Plant Biotechnol Rep (2011) 5:255–263
DOI 10.1007/s11816-011-0180-5

ORIGINAL ARTICLE

Triterpenoid production and phenotypic changes in hairy roots of *Codonopsis lanceolata* and the plants regenerated from them

Ji-Ah Kim · Yun-Soo Kim · Yong-Eui Choi

Received: 9 April 2011 / Accepted: 23 April 2011 / Published online: 13 May 2011

Abstract *Codonopsis lanceolata* (Campanulaceae) has been used in traditional medicines, as its roots contain several kinds of triterpenoid saponin with high medicinal values. In this work, we induced transgenic hairy roots of *C. lanceolata* and analyzed triterpenoid saponins from the hairy roots and hairy root-derived transgenic plants. Hairy roots were obtained from leaf explants by the transformation of *Agrobacterium rhizogenes* R1000. Transgenic hairy root lines were confirmed by the transcriptional activities of *rol*A, B, C, and D genes by RT-PCR. Transgenic root lines actively proliferated on hormone-free medium but not in nontransformed roots. Hairy roots contained richer triterpenoids (lancemaside A, foetidissimoside A, and aster saponin Hb) than nontransformed roots. Transgenic plants were regenerated from the hairy roots via somatic embryogenesis. They showed phenotypic alterations such as shortened shoots and an increased number of axillary buds and adventitious roots. The transgenic plants also contained higher triterpenoid levels than wild-type plants. These results suggest that hairy roots and transgenic plants of *C. lanceolata* could be used as medicinal materials for the production of triterpene saponins.

Keywords *Agrobacterium rhizogenes* · Aster saponin Hb · *Codonopsis lanceolata* Trautv. · Foetidissimoside A · Lancemaside A

J.-A. Kim and Y.-S Kim contributed equally to this work.

J.-A. Kim · Y.-S. Kim · Y.-E. Choi (✉)
Division of Forest Resources, College of Forest Sciences,
Kangwon National University,
Chuncheon 200-701, South Korea
e-mail: yechoi@kangwon.ac.kr

Introduction

Codonopsis lanceolata Trautv. (*Campanulaceae*) is mainly distributed in East Asia, including Korea, Japan, and China. The roots of *C. lanceolata* have been used in traditional medicines as an anti-inflammatory agent, for bronchitis, and for cough (Lee et al. 2002; Xu et al. 2008). The root extracts of this plant can inhibit the reduction of blood testosterone levels caused by immobilization stress in mice (Sekita et al. 2005). Furthermore, a supplement containing the root extracts of *C. lanceolata* functions to alleviate partial androgen deficiency of the aging male (PADAM)-like symptoms (Morales and Lunenfeld 2002; Ushijima et al. 2007). The roots contain triterpenoids and phenylpropanoid glycosides (Li et al. 2007; Shirota et al. 2008). To date, seven 3,28-bidesmosidic triterpenoid saponins have been identified from the *C. lanceolata* roots (Sekita et al. 2005; Shirota et al. 2008; Ichikawa et al. 2009). Among them, lancemaside A is a major compound, and this aids the recovery of decreased testosterone levels in the blood (Sekita et al. 2005; Komoto et al. 2010).

Recently, the demand for *C. lanceolata* roots has been accelerating, along with its increasing reputation in medicinal plant markets of Asia (Li et al. 2003). However, the plants are mostly cultivated on mountains and wild hills, and they are harvested 3–4 years after planting them in the soil. These cultivation traits are a major obstacle to obtaining useful compounds from their roots. As described above, lancemaside A is considered a major compound of *C. lanceolata*, and this compound has been reported to have anti-inflammatory and anti-tumor effects (Joh and Kim 2010; Joh et al. 2010). These facts imply that the demand for lancemaside A is set to grow rapidly. Therefore, an efficient method of producing lancemaside A production is very desirable.

Hairy roots are tumorous outgrowths that are induced on many dicots by infection with *Agrobacterium rhizogenes*, and are characterized by fast and high growth rates (Tepfer 1990; Giri and Narasu 2000). They can also synthesize a variety of secondary metabolites in their tissues (Bulgakov et al. 1998; Palazón et al. 1998; Sevón and Oksman-Caldentey 2002; Choi et al. 2005; Kumar and Gupta 2009). Furthermore, hairy roots can be regenerated into plants either spontaneously or by transferring the roots to hormone-containing medium. The regenerated plants often exhibit several phenotypic changes, including wrinkled leaves, increasing branching, shortened internodes, reduced apical dominance, increasing rooting, and altered flowering (Tepfer 1990; Christey 2001). In some cases, it has been reported that transgenic plants that were regenerated from hairy roots produced higher levels of secondary metabolites in their plant bodies (Pellegrineschi et al. 1994; Palazón et al. 1998). Genetic transformation of *C. lanceolata* by a special *A. tumefaciens* strain led to the production of a transgenic *C. lanceolata* that overexpressed γ-tocopherol methyltransferase (Ghimire et al. 2008). However, there is still no report on the production of transgenic hairy roots in *C. lanceolata* by *Agrobacterium rhizogenes.*

In the present work, we first generated hairy roots from *C. lanceolata* infected with *A. rhizogenes*, and then analyzed the production of triterpenoid saponin in both the hairy roots and the regenerated transgenic plants derived from the hairy roots.

Materials and methods

Plant materials

Codonopsis lanceolata plants were collected from Hoengseong, Korea. The stem explants, including lateral buds, were surface-sterilized by 70% ethanol for 1 min, and disinfected by immersion in 2% sodium hypochlorite for 15 min. After rinsing 5 times with sterilized water, the explants were cultured on MS (Murashige and Skoog 1962) medium supplemented with 30 g l^{-1} sucrose and 3 g l^{-1} gelrite (Duchefa Biochemie, The Netherlands). New shoots that emerged from the lateral buds were used for further works. All media were adjusted to pH 5.8 before autoclaving at 121°C for 20 min. Cultures were placed under 16 h of artificial light (40 μmol m^{-2} s^{-1}) at 24–26°C.

Induction of hairy roots

Leaf explants (0.5 cm^2) were immersed in a diluted *A. rhizogenes* strain R1000 (OD = 1.0) for 15 min. The explants were blotted on sterile filter paper, and then placed onto a hormone-free 1/2 strength WPM (Lloyd and McCown 1980) medium for co-cultivation. After 3 days of culture, the explants were transferred to fresh medium containing 30 g l^{-1} sucrose and 300 mg l^{-1} cefotaxime (Duchefa Biochemie, The Netherlands) to suppress *A. rhizogenes*. Putative hairy roots (about 15 lines) were induced from the excised margins of leaf segments, and these roots were detached from explants and subcultured onto the same medium containing sucrose and cefotaxime.

Reverse transcription (RT)-PCR analysis

Total RNAs were extracted from hairy root lines using an RNeasy Plant Mini Kit (Qiagene, USA), and were then reverse-transcribed by the Improm-II Reverse Transcription system (Promega, USA). First-strand cDNA was used as a template for the RT-PCR reaction. The primer sets that were used to amplify the *rol*A, B, C, D, and 18S rRNA genes are detailed in Table 1. PCR was carried out for 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. After fractionation on agarose gel electrophoresis, products were identified by visualization with ethidium bromide staining.

Table 1 Primers used for RT-PCR analysis of *rol* genes in hairy roots of *Codonopsis lanceolata*

Genes	Primers	PCR product size (bp)	Accession number
*rol*A	5′-ACGGTGAGTGTGGTTGTAGG-3′	403	K03313
	5′-GCCACGTGCGTATTAATCCC-3′		
*rol*B	5′-TCAGGTTTACTGCAGCAGGC-3′	696	GU182968
	5′-AACCTATTCGAGGGGATCCG-3′		
*rol*C	5′-TGTGACAAGCAGCGATGAGC-3′	480	X03433
	5′-AAACTTGCACTCGCCATGCC-3′		
*rol*D	5′-CCTTACGAATTCTCTTAGCGGCACC-3′	477	DQ852612
	5′-GAGGTACACTGGACTGAATCTGCAC-3′		
18S rRNA	5′-GACGTGAACAACATCGGAGA-3′	330	AY548195
	5′-CCCGAACCATTGAGTCTT-3′		

Growth of hairy roots on different sucrose and IBA contents

To optimize the growth of hairy roots, different concentrations of sucrose (30 or 50 g l^{-1}) and indole-3-butyric acid (0 or 0.2 mg l^{-1} IBA) were added to half-strength WPM liquid medium. The nontransformed adventitious roots were used as a control, and were cultured on the same strength WPM containing IBA (0 or 0.2 mg l^{-1}) and 30 or 50 g l^{-1} sucrose. All cultures were accomplished in 250 ml flasks containing 100 ml liquid medium, and incubated in darkness at 25°C on a gyratory shaker at 120 rpm.

Production of transgenic plants through regeneration from hairy roots via somatic embryogenesis

For the induction of embryogenic callus, 10 mm root segments of wild-type and transformed hairy roots were cultured on MS medium supplemented with 2.0 mg l^{-1} 2,4-D, 20 g l^{-1} sucrose, and 3 g l^{-1} gelrite. The culture was maintained at 25 ± 2°C in the dark for 6 weeks, and then the induced embryogenic calli were transferred to 1/2MS medium supplemented with 50 g l^{-1} sucrose and 3 g l^{-1} gelrite for somatic embryo development. Cotyledonary somatic embryos were transferred to 1/2MS medium supplemented with 20 g l^{-1} sucrose and 3 g l^{-1} gelrite for germination. The culture was maintained at 25 ± 2°C and under a 16 h photoperiod with white fluorescent light at 40 µmol m^{-2} s^{-1}. Plantlets with well-developed leaves and roots were transplanted into small plastic pots (10 × 18 cm) containing an artificial soil mixture [peat moss and perlite (3:1, v/v)] and covered with a polyvinyl bag for the first 2 weeks.

Analysis of triterpenoids by liquid chromatography–mass spectrometry (LC–MS)

Triterpenoid saponins were extracted and purified from plant samples dried at 60°C for 48 h. The supernatants were analyzed by LC–MS (Ichikawa et al. 2008; Ichikawa et al. 2009). Briefly, powdered sample (0.1 g) was added to 80% methanol and extracted using a 80°C water bath with sonication for 40 min, followed by centrifugation at 11,000×*g* for 5 min. The supernatant was filtered through a Sepak C18 column (Waters, USA), and the filtered extract was evaporated to dryness and dissolved in 20% acetonitrile. The water-soluble extract was passed through a 0.45 µm membrane filter (Waters, USA). The extract was used for LC–MS/MS analysis (TSQ Quantum Ultra, Thermo Scientific, USA). LC separation was performed on a YMC-Pack Pro C18 RS column (3 µm particle size, 2.0 mm i.d. × 100 mm length; YMC, Kyoto, Japan). The mobile phase comprised a mixture of 0.1% formic acid and acetonitrile (67:33, v/v), and it was delivered at a flow rate of 0.2 ml/min. The injection volume was 2 µl. The mass spectrometer was operated in the negative ion and selected ion monitoring (SIM) modes. Electrospray ionization (ESI) was conducted using a spray voltage of 4.5 kV. The capillary voltage and the tube lens offset were fixed at −40 and −130 V, respectively. The heated capillary temperature was fixed at 350°C.

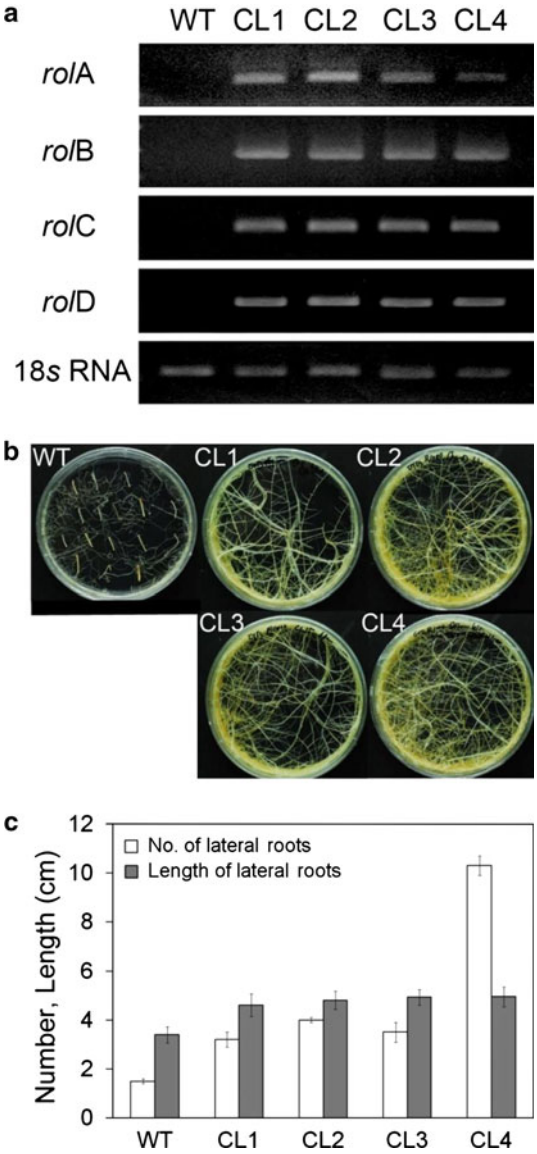

Fig. 1 Transgene expression and phenotypic evaluation in wild-type and hairy roots. **a** Expression of *rol* genes in wild-type (*WT*) and transformed hairy root lines (CL1–CL4). 18S rRNA is used as an internal standard. **b** Phenotypes of WT and transformed hairy roots after 42 days of culture in 1/2 WPM medium supplemented with 30 g l^{-1} sucrose. **c** Number and length of lateral roots in WT and transformed hairy roots. The values are expressed as means of six replicates with standard deviations

Table 2 Effect of IBA and sucrose on root growth and triterpenoid accumulation in the wild-type (WT) and hairy root line CL4 after 42 days of culture

Treatment		Dry weight (g)	Triterpenoid content (mg g^{-1} dry weight)		
IBA (mg g^{-1})	Sucrose (g l^{-1})		Lancemaside A	Foetidissimoside A	Aster saponin Hb
WT					
0.2	30	0.23 ± 0.05	0.4 ± 0.03	0.06 ± 0.001	ND
0.2	50	0.42 ± 0.04	0.6 ± 0.04	0.05 ± 0.001	ND
CL4					
0	30	0.27 ± 0.09	2.0 ± 0.09	0.42 ± 0.03	0.13 ± 0.004
0.2	30	0.36 ± 0.05	2.2 ± 0.11	0.47 ± 0.01	0.12 ± 0.009
0	50	0.40 ± 0.10	1.8 ± 0.06	0.68 ± 0.02	0.23 ± 0.007
0.2	50	0.70 ± 0.15	1.7 ± 0.07	0.44 ± 0.02	0.16 ± 0.003

All data were obtained in 50 ml shake flasks containing 100 ml liquid media. Each value is the mean of six replicates along with its standard deviation

ND not detected

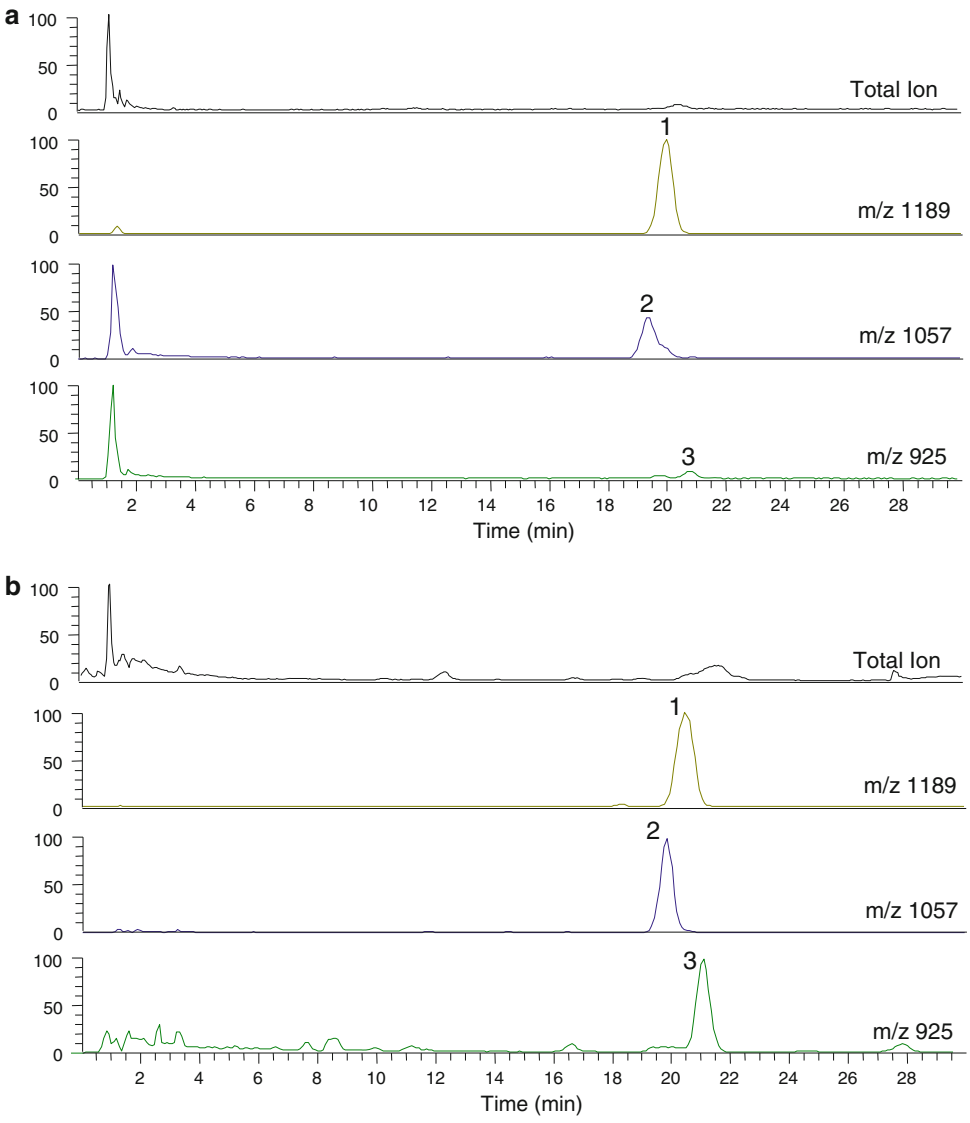

Fig. 2 Total ion and mass chromatograms obtained from a methanol extract of the hairy root line CL4 (**a**) and the standard control (**b**). *Peak 1* lancemaside A, *peak 2* foetidissimoside A, *peak 3* aster saponin Hb

Results and discussion

Induction of hairy roots

Leaf segments of plants maintained in vitro were infected with *A. rhizogenes* R1000. Two or three putative hairy roots per leaf explant appeared after 4 weeks of culture (data not shown). These roots were excised from the leaf explants and cultured in hormone-free 1/2 WPM medium containing 300 mg l^{-1} cefotaxime and 30 g l^{-1} sucrose at 2 week subculture intervals. Non-transformed roots induced from the wild type showed no elongation and rapidly turned brown. Most of the isolated putative transformed roots showed vigorous elongation with several lateral roots on hormone-free medium. Transgenic lines were confirmed by RT-PCR (Fig. 1a). All four lines (named CL1–CL4) accumulated the transcripts of *rol*A, B, C, and D, while wild-type (WT) roots did not accumulate any transcripts of *rol* genes. These results suggested that the transgenic hairy roots were successfully transformed by *A. rhizogenes* strain R1000 during the culture of leaf discs. The hairy roots actively proliferated in the absence of an exogenous auxin supply. Finally, the four transgenic hairy root lines were selected and maintained. Among the four transgenic lines, the CL4 line showed fast growth and produced more lateral roots than the other three lines (Fig. 1b, c).

Effects of sucrose and IBA on root growth and triterpenoid production

CL4 hairy roots were cultured in 1/2 WPM liquid medium with different concentrations of sucrose and IBA (Table 2). In nontransformed roots, they did not grow on hormone-free medium (data not shown). Thus, IBA treatment was used to proliferate the nontransformed roots. IBA treatment also had a positive effect on the growth of hairy roots (Table 2). The combination of 0.2 mg l^{-1} IBA and 50 g l^{-1} sucrose in hairy root cultures resulted in a 1.8-fold increase (0.7 g l^{-1}) in dry weight compared to treatment with 50 g l^{-1} sucrose. In addition to dry weight, sucrose and IBA positively affected triterpenoid production in both wild-type and hairy roots (Table 2). Triterpenoid accumulation was higher in transgenic lines compared to nontransformed roots. Lancemaside A was highly accumulated as a major compound in both roots compared to foetidissimoside A and aster saponin Hb. Lancemaside A was demonstrated to be the major triterpene saponin in an analysis of ex vitro cultured roots (Ichikawa et al. 2008, 2009). In the transgenic hairy roots, the high-concentration sucrose (50 g l^{-1}) treatment alone slightly deceased the accumulation of triterpenoids, especially lancemaside A (1.8 mg g^{-1} dry weight), but the sucrose with 0.2 mg l^{-1} IBA treatment synergistically increased the accumulation of lancemaside A (2.7 mg g^{-1}

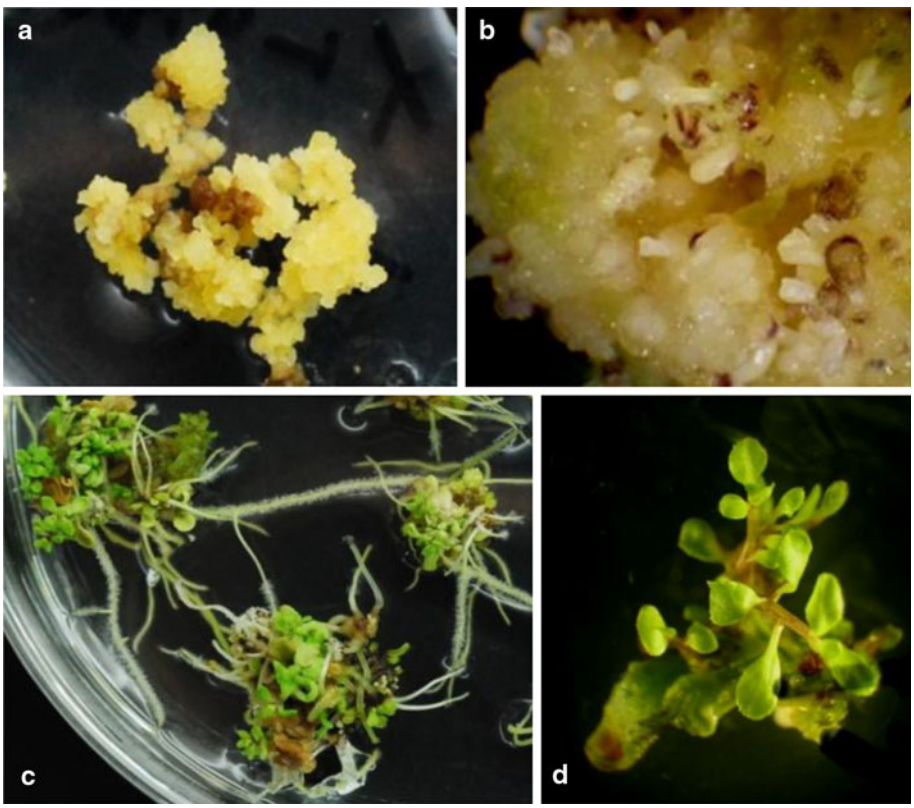

Fig. 3 Somatic embryogenesis and plant regeneration from transgenic hairy roots. **a** Embryogenic calli induced from transgenic roots on 1/2MS medium containing 2 mg l^{-1} 2,4-D after 4 weeks of culture. **b** Somatic embryos developed from the embryogenic callus on 1/2MS medium without 2,4-D after 3 weeks of culture. **c** Cotyledonary embryos germinated into plantlets on 1/2MS medium with 20 g l^{-1} sucrose and 3 g l^{-1} gelrite. **d** Regenerated transgenic plantlets after 1 month of culture on 1/2MS free medium

dry weight). Typical chromatograms of standard control and hairy root CL4 cultured on medium containing IBA and sucrose are shown in Fig. 2. The retention times and mass fragmentations of peaks 1 (lancemaside A), 2 (foetidissimoside A), and 3 (aster saponin Hb) are consistent with those of previous reports (Ichikawa et al. 2008, 2009). These results suggested that sucrose treatment is an effective way to increase the dry weight of *C. lanceolata* hairy roots, as seen in other plant species (Giri and Narasu 2000; Shin et al. 2003). The combination of sucrose and IBA stimulates the root thickness, resulting in an increase in the dry weight of date palm plants (Ibrahim et al. 2009). In *C. lanceolata*, IBA positively affected the growth of roots and lancemaside A accumulation in the transgenic hairy roots. These results support the idea that IBA, a phytohormone, could affect secondary metabolism, particularly of triterpenoid compounds produced from various medicinal plants (Kim et al. 2003; Baque et al. 2010).

Regeneration of transgenic plants via somatic embryogenesis

Root segments of a wild-type and two transgenic hairy root lines (CL3 and CL4) were cultured on MS medium containing 2,4-D (2 mg l^{-1}). Embryogenic calli were induced from wild-type and transgenic roots after four weeks of culture (Fig. 3a). Somatic embryos were developed from the embryogenic callus 3 weeks after transferring them onto 1/2MS medium lacking 2,4-D (Fig. 3b), and were subsequently developed into torpedo and cotyledonary embryos on the same medium. However, the frequency of somatic embryo formation was lower (less than 30%) in the wild type than in the transgenic root lines (more than 80%). In our unpublished data, embryo development was slightly stimulated on medium containing cytokinins such as thidiazuron and 6-benzylaminopurine. However, the transition rate from globular to cotyledonary embryos was rather higher (over 80%) on 1/2MS lacking any plant growth regulators. This result indicates that the physiological changes in transgenic roots caused by the expression of *rol* genes affect the development of somatic embryos. These observations were consistent with an experiment on *Aralia elata* (Kang et al. 2006). On the other hand, inconsistent results were reported in Colt cherry (*Prunus avium* × *P. pseudocerasus*) (Gutierrez-Pesce et al. 1998); in that study, none of the putative transgenic roots grew in hormone-free medium—they all needed growth regulators.

Phenotypic changes in transgenic plants

Cotyledonary embryos germinated into plantlets on 1/2MS medium with 20 g l^{-1} sucrose and 3 g l^{-1} gelrite (Fig. 3c). The transgenic plantlets were maintained on the same medium for further growth (Fig. 3d). When the plantlets reached 5–6 cm in height, they were successfully acclimatized in soils within 2 weeks.

After wild-type and transgenic plantlets had been cultured on 1/2MS medium in culture boxes, phenotypic changes was investigated after 6 weeks of culture. Transgenic plantlets showed distinct differences in the numbers of axillary buds and adventitious roots compared to those of wild-type plantlets (Fig. 4a). In transgenic plantlets, 3–4 axillary buds emerged from a shoot, whereas the wild type did not have any axillary buds (Fig. 4b). The numbers of roots were increased by 2.6- and 4.2-fold in transgenic lines CL3 and CL4, respectively, compared to the wild type (Fig. 4c). The phenotypic changes in transgenic plants after acclimatization in the soil were investigated (Fig. 5). Transgenic plants displayed fibrous roots with abundant lateral roots (Fig. 5b, c, e, f), whereas wild-type plants had a thickened taproot (Fig. 5a, d). The total lengths of the wild-type plants were longer than the total lengths of the transgenic plants (Table 3). The root lengths were slightly longer in the transgenic plants, whereas the shoot lengths were strongly shorter in transgenic plants (approximately 13 cm) than those in the wild type (approximately 30 cm).

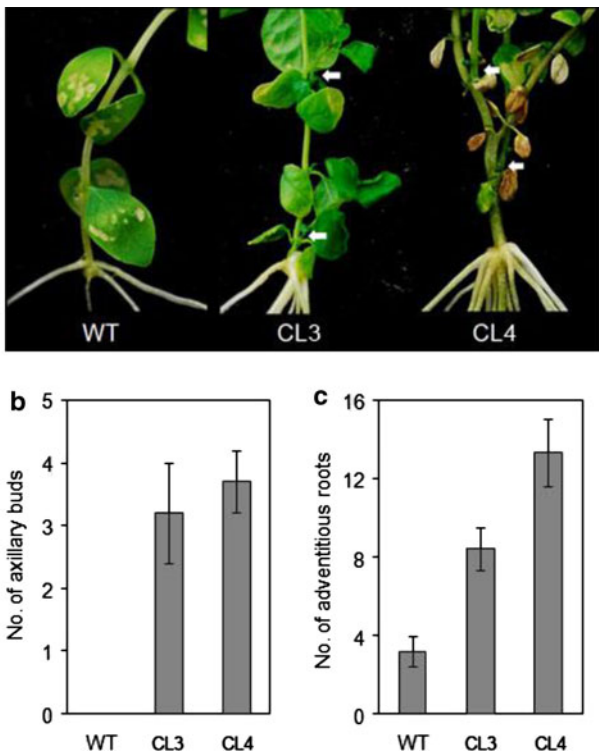

Fig. 4 Phenotypic changes in in vitro wild-type and regenerated transgenic plantlets. **a** Wild-type (*WT*) and transgenic plantlets (CL3 and CL4) cultured in culture boxes containing 1/2MS medium for 6 weeks. **b** Numbers of axillary buds in WT and transgenic lines CL3 and CL4. **c** Numbers of adventitious roots in WT and transgenic lines CL3 and CL4. The data are expressed as the means of six replicates along with their standard deviations

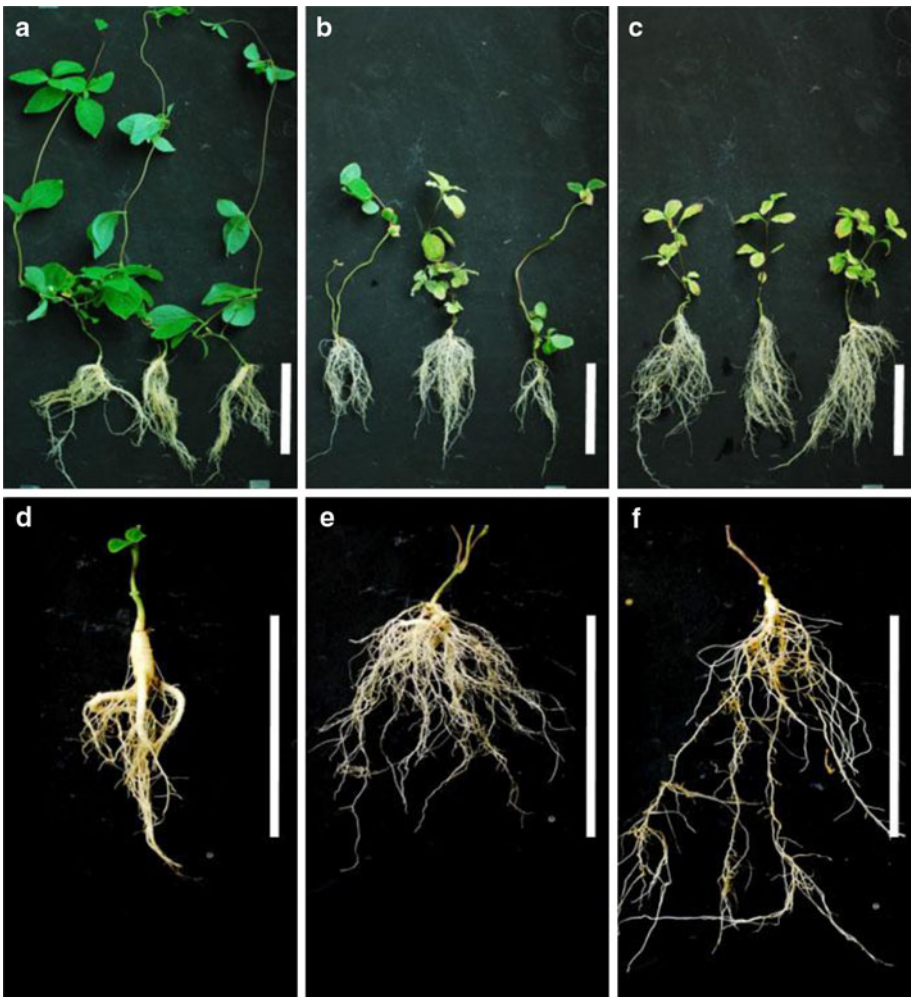

Fig. 5 Phenotypic changes in wild-type and transgenic plants after acclimatization. **a**, **d** Wild-type plants with a thickened taproot. Transgenic plant lines CL3 (**b**, **e**) and CL4 (**c**, **f**) with abundant lateral roots. All *bars* in each figure indicate 10 cm lengths

Table 3 Growth in wild-type (WT) and transgenic plants (CL3 and CL4) cultivated for 6 weeks after transferring to soil in a greenhouse

Type of explants	Length of shoots (cm)	Length of roots (cm)	Total length (cm)
WT	30.1 ± 1.3	10.7 ± 1.3	40.8 ± 1.1
CL3	12.1 ± 1.2	9.5 ± 1.4	21.6 ± 1.2
CL4	14.8 ± 0.8	16.5 ± 1.3	31.3 ± 1.0

The values are expressed as the means of fifteen replicates along with their standard deviations

These results suggested that the transgenic plants that were regenerated from *A. rhizogenes*-transformed roots showed a kind of "hairy root syndrome". It also suggests that the accumulation of the *rol*C gene (see Fig. 1a) in transgenic roots is responsible for the phenotypic alterations (Giri and Narasu 2000; Christey 2001; Casanova et al. 2005).

Production of triterpenoids in transgenic plants

In many species, it has been reported that the expression of *rol* genes in transgenic plants regenerated from hairy roots effects not only a variety of phenotypic changes but also the accumulation of secondary metabolites (Giri and Narasu 2000; Christey 2001; Casanova et al. 2005). It is conceivable that triterpenoid saponins of *C. lanceolata* would be highly accumulated in the transgenic plants regenerated from hairy roots. Thus, we analyzed three kinds of triterpenoids in leaves, stems, and roots of transgenic plants (Fig. 6). The results showed that the triterpenoid accumulations were significantly enhanced in transgenic lines compared to the wild type. Lancemaside A accumulation was highly stimulated in the stems of all tested plants compared to the leaves and roots (Fig. 6a). Similarly, the transcripts of *rol*A, B, and C were highly accumulated in the stems of our *C. lanceolata* transgenic plants compared to those of roots and leaves (data not shown). This observation is consistent with the high nicotine production observed in transgenic tobacco plants regenerated from hairy roots (Palazón et al. 1998). The nicotine production is more enhanced in transgenic plants containing three *rol* genes (*rol*A, B, and C) than plants containing only *rolC*. Pellegrineschi et al. (1994) reported

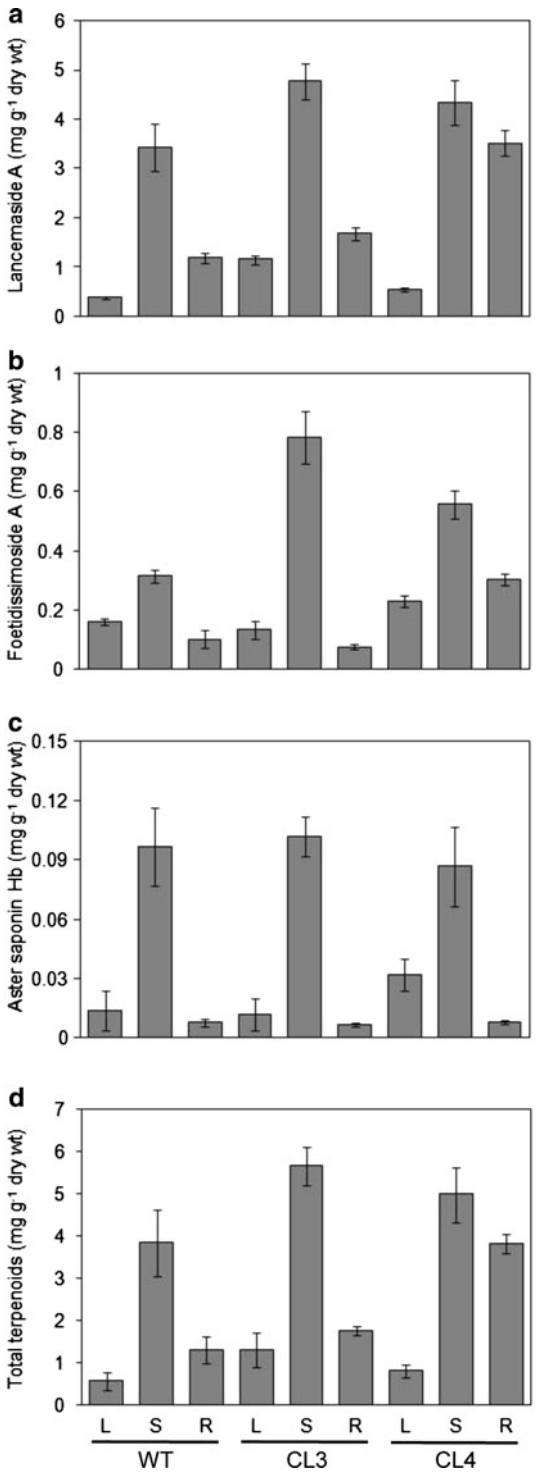

Fig. 6 Triterpenoid accumulations in leaves (*L*), stems (*S*), and roots (*R*) of wild-type and transgenic plants. **a** Lancemaside A accumulation in each organ of wild-type (*WT*) and transgenic lines CL3 and CL4. Accumulations of foetidissimoside A (**b**) and aster saponin Hb (**c**) in each organ of WT plants and the transgenic lines CL3 and CL4. **d** Total contents of triterpenoids in each organ of the WT plants and transgenic lines; the contents were calculated by adding the content of lancemaside A, foetidissimoside A, and aster saponin Hb. The data are expressed as the means of three replicates along with their standard deviations

that fragrance production in lemon was highly enhanced in leaves of transgenic plants expressing *rol* genes. Together with lancemaside A, foetidissimoside A (Fig. 6b) and aster saponin Hb (Fig. 6c) were highly accumulated in stems of transgenic lines compared to the wild type, although the amounts were much lower than lancemaside A. Consequently, the content of total triterpenoids was enhanced in transgenic lines compared to the wild type, particularly in stems of transgenic CL3 line (Fig. 6d). These results indicated that triterpenoid production in transgenic *C. lanceolata* plants regenerated from hairy roots was enhanced by the expression of *rol* genes.

In conclusion, we generated *C. lanceolata* hairy roots using *A. rhizogenes*-mediated transformation, and achieved enhanced production of triterpenoid saponins compared to the wild type. The amounts of the three major triterpenoids were similar to those seen in roots harvested from soil. Transgenic plants regenerated from the hairy roots showed phenotypic alterations such as shortened shoots, increased axillary buds and adventitious roots, and bushy roots, as well as increased triterpenoid production (including lancemaside A, foetidissimoside A, and aster saponin Hb). Based on these observations, we suggest that *rol* gene-mediated transformation is attractive for not only the production of secondary metabolites in medicinal plants but also molecular breeding involving morphological changes in floricultural crops.

Acknowledgments This study was carried out with the support of Forest Science and Technology Projects (project nos. S211010L020100 and S211010L20130) provided by the Korea Forest Service, and from the WCU project (R33-10157) of the Ministry of Education, Science & Technology (MEST), Republic of Korea.

References

Baque MA, Hahn EJ, Paek KY (2010) Growth, secondary metabolite production and antioxidant enzyme response of *Morinda citrifolia* adventitious root as affected by auxin and cytokinin. Plant Biotechnol Rep 4:109–116

Bulgakov VP, Khodakovskaya MV, Labetskaya NV, Chemoded GK, Zhuravlev YN (1998) The impact of plant *rolC* oncogene on ginsenoside production by ginseng hairy root cultures. Phytochem 49:1929–1934

Casanova E, Trillas MI, Moysset L, Vainstein A (2005) Influence of *rol* genes in floriculture. Biotechnol Adv 23:3–39

Choi DW, Jung JD, Ha YI, Park HW, In DS, Chung HJ, Liu JR (2005) Analysis of transcripts in methyl jasmonate-treated ginseng hairy roots to identify genes involved in the biosynthesis of ginsenosides and other secondary metabolites. Plant Cell Rep 23:557–566

Christey MC (2001) Use of Ri-mediated transformation for production of transgenic plants. In Vitro Cell Dev Biol Plant 37:687–700

Ghimire BK, Seong ES, Lim JD, Heo K, Kim MJ, Chung IM, Juvik JA, Yu CY (2008) *Agrobacterium*-mediated transformation of *Codonopsis lanceolata* using the γ-TMT gene. Plant Cell Tissue Organ Cult 95:265–274

Giri A, Narasu ML (2000) Transgenic hairy roots; recent trends and applications. Biotechnol Adv 18:1–22

Gutierrez-Pesce P, Taylor K, Muleo R, Rugini E (1998) Somatic embryogenesis and shoot regeneration from transgenic roots of the cherry root stock colt (*Prunus avium* × *P. pseudocerasus*) mediated by pRi 1855 T-DNA of *Agrobacterium rhizogenes*. Plant Cell Rep 17:574–580

Ibrahim K, Alromaihi KB, Elmeer KMS (2009) The combined role of sucrose with IBA and NAA in rooting of date palm somatic embryos cv. Khanaizi. Plant Tissue Cult Biotechnol 19:127–132

Ichikawa M, Ohta S, Komoto N, Ushijima M, Kodera H, Hayama M, Shirota O, Sekita S, Kuroyanagi M (2008) Rapid identification of triterpenoid saponins in the roots of *Codonopsis lanceolata* by lipuid chromatography-mass spectrometry. J Nat Med 62:423–429

Ichikawa M, Ohta S, Komoto N, Ushijima M, Kodera H, Shirota O, Sekita S, Kuroyanagi M (2009) Stimulateous determination of seven saponins in the roots of *Codonopsis lanceolata* by liquid chromatography-mass spectrometry. J Nat Med 63:52–57

Joh EH, Kim DH (2010) Lancemaside A inhibits lipopolysaccharide-induced inflammation by targeting LPS/TLR4 complex. J Cell Biochem 111:865–871

Joh EH, Lee IA, Han SJ, Chae S, Kim DH (2010) Lancemaside A ameliorates colitis by inhibiting NF-κB activation in TNBS-induced colitis mice. Int J Colorectal Dis 25:545–551

Kang HJ, Anbazhagan VR, You XL, Moon HK, Yi JS, Choi YE (2006) Production of transgenic *Aralia elata* regenerated from *Agrobacterium rhizogenes*-mediated transformed roots. Plant Cell Tissue Organ Cult 85:187–196

Kim YS, Hahn EJ, Yeung EC, Paek KY (2003) Lateral root development and saponin accumulation as affected by IBA or NAA in adventitious root cultures of *Panax ginseng* C.A. Meyer. In Vitro Cell Dev Biol Plant 39:245–249

Komoto N, Ichikawa M, Ohta S, Nakano D, Nishihama T, Ushijima M, Kodera Y, Hayama M, Shirota O, Sekita S, Kuroyanagi M (2010) Murine metabolism and absorption of lancemaside A, an active compound in the roots of *Codonopsis lanceolata*. J Nat Med 64:321–329

Kumar J, Gupta PK (2009) Molecular approaches for improvement of medicinal and aromatic plants. Plant Biotechnol Rep 2:93–112

Lee KT, Choi J, Jung WT, Nam JH, Jung HJ, Park HJ (2002) Structure of a new echinocystic acid bisdesmoside isolated from *Codonopsis lanceolata* roots and the cytotoxic activity of prosapogenins. J Agric Food Chem 50:4190–4193

Li YS, Li HL, An JH, Li LH, Shu YM (2003) Morphology and germination of *Codonopsis lanceolata*. J Agri Sci Yanbian Uni 25:155–158

Li JP, Liang ZM, Yuan Z (2007) Triterpenoid saponins and anti-inflammatory activity of *Codonopsis lanceolata*. Pharmazie 62:463–466

Lloyd G, McCown BH (1980) Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb Proc Int Plant Prop Soc 30:421–427

Morales A, Lunenfeld B (2002) Investigation, treatment and monitoring of late-onset hypogonadism in males. Aging Male 5:74–86

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497

Palazón J, Cusidó RM, Roig C, Piñol MT (1998) Expression of the *rolC* gene and nicotine production in transgenic roots and their regenerated plants. Plant Cell Rep 17:384–390

Pellegrineschi A, Damon JP, Valtorta N (1994) Improvement of ornamental characters and fragrance production in lemon-scented geranium through genetic transformation by *Agrobacterium rhizogenes*. Biotechnology 12:64–68

Sekita S, Kuroyanagi M, Yasuda K, Mizuno I, Ushijima M, Hayama M, Ichikawa M, Sumihiro M (2005) Drug for ameliorating male climacteric disorder. Patent WO200515426

Sevón N, Oksman-Caldentey K-M (2002) *Agrobacterium rhizogenes*-mediated transformation: root cultures as source of alkaloids. Planta Med 68:859–868

Shin KS, Chakrabarty D, Ko JY, Han SS, Paek KY (2003) Sucrose utilization and mineral nutrient uptake during hairy root growth of red beet (*Beta vulgaris* L.) in liquid culture. Plant Growth Regul 39:187–193

Shirota O, Nagamatsu K, Sekita S, Komoto N, Kuroyanagi M, Ichikawa M, Ohta S, Ushijima M (2008) Preparative separation of the saponin lancemaside A from *Codonopsis lanceolata* by centrifugal partition chromatography. Phytochem Anal 19:403–410

Tepfer D (1990) Genetic transformation using *Agrobacterium rhizogenes.* Physiol Plant 79:140–146

Ushijima M, Mizuno I, Suzuki E, Amayasu R, Ishii S, Nishihama T, Morihara N, Kashimoto N, Mouri Y, Sumioka I, Kuroyanagi M, Sekita S, Hayama M (2007) Improvement of PADAM-like symptoms in middle-aged men by a designer food containing *Codonopsis lanceolata*. Pharmacometrics 72:23–30

Xu LP, Wang H, Yuan Z (2008) Triterpenoid saponins with anti-inflammatory activity from *Codonopsis lanceolata*. Planta Med 74:1412–1415