1} D.W.). For both putrescine and spermidine, concentrations up to 50 mg·L^{-1} increased the dry weight and rosmarinic acid content; however, higher concentrations decreased these effects. In contrast, increasing concentrations of spermine monotonically increased the dry weight and rosmarinic acid content.

Discussion

The enhancement of catnip hairy root growth and rosmarinic production upon treatment with auxins and polyamines is consistent with the results of previous studies showing that exogenous auxins and polyamines increase growth and production of secondary metabolites in hairy root cultures of several plant species. Auxins are important for stimulating plant growth and root development in plants. For example, exogenous auxins increase growth and hernandulcin production in hairy root cultures of *Lippia dulcis* (Sauerwein *et al*., 1991). Similarly, exogenous IAA or NAA increases the biomass and lobeline production in hairy roots of *Lobelia inflata* (Bálványos *et al.*, 2001). Likewise, the amount of podophyllotoxin and 6-methoxypodophyllotoxin in hairy roots of *Linum album* increased by 1.86 times and 1.45 times, as a result of the addition of IAA and 2,4-D to the basal medium (Farkya and Bisaria, 2008). Polyamines regulate developmental processes, such as root development, in plants (Couée et al., 2004). Similar to auxins, exogenous polyamines also stimulate growth and production of secondary metabolites in hairy root cultures. For example, putrescine increased growth and production of esculin and esculetin in hairy root cultures of witloof chicory (Bais and Ravishankar, 2003; Bais et al., 1999). Moreover, putrescine is 1 of the most effective polyamines that stimulate hairy root growth and synthesis of coumarin and betalaine in *Cichorium intybus* and *Beta vulgaris* (Bais *et al.*, 2004; Suresh *et al.*, 2004).

Conclusion

Our results indicate that exogenous auxins and polyamines can enhance growth and rosmarinic acid production in *N. cataria*. As a result, hairy root cultures of *N. cataria* may be a valuable alternative approach for producing rosmarinic acid. Since other

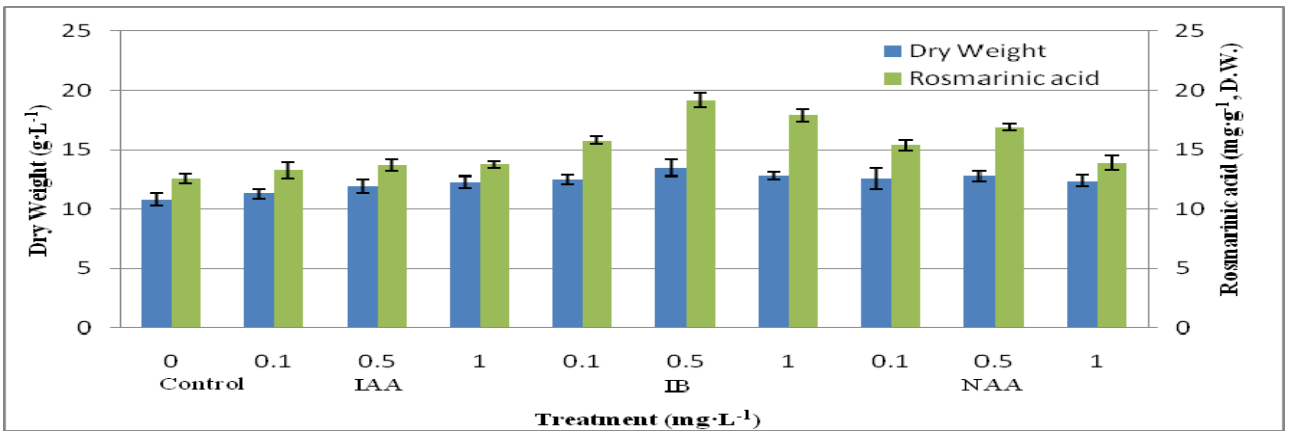

Fig 2. Effect of different concentration of auxins on growth and production of rosmarinic acid in hairy roots of *N. cataria*. Data are expressed as mean ± SD (standard deviation)

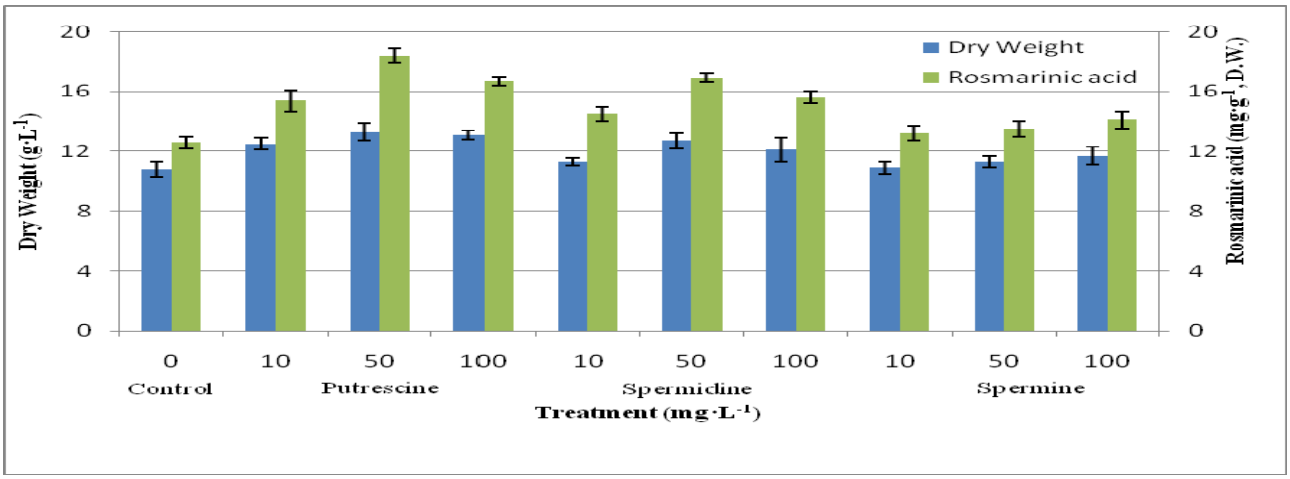

Fig 3. Effect of different concentration of polyamines on growth and production of rosmarinic acid in hairy roots of *N. cataria*. Data are expressed as mean ± SD (standard deviation)

plant hormones may be more potent stimulators of rosmarinic acid production in hairy root cultures of *N. cataria*, further research is needed.

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