

## ***In vitro* induction of hairy root from isoflavones-producing Korean wild arrowroot *Pueraria lobata***

Soojung Kim · Min-Seok Cha · Eunji Lee · Inhye Kim · Jung Eun Kwon · Se Chan Kang · Tae-Ho Park

Received: 19 September 2012 / Accepted: 21 September 2012  
© Korean Society for Plant Biotechnology

**Abstract** *Pueraria lobata* is a perennial legume plant, widely distributed in the countries of East Asia. It is a medicinally important leguminous plant and produces various isoflavones such as puerarin, daidzein etc which have potential for preventing several chronic diseases including osteoporosis, cardiovascular disease and cancer. In this study, we tried to induce hairy roots *in vitro* from Korean wild arrowroot *P. lobata* and investigated the effects of hormones and light conditions. Initially leaf and stem segments were infected with *Agrobacterium rhizogenes* and incubated in different conditions. Hairy roots were induced from only stem segments and the induction was best at dark condition and the presence of IBA during incubation. Secondary roots were also significantly much more induced at the dark condition than at the 16 hours light condition. Among plant growth regulators of auxin, IBA was best for secondary root formation while 2,4-D, IAA and NAA produced callus or less hairy roots. The presence of the foreign gene *rolC* transferred by *A. rhizogenes* that plays a major role in hairy root induction was confirmed by PCR. The accumulation of isoflavones such as puerarin and daidzin was also confirmed. These results will facilitate mass production of hairy root and can be used for the production of functional substances from wild arrowroots.

### **Introduction**

The genus *Pueraria* is a perennial legume plant widely distributed in tropical and subtropical countries of East Asia and one of the oldest medicinal plants in Korea, China and Japan (Chen and Li 2007; Kim et al. 2009). These plants possess estrogen-like effects and have been used for the treatment of angina pectoris, hypertension, deafness, optic nerve and atrophy and as antipyretic, antidiarrheic, diaphoretic and antiemetic substances (Kinjo et al. 1987; Sato et al. 1992; Thiem 2003; Cherdshewasart et al. 2007). One of the genus *Pueraria*, *P. lobata* commonly called arrowroot or kudzu grows in Korea and is a Korean indigenous herb with the local name of ‘Chihk’. Its tuberous root yields phenolic compounds, various isoflavonoids of high antioxidant properties such as daidzin, daidzein, genistin, and puerarin (Kinjo et al. 2000; Chansakaow et al. 2000; Kim et al. 2003; Pandey et al. 2007). The contents of the isoflavonoids in the tuber are variable due to environmental conditions depending on the regions and seasons resulting different quality of tubers (Pappert et al. 2000; Cherdshewasart and Sriwatcharakul 2008) and phenolic compounds in medicinal plants have been reported to be unstable depending on factors like temperature, light and oxygen (Santos-Gomes et al. 2002). These problems led to establish plant tissue technologies to standardize a method for the production of such secondary metabolites.

Previous investigation of plant tissue cultures with the genus *Pueraria* was mostly limited to cell suspension and callus cultures. Boonsongcheep et al. (2010) and Korsangruang et al. (2010) have developed a system of cell suspension culture and investigated its growth and isoflavonoid accumulation for *P. candollei* and Thanonkeo and Panichajakul (2006) have also reported on successful callus cultures of *P. candollei* while remarkable efforts have been reported on cell suspension cultures and enhancement of isoflavonoid accumulation for *P. tuberosa* (Vaishnav et al. 2006; Goyal

---

S. Kim · M.-S. Cha · E. Lee · T.-H. Park (✉)  
Department of Horticulture, Daegu University, Gyeongsan  
712-714, Republic of Korea  
e-mail: thzoo@daegu.ac.kr

I. Kim  
Functional Food & Nutrition Division, National Academy of  
Agricultural Science, RDA, Suwon 441-853, Republic of Korea

J. E. Kwon · S. C. Kang  
Department of Life Science, Gachon University, Seongnam  
461-701, Republic of Korea

and Ramawt 2007, 2008; Rathore and Shekhawat 2009). Moreover, Sharma et al. (2009, 2011) tried to scale up production of isoflavonoids in cell suspension cultures of *P. tuberosa* using a bioreactor. For *P. lobata*, a few results related to both tissue culture and isoflavone production were reported (Barbara 2003; Thiem 2003; Reppert et al. 2008). In addition to these, hairy root culture was used to improve isoflavonoid production from *P. candollei* (Udomsuk et al. 2011) and *P. phaseoloides* (Shi and Kintzios 2003) as the induction of a stable and high rate of secondary metabolite production have been reported using hairy roots (Giri and Narasu 2000). Hwang (2006) also reported that hairy roots obtained through the transformation of higher plants using *Agrobacterium rhizogenes* produced various higher amounts of secondary metabolites than normal field-grown plants did. This technology has successfully been applied to other plant species (Bonhomme et al. 2000; Villalobos-Amador et al. 2002; Limpens et al. 2004; Bulgakov et al. 2005; Crane et al. 2006; Ko et al. 2007). The technology in which plant tissue culture is combined with hairy root induction has not been developed for *P. lobata*, especially Korean wild arrowroot although it has advantages for the production of secondary metabolites. In the present study, therefore, we set up a system of hairy root induction and culture and investigate the effects of plant growth regulators and light conditions for Korean wild arrowroot (Chihk), *P. lobata*. In addition to these, we compared the accumulation of isoflavones in the produced hairy root with that in tubers of Korean wild arrowroot.

## Material and methods

### Plant materials

Two different lines of Korean wild arrowroot, *P. lobata* collected in Mungyeong and Daejeon early spring were used. The collected plants were planted to clay pots and maintained in a greenhouse at Daegu University. When the plants were sprouted, stem and leaf segments were dissected. Twelve 0.5–1 cm long stem segments and twelve 0.5 cm x 0.5 cm leaf segments from two collected lines were used. They were surface-sterilized with 70% ethanol for 2 minutes and commercial bleach containing 1.5% sodium hypochlorite for 20 minutes and rinsed four times in sterile distilled water.

### Hairy root induction and culture

A single strain of *A. rhizogenes* (KCTC2744), which were

kindly provided by Korean Collection for Type Culture (KCTC), were used to infect two *P. lobata* lines. The strain was grown in 100 ml NB medium and collected by the centrifugation at 3,200 rpm for 10 minutes, followed by resuspension in growth regulator-free liquid MS medium (Murashige and Skoog 1962) for inoculation. The stem and leaf segments excised from the greenhouse plant of two lines were infected by dipping into the inoculum and the infected explants were cultured on MS medium in the dark at 23±2°C. After 48 hours of co-cultivation, the explants were transferred to 1/2 MS medium with 500 mg cefotaxime (CF)/L supplemented with or without 0.5 mg/L indole-3-butyric acid (IBA) and cultured in the dark or in the 16 hours light and 8 hours dark condition. At 2-week intervals, infected segments were transferred to 1/2 MS medium with 300 and 100 mg CF/L. Medium and light conditions were continuously identical to the initial treatment. When the hairy roots were induced, they were subcultured on 1/2 MS medium containing 0.5 mg/L IBA and cultured in the dark or in the 16 hours light and 8 hours dark condition for two weeks to investigate the effect of light condition on the induction of secondary roots from the primary hairy roots. In addition to these, transformed hairy roots of *P. lobata* were subcultured on 1/2 MS medium supplemented with 0.5 mg/L of 2,4-dichlorophenoxy acetic acid (2,4-D), 1-naphthalene acetic acid (NAA), indole 3-butyric acid (IBA) and indole acetic acid (IAA) each and incubated in the dark at 23±2°C to examine the effect of different auxins.

### DNA isolation and PCR analysis

Colonies of bacterial strain *A. rhizogenes* KCTC2744 and genomic DNA of transformed and untransformed plant materials were used to analyze whether or not induced roots contained hairy root induction gene. Genomic DNA was isolated from hairy roots and from the control roots frozen in liquid nitrogen by the method slightly modified from Doyle and Doyle (1990), Bousquet et al. (1990) and Park and Jones (2009). The polymerase chain reaction was used to confirm the presence of the *rolC* gene in the bacterial strain and roots. PCR experiments were performed using primers at the position 51–69 (5'-TGTGACAAGCAGCGA TGAG-3') and 550–532 (5'-GATTGCAAACCTTGCACTCG-3'), designed for the amplification of a 490 bp *rolC* fragment (Bonhomme et al. 2000). Samples for PCR reaction were subjected to 35 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 56°C and 1 min extension at 72°C. Amplified DNAs were detected on 1% agarose gels.

## Isoflavones extraction and analysis

To analyze the accumulation of isoflavones (puerarin and daidzin) in the produced hairy root, tubers and callus of Korean wild arrowroot, two lines were used. The following reagents were used as standard substances: puerarin, P-5555 Sigma-Aldrich (MG, USA); and daidzin, D-30408 Sigma-Aldrich (MG, USA). An SIL-20A equipped with SPD-M20A PDA and CTO-20A column oven (SHIMDZU Corp., Kyoto, Japan) was used for HPLC analysis. Data were analyzed using the attached software. The fractions were separated at 40°C on a ACE-121-2546 (Advanced Chromatography Technologies Corp., Aberdeen, Scotland) using a one-step linear gradient. Mobile phase A [water : acetic acid (100:1)] and B [water : acetonitrile : acetic acid (50:50:1)] ratios were changed after 5 minutes from 7:3 to 3:7 for 40 minutes at a flow rate of 1.0 mL/min.

## Statistical analysis

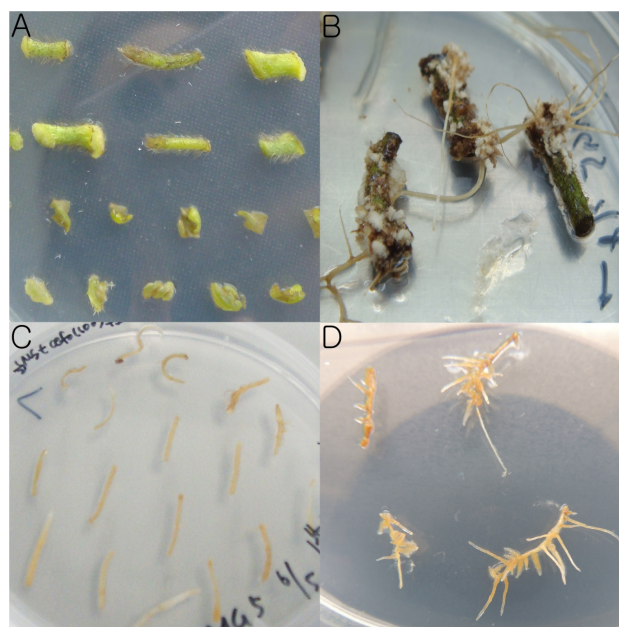
Counts of the stem segments at which hairy roots and secondary root were induced were made every week. To analyze the effects of each treatment, the number of stem segments and secondary roots scored six and two weeks after initial culture were analyzed using analysis of variance (ANOVA), respectively. After defining the effects of two or one factors and interactions between factors, Duncan's multiple-range test (DMRT) were applied to analyze the effects of each treatment.

## Results and discussion

For setting up the system of hairy root induction and culture from Korean wild arrowroot *P. lobata*, stem and leaf segments of two lines collected from two different regions were infected with *A. rhizogenes* strain KCTC2744 as the pathogen has been known to be able to induce hairy roots due to its root-inducing plasmid (Bonhomme et al. 2000; Villalobos-Amador et al. 2002; Bulgakov et al. 2005; Crane et al. 2006; Kim et al. 2010). Continuous culture of infected stem segments generated hairy roots while leaf segments did not (Fig. 1A and 1B) and secondary roots were successfully induced and elongated by subculture of the primary hairy roots (Fig. 1C and 1D).

For the investigation of effects of IBA and light on hairy root induction, 12 infected stem explants of each line were incubated at different conditions: 1) continuous dark with 0.5 mg/L IBA in the medium, 2) continuous dark without IBA in the medium, 3) 16/8 hours light/dark

with 0.5 mg/L IBA in the medium and 4) 16/8 hours light/dark without IBA in the medium as each condition was applied to induce or culture hairy roots in *Pueraria* species (Thiem 2003; Reppert et al. 2008; Rathore and Shekhawat 2009; Udomsuk et al. 2011) and other plant species (Ko et al. 2007; Ono et al. 2012; Swain et al. 2012). The data on hairy root induction scored six weeks after initial incubation were used for statistical analysis. As shown in Table 1, the efficiency of hairy root induction on the dark condition and the supplement of 0.5 mg/L IBA in the medium was 91.7% and the number of explants with the induced hairy root was significantly higher than those on other treatments. Successive experiment for the induction of secondary roots resulted that the continuous dark condition was much better than the 16 hours light and 8 hours dark condition (Table 2) and average numbers of the secondary roots were 32.5 and 14.6, respectively. In most cases of experiments for hairy root induction, the infected explants were transferred to fresh medium with reduced antibiotics and without plant hormones every two or three weeks and routinely incubated at the dark condition or under continuous light for 16 hours (Crane et al. 2006; Ko et al. 2007; Prasain et al. 2007; Kim et al. 2010; Udomsuk et al. 2011). However, the effects of auxin-type hormones and light conditions were not investigated for the initial induction of hairy roots from *P. lobata*. The results obtained



**Fig. 1** Hairy root induction of Korean wild arrowroot, *P. lobata*. A: Stem and leaf segments grown in root induction medium, B: Hairy root induced from stem segments after four weeks of culture, C: Hairy root explants, D: Secondary roots initiated from hairy root explants after two weeks of subculture

**Table 1** Effects of IBA and light conditions on hairy root induction from stem segment of *P. lobata* in 1/2 MS medium six weeks after initial incubation

Light condition	IBA (0.5 mg/L)	Number of incubated explants	Number of explants with hairy root <sup>1</sup>	Efficiency of hairy root induction (%)
Dark	Presence	24	11 ± 1.4 <sup>a</sup>	91.7
Dark	Absence	24	2 ± 0 <sup>b</sup>	16.7
Light	Presence	24	2 ± 2.8 <sup>b</sup>	16.7
Light	Absence	24	0.5 ± 0.7 <sup>b</sup>	4.2

<sup>1</sup>The values represent mean ± standard deviation. Mean values with a different letter are significantly different at P=0.05 by DMRT.

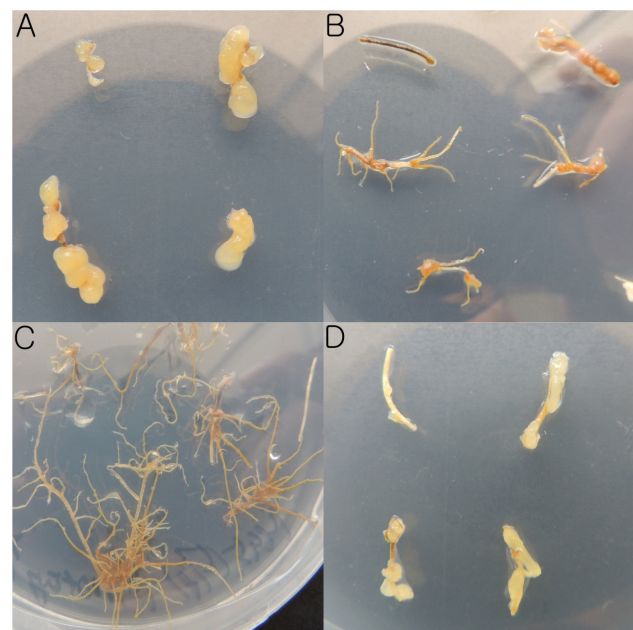
**Table 2** Effect of light condition on the induction of secondary roots from the primary roots of *P. lobata* in 1/2 MS medium containing 0.5 mg/L IBA two weeks after subculture

Light condition	Number of incubated explants	Number of secondary roots <sup>1</sup>
Dark	19	32.5 ± 5.7 <sup>a</sup>
Light	25	14.6 ± 3.8 <sup>b</sup>

<sup>1</sup>The values represent mean ± standard deviation. Mean values with a different letter are significantly different at P=0.05 by DMRT.

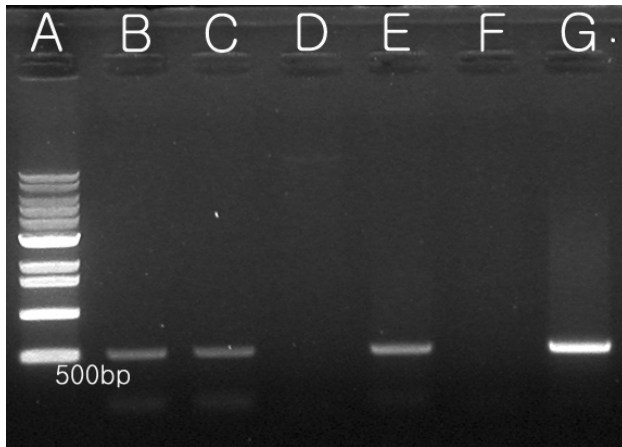
in this study could conclude that the supplement of IBA during medium refreshment and the incubation under the continuous dark condition during the primary hairy root induction and secondary root induction were effective (Table 1 and 2). Additionally, plant growth regulators of auxin including IAA, IBA and NAA were often tested to accelerate the growth of roots, when induced hairy roots or adventitious roots were subcultured (Thiem 2003; Reppert et al. 2008; Uddin et al. 2012). We also tried to select the optimal plant hormone of auxin for secondary root formation and growth. As shown in Fig. 2, both IBA and IAA formed secondary roots, but IBA was better than IAA on the number and growth of secondary roots. 2,4-D and NAA mainly formed callus. These results were contradictory to those of some other researches. Thiem (2003) reported that IAA is clearly more effective in promoting root induction from the regenerated shoots than NAA or IBA and Reppert et al. (2008) used NAA for root suspension culture. However, IBA has commonly been used in several plant species and was identified as the best auxin-type hormone for root induction and root growth (Verma et al. 2002; Ko et al. 2007; Rathore et al. 2009; Swain et al. 2012; Uddin et al. 2012).

*A. rhizogenes* is a plant pathogen capable of inducing hairy roots in a variety of plants (Cardarelli et al. 1987). Therefore this bacterium has often been used to induce hairy roots. The integration of the root-inducing plasmid into the plant genome was successfully performed and confirmed using hairy root induction-related genes in various plant species such as *Atropa belladonna* (Bonhomme et al. 2000), the genus *Pinus* (Villalobos-Amador et al. 2002),



**Fig. 2** Effect of different plant growth regulators of auxin on secondary root formation. A: 0.5 mg/L 2,4-D, B: 0.5 mg/L IAA, C: 0.5 mg/L IBA, D: 0.5 mg/L NAA

*Pueraria phaseoloides* (Shi and Kintzios 2003), *Medicago truncatula* (Limpens et al. 2004; Crane et al. 2006), *Eritrichium sericeum* and *Lithospermum erythrorhizon* (Bulgakov et al. 2005), *Panax ginseng* (Ko et al. 2007) etc. In this study, stem explants of two different lines of *P. lobata* were infected with the *A. rhizogenes* strain KTCT2744. After hairy roots were induced from the infected stem explants, DNA was isolated from both transformed and untransformed roots. PCR analysis (Fig. 3) showed that the *A.*



**Fig. 3** PCR amplified DNA fragments of the *rolC* gene. Lane A: Marker ladder, lanes B-C: DNA from *A. rhizogenes* KCTC2744 as positive controls, lane D: DNA from the intact roots of *P. lobata* line I as a negative control, lane E: DNA from hairy roots of *P. lobata* line I incubated with *A. rhizogenes* KCTC 2744, F: DNA from the intact roots of *P. lobata* line II as a negative control, G: DNA from hairy roots of *P. lobata* line II incubated with *A. rhizogenes* KCTC2744

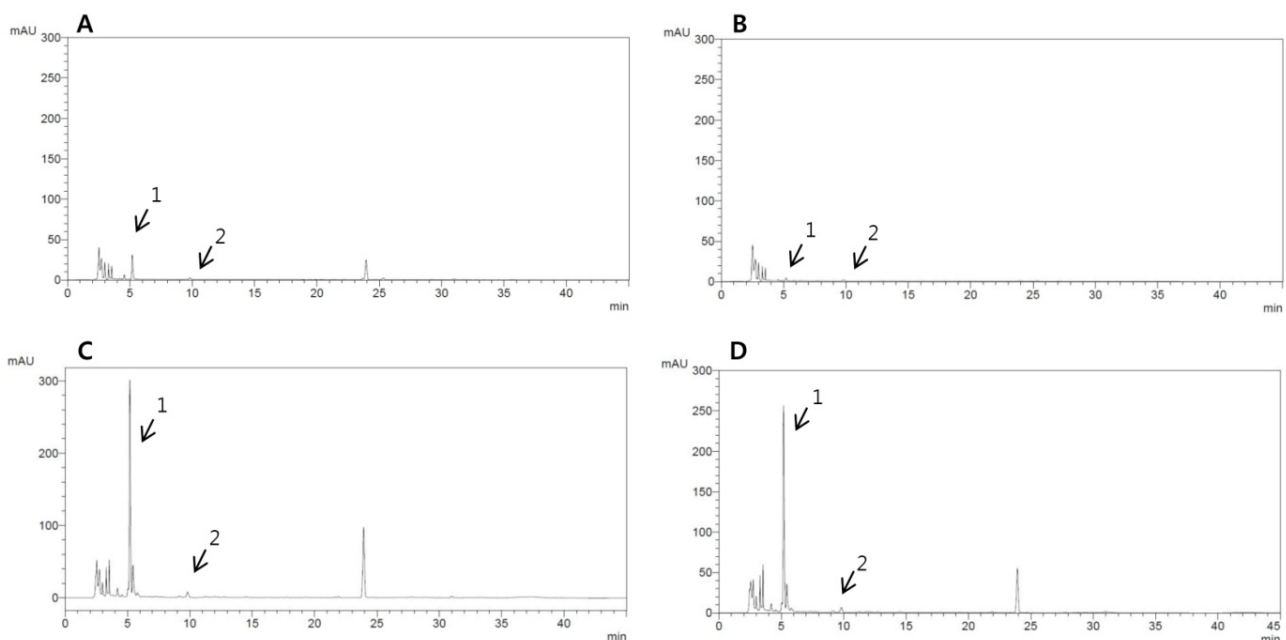
*rhizogenes* strain and the transformed lines contained the *rolC* gene (lanes B, C, E and G) and no band was observed for the untransformed roots (lanes D and F). Amplification with the *rolC* primers showed bands around the 500 bp size marker. The size of the bands is coincident with the expected size of *rolC*. This result indicated that the hairy root inducible gene from *A. rhizogenes* has properly been integrated to the genome of *P. lobata*.

To investigate a possibility of producing functional substances from the produced hairy roots, isoflavones such as puerarin and daidzin were analyzed using a HPLC method in two different lines of Korean wild arrowroot. As shown in Table 3 and Fig. 4, both of the substances were detected in the hairy root. The amounts of them were less than those in wild tubers. However, the accumulation of isoflavones in tissue-cultured *Pueraria* has been reported to be variable depending on the treatment of abiotic and biotic elicitors (Korsanguang et al. 2010; Udomsuk et al. 2011). Therefore, the accumulation of isoflavones in hairy root could be

**Table 3** Isoflavone contents in different tissues of Korean wild arrowroot

	Line I		Line II	
	Puerarin	Daidzin	Puerarin	Daidzin
Tuber	1.170	0.720	0.660	0.270
Callus	0.041	0.006	0.002	0.004
Hairy root	0.382	0.026	0.310	0.021

Puerarin and daidzin content are given in milligram per gram dry weight.



**Fig. 4** HPLC-PDA analysis of puerarin (1) and daidzin (2) from callus and hairy root of Korean wild arrowroot. A: Callus of line I B: Callus of line II C: Hairy root of line I D: Hairy root of line II



expected to be enhanced and the system of hairy root induction and culture developed in this study can be applied.

In conclusion, we developed an *in vitro* system for hairy root induction and secondary root formation from Korean wild arrowroot, *P. lobata*. The addition of IBA to the culture media remarkably improved hairy root induction and secondary root formation under the continuous dark condition. The accumulation of puerarin and daidzin in the hairy root was also confirmed. These root induction and growth systems will facilitate the mass production of arrowroots that could be used for a wide variety of purposes such as an extraction of functional substances and a development of functional foods and cosmetics.

### Acknowledgements

This study was carried out with the support of “Cooperative Research Program for Agricultural Science & Technology Development (Project No. PJ008398)”, Rural Development Administration, Republic of Korea and supported by the Daegu University Research Scholarship Grants.

### References

- Barbara T (2003) *In vitro* propagation of isoflavone-producing *Pueraria lobata* (Willd.) Ohwi. *Plant Sci* 165:1123-1128
- Bonhomme V, Laurain-Mattar D, Lacoux J, Fliniaux M-A, Jacquin-Dubreuil A (2000) Tropane alkaloid production by hairy roots of *Atropa belladonna* obtained after transformation with *Agrobacterium rhizogenes* 15834 and *Agrobacterium tumefaciens* containing *rol A, B, C* genes only. *J Biotechnol* 81:151-158
- Boonsongcheep P, Korsangruang S, Soonthornchareonnon N, Chintapakorn Y, Saralamp P, Prathantharurug S (2010) Growth and isoflavonoid accumulation of *Pueraria candollei* var. *candollei* and *P. candollei* var. *mirifica* cell suspension cultures. *Plant Cell Tiss Cult* 101:119-126
- Bousquet J, Simon L, Lalonde M (1990) DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. *Can J For Res* 20:254-257
- Bulgakov VP, Veselova MV, Tchernoded GK, Kiselev KV, Fedoreyev SA, Zhuravlev YN (2005) Inhibitory effect of the *Agrobacterium rhizogenes rolC* gene on rabsosin and rosmarinic acid production in *Eritrichium sericeum* and *Lithospermum erythrorhizon* transformed cell cultures. *Plant* 221:471-478
- Cardarelli M, Mariotti D, Pomponi M, Spanó L, Capone I, Costantino P (1987) *Agrobacterium rhizogenes* T-DNA gene capable of inducing hairy root phenotype. *Mol Gen Genet* 209:475-480
- Chansakaow S, Ishikawa T, Sekine K, Okada M, Higushi Y, Kudo M, Chaichantipyuth C (2000) Isoflavonoids from *Pueraria mirifica* and their estrogenic activity. *Planta Med* 66: 572-574
- Chen G, Li L (2007) Nutrient consumption and production of isoflavones in bioreactor cultures of *Pueraria lobata* (Willd.). *J Environ Biol* 28:321-326
- Cherdshewasart W, Sriwatcharakul S (2008) Metabolic activation promotes estrogenic activity of the phytoestrogen-rich plant. *Maturitas* 59:128-136
- Cherdshewasart W, Subtang S, Dahlan W (2007) Major isoflavonoid contents of the phytoestrogen rich-herb *Pueraria mirifica* in comparison with *Pueraria lobata*. *J Pharm Biomed Anal* 43:428-434
- Crane C, Wright E, Dixon RA, Wang Z-Y (2006) Transgenic *Medicago truncatula* plants obtained from *Agrobacterium tumefaciens*-transformed roots and *Agrobacterium rhizogenes*-transformed hairy roots. *Planta* 223:1344-1354
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13-15
- Giri A, Narasu ML (2000) Transgenic hairy roots: recent trends and applications. *Biotechnol Adv* 18:1-22
- Goyal S, Ramawat KG (2007) Effect of chemical factors on production of isoflavones in *Pueraria tuberosa* (Roxb.ex. Willd.) DC suspension culture. *Indian J Exp Biol* 45:1063-1067
- Goyal S, Ramawat KG (2008) Increased isoflavonoids accumulation in cell suspension cultures of *Pueraria tuberosa* by elicitors. *Indian J Biotechnol* 7:378-382
- Hwang SJ (2006) Baicalin production in transformed hairy root clones of *Scutellaria baicalensis*. *Biotechnol Bioprocess Eng* 11:849-853
- Kim C, Shin S, Ha H, Kim JM (2003) Study of substance changes in flowers of *Pueraria thunbergiana* Benth. During storage. *Arch Pharm Res* 26:210-213
- Kim D-K, Jang DS, Kim JS, Kim J-H (2009) Genetic variations and phylogenetic relationship of and *Pueraria lobata* Ohwi (Fabaceae) and related taxa by RAPD makers. *Korean J Plant Res* 22:446-453
- Kim JS, Oh EJ, Lee SK (2010) Influence of different strains of *Agrobacterium rhizogenes* on hairy root induction and rosmarinic acid production in *Agastache rugosa* Kuntze. *Korean J Plant Res* 23:14-18
- Kinjo J-E, Furusawa J-I, Baba J, Takeshita T, Yamasaki M, Nohara T (1987) Studies on the constituents of *Pueraria lobata*. III: Isoflavonoids and related compounds in the roots and the voluble stems. *Chem Pharm Bull* 35:4846- 4850
- Kinjo J, Ikeda T, Okawa M, Udayama M, Hirakawa T, Shii Y, Nohara T (2000) Hepatoprotective and hepatotoxic activities of Soporadiol analogs on rat primary liver cell cultures. *Biol Chem Bull* 23:1118-1121
- Ko SM, In DS, Chung H-J, Choi D-W, Liu JR (2007) Mass production of gain-of-function mutants of hair roots in Ginseng. *J Plant Biotechnol* 34:285-591
- Korsangruang S, Soonthornchareonnon N, Chintapakorn Y, Saralamp P, Prathantharurug S (2010) Effects of abiotic and biotic elicitors on growth and isoflavonoid accumulation in

- Pueraria candollei* var. *candollei* and *P. candollei* var. *mirifica* cell suspension cultures. *Plant Cell Tiss Organ Cult* 103: 333-342
- Limpens E, Ramos J, Franken C, Raz V, Compaan B, Franssen H, Bisseling T, Geurts R (2004) RNA interference in *Agrobacterium rhizogenes*-transformed roots of *Arabidopsis* and *Medicago truncatula*. *J Exp Bot* 55:983-992
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* 15:473-497
- Ono NN, Bandaranayake PCG, Tian L (2012) Establishment of pomegranate (*Punica granatum*) hairy root cultures for genetic interrogation of the hydrolysable tannin biosynthetic pathway. *Planta* 236:931-941
- Pandey N, Chaurasia JK, Tiwari OP, Tripathi YB (2007) Antioxidant properties of different fractions of tubers from *Pueraria tuberosa* Linn. *Food Chem* 105:219-222
- Pappert RA, Hamrich JL, Donovan LA (2000) Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, invasive plant of the southeastern United States. *Am J Bot* 87:1240-1245
- Park T-H, Jones JDG (2009) A new resistance gene to powdery mildew identified in *Solanum neorossii* has been localized on the short arm of potato chromosome 6. *Euphytica* 166: 331-339
- Prasain JK, Reppert A, Jones K, Moore DR, Barnes S, Lila MA (2007) Identification of isoflavones glycosides in *Pueraria lobata* cultures by tandem mass spectrometry. *Phytochem Anal* 18:50-59
- Rathore MS, Shekhawat NS (2009) Micropropagation of *Pueraria tuberosa* (Roxb. Ex Willd.) and determination of puerarin content in different tissues. *Plant Cell Tiss Organ Cult* 99:327-334
- Reppert A, Yousef GG, Rogers RB, Lila MA (2008) Isolation of radiolabeled isoflavones from Kudzu (*Pueraria lobata*) root cultures. *J Agric. Food Chem* 56:7860-7865
- Santos-Gomes PC, Seabra RM, Andrade PB, Fernandes-Ferreira M (2002) Phenolic antioxidant compounds produced by *in vitro* shoots of sage (*Salvia officinalis* L.). *Plant Sci* 162: 981-987
- Sato T, Kawamoto A, Tamura A, Tatsumi Y, Fujii T (1992) Mechanism of antioxidant action of pueraria glycoside (PG)-1 (an isoflavonoid) and mangiferin (a xanthonoid). *Chem Pharm Bull* 40:721-724
- Sharma V, Goyal S, Ramawat KG (2009) Scale up production of isoflavonoids in cell suspension cultures of *Pueraria tuberosa* grown in shake flasks and bioreactor. *Eng Life Sci* 9:267-271
- Sharma V, Goyal S, Ramawat KG (2011) Increased puerarin biosynthesis during *in vitro* shoot formation in *Pueraria tuberosa* grown in growtek bioreactor with aeration. *Physiol Mol Biol Plants* 17:87-92
- Shi H-P and Kintzios S (2003) Genetic transformation of *Pueraria phaseoloides* with *Agrobacterium rhizogenes* and puerarin production in hairy roots. *Plant Cell Rep* 21:1103-1107
- Swain SS, Sabu L, Pal A, Barik D.P, Pradhan C, Chand P.K (2012) Hairy root cultures of butterfly pea (*Clitoria ternatea* L.): *Agrobacterium* × plant factors influencing transformation. *World J Microbiol Biotechnol* 28:729-739
- Thanonkeo S, Panichajakul S (2006) Production of isoflavones, daidzein and genistein in callus cultures of *Pueraria candollei* Wall. Ex Benth. Var. *mirifica*. *Songklanakarin J Sci Technol* 28:45-53
- Thiem B (2003) *In vitro* propagation of isoflavone-producing *Pueraria lobata* (Willd.) Ohwi. *Plant Sci* 165:1123-1128
- Uddin MR, Li X, Won OJ, Park SU, Pyon JY (2012) Herbicidal activity of phenolic compounds from hairy root cultures of *Fagopyrum tataricum*. *Weed Res* 52:25-33
- Udomsuk L, Jarukamjorn K, Tanaka H, Putalun W (2011) Improved isoflavonoid production in *Pueraria candollei* hairy root cultures using elicitation. *Biotechnol Lett* 33: 369-374
- Vaishnav K, Goyal S, Ramawat KG (2006) Isoflavonoids production in callus culture of *Pueraria tuberosa*, the Indian kudzu. *Indian J Exp Biol* 44:1012-1017
- Verma P, Singh D, Rahman L, Gupta MM, Banerjee S (2002) *In vitro*-studies in *Plumbago zeylanica*: rapid micropropagation and establishment of higher plumbagin yielding hairy root cultures. *J Plant Physiol* 159:547-552
- Villalobos-Amador E, Rodriguez-Hernández G, Pérez-Molphe-Balch E (2002) Organogenesis and *Agrobacterium rhizogenes*-induced rooting in *Pinus maximarinenzii* Rzedowsky and *P. pinceana* Gordon. *Plant Cell Rep* 20:779-785