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Research Note

Influence of media and auxins on growth and falvone production in hairy root cultures of baikal skullcap, *Scutellaria baicalensis*

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Abstract

The hairy root culture of *Scutellaria baicalensis* was studied using different media, strength of medium and addition of various concentrations of auxins to the culture media to optimize the growth and flavone production. Hairy roots grown in full-strength SH medium showed the highest levels of hairy root growth (0.32 g/30 mL). However, the levels of the flavones baicalin, baicalein, and wogonin were higher in the hairy root cultures using half-strength B_5 than those of the other media used in this study. The growth rates of the hairy roots did not vary significantly between auxin treatments. However, the auxins were observed to increase flavone production in *S. baicalensis* hairy root culture. The auxin indole acetic acid (IAA) at 1 mg/L performed the best for the accumulation of baicalin and baicalein. Meanwhile, the highest levels of wogonin were observed for hair root cultures in the presence of indolebutyric acid at 1 mg/L, followed by IAA at 0.1 mg/L. These findings indicate that hairy root cultures of *S. baicalensis* using half-strength B5 media supplemented with auxin could be a valuable alternative approach for flavonoid production.

Key words: Flavones; auxin; medium; hairy root culture; *Scutellaria baicalensis.*
Abbreviation: B5 media - Gamborg B5 medium; IAA- indole acetic acid; IBA- indole-3-butyric acid; MS media - Murashige & Skoog medium; NAA- 1-naphthaleneacetic acid; HPLC- High performance liquid chromatography; SH media - Schenk & Hildebrandt medium.

Introduction

Scutellaria baicalensis is a species of flowering plant in the Lamiaceae family and is one of the 50 fundamental herbs used in traditional Chinese medicine. The roots of *S. baicalensis* have been clinically used to treat bacterial and viral infections, reduce total cholesterol levels, and depress blood pressure and has been used as an anti-inflammation and anticancer agent (Li et al., 2004; Zhang et al., 2003). *S. baicalensis* contains a variety of flavones, phenylethanoids, amino acids, sterols, and essential oils. The dried root of *S. baicalensis* is rich in flavonoids, containing over 30 different kinds of flavonoids. Phytochemical investigations revealed that flavonoids primarily comprise baicalin, baicalein, and wogonin (Fig. 1). Baicalin is the glucuronide of baicalein and is commonly used in the treatment of chronic hepatitis in Japan and China (Wan et al., 2008). Baicalin has been reported to function as an anti-oxidant (Waisundara et al., 2009) but also to cause cytotoxic effects (Ueda et al., 2002). Baicalin also has an anti-viral effect through its inhibition of reverse transcriptase (Kitamura et al., 1998). Baicalein was isolated originally from the roots of *S. baicalensis* and later from *Oroxylum indicum* or the Indian trumpet flower. Baicalein has been shown to inhibit certain types of lipoxygenases and to act as an anti-inflammatory agent (Cui et al., 2010; Yang et al., 2008). Moreover, baicalein is an inhibitor of CYP2C9, an enzyme in the cytochrome P450 system that metabolizes drugs in the body (Si et al., 2009). Wogonin is an *O*-methylated flavone that was found in *S. baicalensis* (Hui et al., 2002). Wogonin has been reported to have anti-oxidant activity, which may, in part, underlie its antiinflammatory, anti-cancer, antiviral and neuroprotective actions (Tai et al., 2005). Hairy root cultures have widely proven to be an efficient alternative system for the production of secondary metabolites in many plant species because of their genetic and biochemical stability, rapid growth rate, and ability to synthesize natural compounds at levels comparable to *in vivo* grown plants (Giri and Narasu, 2000; Guillon et al., 2006). A number of factors, including temperature, light, pH, medium composition, and exogenous treatment with plant growth regulators, affect the production of secondary metabolites in the hairy root cultures of various plants (Bourgaud et al., 2001). Recently, we reported the development of a hairy root culture of *S. baicalensis* for flavone production (Park et al., 2011). However, the influence of media and auxin treatment on flavone production in *S. baicalensis* hairy root culture has not been reported. In this paper, we describe the effects of 3 standard media at different strengths and of auxin on flavone production and growth in hairy root cultures of *S. baicalensis*.

Result and discussion

Secondary metabolite biosynthesis in transformed roots is largely controlled genetically but can be affected by nutritional and environmental factors. For examining the effect of different

Table 1. The effects of media on flavone production in hairy root cultures of *S. baicalensis* grown for 21 days.

Medium	Flavones (μg/mg)		
	Baicalin	Baicalein	Wogonin
B_5	39.90 ± 0.78	11.55 ± 1.24	3.71 ± 0.27
1/2 B_5	59.76 ± 0.68	22.61 ± 1.98	4.47 ± 1.08
MS	23.92 ± 1.21	13.01 ± 1.63	3.06 ± 0.81
1/2 MS	37.29 ± 0.73	10.99 ± 1.19	2.98 ± 0.55
SH	40.66 ± 0.34	12.78 ± 0.85	3.63 ± 0.38
1/2 SH	35.68 ± 0.51	15.49 ± 0.75	4.00 ± 0.36

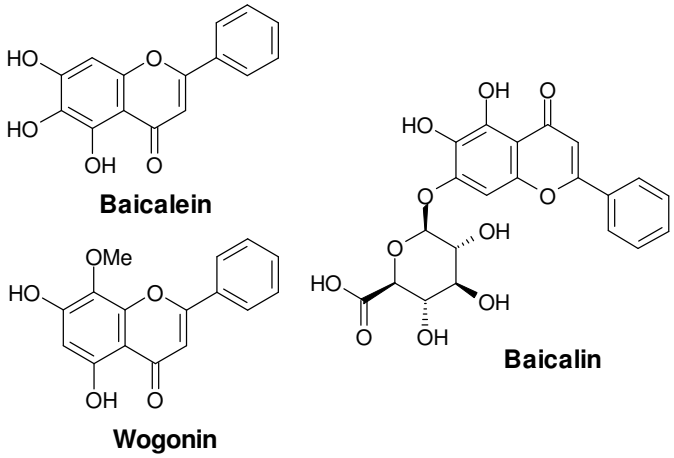

Fig 1. Structures of baicalin, baicalein, and wogonin

media on hairy root growth and flavone production, *S. baicalensis* was cultured for 3 weeks in full- and half-strength MS, B5, or SH basal media supplemented with 3% sucrose. Among these different media, both full- and half-strength SH media performed the best for the production of hairy roots (Fig. 2). In particular, hairy roots grown in full-strength SH medium showed the highest levels of growth (0.32 g/30 mL). The amount of hairy root production with half strength SH was close (0.31 g/30 mL) to that of full-strength SH. The full- and half-strength B_5 media produced slightly lower growth values (0.28–0.27 g/30 mL). Both full- and half-strength MS media did not perform well for the production of hairy roots. The full-strength MS media produced the lowest amount of hairy roots (0.10 g/30 mL). The type of media had a significant influence on flavone production in *S. baicalensis*. Baicalin levels in the hairy roots of *S. baicalensis* were much higher with half-strength B_5 than those with other media did. Baicalin was present at 59.76 μg/mg when half-strength B_5 media was used; this value was 2.5, 1.7, 1.6, and 1.5 times higher than full-strength MS, half-strength SH, half-strength MS, and full-strength SH, respectively. Similar trends were observed for the accumulation of baicalein and wogonin using half-strength B5. Baicalein was present at 22.61 μg/mg, which was 2.1, 2.0, 1.8, 1.7, and 1.5 times higher than that of half-strength MS, full-strength B_5, full-strength SH, full-strength MS, and half-strength SH, respectively. The highest amount of wogonin (4.47 μg/mg) was accumulated using half-strength B_5 and was 1.5 times higher than the lowest Wogonin levels, which were observed for half-strength MS media. Hairy roots of *S. baicalensis* were allowed to grow for 3 weeks in half-strength B_5 media supplemented with various concentrations of different auxins to study the effects on growth and flavone production. Three different auxins (i.e., IAA, IBA, and NAA) at 3 different concentrations (i.e., 0.1, 0.5, and 1 mg/L) were used.

Our results revealed that the growth rates of the hairy roots did not vary significantly between the various auxin treatments (Fig. 3). Moreover, IBA at 0.1 and 0.5 mg/L and IAA at 1 mg/L produced slightly higher amounts of hairy roots compared to the control. IAA at 1 mg/L resulted in the highest amount of baicalin (72.74 μg/mg) and baicalein (26.27 μg/mg) (Table 2). The second highest baicalin levels accumulated in the presence of IAA at 0.1 mg/L. With the exception of these treatments, no other auxin treatment showed higher flavone levels than the control. Baicalin accumulation with IAA at 1 mg/L was 1.2 times higher as compared to that of the control. The lowest amount of baicalin was accumulated in the presence of NAA at 0.5 mg/L. Baicalein accumulation increased with all the auxin treatments, with the exception of IAA at 0.5 mg/L, IBA at 1 mg/L, and NAA at 0.1 mg/L (Table 2). Baicalein accumulation with IAA at 1 mg/L was also 1.2 times higher as compared that of the control. The lowest amount of baicalein was found at an IAA concentration of 0.5 mg/L. Wogonin levels varied considerably within the auxin treatments. The levels of this flavone increased with all auxin treatments except for NAA at 0.1 and 1 mg/L (Table 2). The highest level of wogonin was produced in the presence of IBA at 1 mg/L and was 1.4 times higher relative to that of the control. IBA at 0.1 mg/L increased the accumulation of wogonin 1.3 fold compared to the control. For producing secondary metabolites in hairy root cultures, the optimization of the medium can play an important role in the growth of the roots and the production of secondary metabolites. These findings are consistent with prior studies indicating that the growth and secondary metabolite biosynthesis in hairy root cultures of *Lobelia inflata* (Yonemitsu et al., 1990), *Centranthus rubber* (Granicher et al., 1995), *Fagopyrum esculentum* (Lee et al., 2007), and *Withania somnifera* (Murthy et al., 2008). Auxins play important roles in plant growth and root development. The enhancement of hairy root growth and secondary metabolite is similar to the results of

Table 2. The effects of auxin on flavones production in hairy cultures of *S. baicalensis* grown for 21 days

Auxin		Flavones (μg/mg)		
		Baicalin	Baicalein	Wogonin
Control		59.76 ± 0.07	22.61 ± 0.76	5.00 ± 0.23
	0.1	63.00 ± 0.37	23.09 ± 0.38	5.87 ± 0.02
IAA	0.5	53.07 ± 0.29	19.83 ± 0.56	5.95 ± 0.12
	1	72.74 ± 2.65	26.27 ± 3.09	5.69 ± 0.41
	0.1	56.49 ± 0.84	23.02 ± 0.47	6.46 ± 0.07
IBA	0.5	57.76 ± 1.20	25.87 ± 1.18	5.90 ± 0.20
	1	55.54 ± 2.06	22.19 ± 0.01	6.99 ± 0.00
	0.1	53.92 ± 0.32	21.47 ± 0.48	4.78 ± 0.23
NAA	0.5	51.40 ± 0.53	23.01 ± 0.38	5.08 ± 0.12
	1	54.14 ± 0.41	23.52 ± 0.56	4.92 ± 0.03

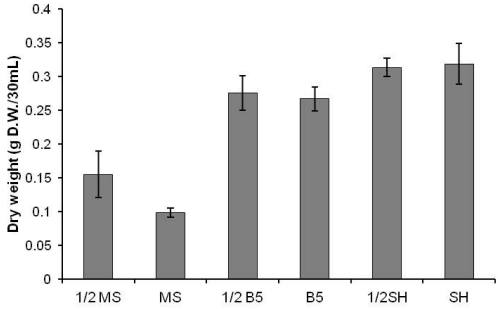

Fig 2. The effects of media on growth in hairy root cultures of *S. baicalensis* grown for 21 days.

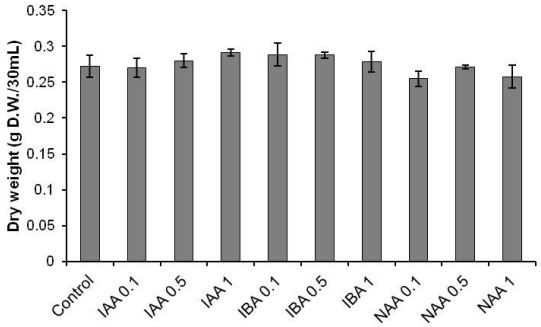

Fig 3. The effects of auxin on growth in hairy cultures of *S. baicalensis* grown for 21 days.

previous reports showing that exogenous auxin treatments enhanced growth and natural compound production in hairy root cultures of *Lippia dulcis* (Sauerwein et al., 1991), *Lobelia inflata* (Bálványos et al., 2001), and Panax hybrid (Washida et al., 2004). Our findings indicate that *S. baicalensis* hairy culture can be a valuable alternative approach for the production of flavones. By using a selective culture (i.e., half-strength B5 medium) and exogenous auxin treatments, a relatively high flavone production and improved root growth can be achieved. Further investigations for the improvement of flavone production in hairy root cultures of *S. baicalensis* are in progress in our laboratory.

Materials and methods

Maintenance of hairy root culture

We previously established a hairy root culture of *S. baicalensis* for producing flavones. The establishment and maintenance of hairy root cultures was performed according to Park et al. (2011). Hairy roots of *S. baicalensis* were subcultured on fresh agar-solidified MS medium every month. They were transferred to MS liquid culture medium and used for the initial experiments. Hairy root cultures were maintained in MS liquid medium and subcultured every 21 days. Root cultures were maintained at 25 °C on a gyratory shaker (100 rpm) in a growth chamber under standard cool white fluorescent tubes, with a flux rate of 35 mol·s^{-1} m^{-2} and a 16-h photoperiod.

Optimization of culture conditions

For the selection of optimal medium conditions for hairy root growth and flavone production, the effects of full- and half-strength B5 (Gamborg et al., 1968), MS (Murashige and Skoog, 1962), and SH (Schenk and Hildebrandt, 1972) media were tested. The addition of various concentrations (0.0, 0.1, 0.5, and 1.0 mg/L) of the auxins indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA) to the culture media were tested to promote the growth of hairy roots and the biosynthesis of flavones. After 21 days of culture, the hairy roots were harvested, and the dry weights and flavone

contents were determined. Three flasks were used for each culture condition, and the experiments were performed in duplicate.

High performance liquid chromatography (HPLC) analysis

Suspension cells (0.05 g) were frozen in liquid nitrogen, ground to a fine powder using a mortar and pestle, and extracted with 10 mL of 70% ethanol for 1 h at 60 °C. After centrifugation, the supernatant was filtered through a 0.45-μm poly filter and analyzed by HPLC. The analysis was monitored at 275 nm and performed using a C_{18} column (250 mm × 4.6 mm, 5 μm; RStech, Daejon, Korea). The mobile phase was a gradient prepared from mixtures of acetonitrile, methanol, and 0.2% acetic acid; the column was maintained at 30 °C. The flow rate was set at 1.0 $mL \cdot min^{-1}$, and the injection volume was 20 μL. Results were calculated using a standard curve.

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