

CUCUMOPINE TYPE *AGROBACTERIUM RHIZOGENES* K599 (NCPB2659) T-DNA MEDIATED PLANT TRANSFORMATION AND ITS APPLICATION

TAIHE XIANG*¹, SHASHA WANG, PAN WU, YAFEI LI, TING ZHANG,
DAZE WU AND SHIYI ZHOU

College of Life and Environment Sciences, Hangzhou Normal University,
Hangzhou-310036, China

Key words: Cucumopine type, *Agrobacterium rhizogene* K599, T-DNA, Plant transformation

Abstract

Agrobacterium rhizogenes infects plants to induce hairy roots. It is classified into four different types such as agropine, mannopine, cucumopine and mikimopine. *A. rhizogenes* K599, also known as NCPB2659, is a representative strain of the cucumopine type. In this review, we discussed the functions of 11 genes on the T-DNA of Ri plasmid pRi2659 in K599. We summarized the infectivity of K599 on a variety of plants and reviewed the applications of K599-induced hairy roots. These applications include analyses of gene and recombinant plasmid functions, direct employment as root parasite host and model system for the interaction between root and its parasite, production of herbal active ingredients as bioreactor, and the regeneration of transgenic plant. We also prospected the application potentials of *A. rhizogenes* K599 in transgenic plant and the mechanism of K599-mediated transgenic plant.

Introduction

Agrobacterium rhizogenes is a gram-negative soil bacterium that belongs to the genus *Agrobacterium* and family *Rhizobiaceae* with an optimum growth temperature of 28°C (White and Nester 1980). It infects plants to induce hairy roots from the infection site, which is a result of the integration of T-DNA of the Ri plasmid of *A. rhizogenes* into plant genome (Chilton *et al.* 1982; Giri and Narasu 2000). The hairy roots harbor certain morphological characters, which roots are branched, pleiotropic and grow quickly on medium without plant hormones. Besides, hairy roots are maintaining the characteristics of the ordinary root system anatomically, and possesses highly stable hereditary since they originate from single-cells containing no chimeras (Banerjee *et al.* 2012, Peebles *et al.* 2009). According to opines production after infection, *A. rhizogenes* is classified into four different types, namely agropine, mannopine, cucumopine, and mikimopine. The Ri plasmid in the agropine type *A. rhizogenes* has two T-DNA regions TL-DNA (15-20 kb) and TR-DNA region (more than TL-DNA in length) that are linked by non-insertion region. The other three types of T-DNA is one-single continuous region in Ri plasmid containing *rol* genes and opine synthesis genes but no auxin synthesis genes (Combard *et al.* 1987, Kiyokawa *et al.* 1994, Guillon *et al.* 2006).

Genes and their functions on the T-DNA of Ri plasmid of A. rhizogenes K599: K599 as a representative strain of cucumopine type *A. rhizogenes*, was isolated from soil by an Australian scholar, Allen Kerr (Savka *et al.* 1990). It contains endogenous Ri plasmid pRi2659 which is 185,462 bp in length with a 14,982 bp T-DNA (GenBank accession number: EU186381). The T-DNA region contains a total of 11 genes/ORFs, which are *orf2*, *orf3*, *orf4*, *orf8*, *rolA*, *rolB*, *rolC*, *rolD* (*orf13*), *rolE* (*orf13a*), *orf14* and *cus*, with *orf4* being nested in *orf3* (Fig. 1) (Mankin *et al.* 2007).

*Author for correspondence. <xthcn@163.com; xthen@hznu.edu.cn>. ¹Zhejiang Provincial Key Laboratory for Genetic Improvement and Quality Control of Medicinal Plants, Hangzhou-310036, China.

It has been indicated that *rolA* (*rolα*), *rolB* (*rolβ*), and *rolC* (*rolγ*) genes regulate formation and development of hairy roots induced by K599, and *cus* gene encodes the enzyme for cucumopine synthesis (Failla *et al.* 1990; Serino *et al.* 1994). Many reports have shown that homologous *rolA*, *rolB*, *rolC*, and *rolD* genes in other *A. rhizogenes* are also involved in hair root induction (Casanova *et al.* 2005). Hansen *et al.* (1994) proposed that *rolE* (*orf13a*) in mannopine type *A. rhizogenes* 8196 is a class of regulatory proteins, while Aoki *et al.* (1994) believed that *orf14* assisted *rol* hairy root induction. Otten and Helfer (2001) reported that *orf8* gene in *A. rhizogenes* affected glucose metabolism in plants, while Umber *et al.* (2005) found that *orf8* transgenic tobacco plants appeared shorter and variegated. We cloned *orf3* gene from K599 and determined

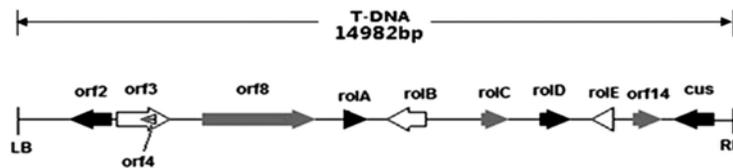


Fig. 1. T-DNA structure of Ri plasmid of cucumopine type *Agrobacterium rhizogenes* K599.

that its open reading frame (ORF) is 1,479 bp encoding a protein of 492 amino acids. The protein sequence deduced from K599 *orf3* is highly homologous to the protein sequence of *orf3* encoded by the *orf3* gene on *A. rhizogenes* Ri plasmid, pRi1724, reported by Moriguchi *et al.* (2001). We investigated the function of *orf3* cloned from K599 by *A. tumefaciens*-mediated transgenic technology and observed decreased internode length and more branching in transgenic tobacco and *Arabidopsis* plants (Wang *et al.* 2016). However, no studies on the functions of *orf2* and *orf4* in K599 have been reported yet. The T-DNA sequences in pRi2659, mikimopine type Ri plasmid pRi1724 (GenBank accession number: AP002086.1, NC_002575), and agropine type *A. rhizogenes* Ri plasmid pRiA4 (GenBank accession number: K03313, X03433, X51338, X12579, X04833) (Slightom *et al.* 1986) are somewhat similar (Table 1).

Table 1. Comparison of K599 Ri plasmid pRi2659, mikimopine type MAFF03-01724 plasmid pRi1724, and agropine type A4 plasmid pRiA4.

Gene	pRi2659 in K599		pRi1724 in MAFF03-01724		pRiA4 in A4	
	ORF length (bp)	No. of amino acids	ORF length (bp)/maximum matching percentage with pRi2659	No. of amino acids/maximum matching percentage with pRi2659	ORF length (bp)/maximum matching percentage with pRi2659	No. of amino acids/maximum matching percentage with pRi2659
<i>orf2</i>	825	274	825/100%	274/100%	-	-
<i>orf3</i>	1479	492	1479/99%	492/99%	1080/82%	359/46.1%
<i>orf4</i>	366	121	366/99%	121/98.3%	366/86%	121/68.6%
<i>orf8</i>	2343	780	2286/99%	761/83.8%	2300/82%	766/70.8%
<i>rolA</i>	282	93	282/99%	93/99%	303/73%	100/60%
<i>rolB</i>	840	279	840/98%	279/98%	780/44.6%	259/75%
<i>rolC</i> (<i>orf12</i>)	543	180	543/99%	180/99%	543/87%	180/74%
<i>rolD</i> (<i>orf13</i>)	597	198	597/97%	198/93%	603/91%	200/84.8%
<i>rolE</i> (<i>orf13a</i>)	273	90	273/99%	90/99%	-	-
<i>orf14</i>	567	188	567/98%	188/98%	552/86%	183/75.5%
<i>cus/mis/ags</i>	792	263	987	294	1191	397

Plant hairy root formation induced by A. rhizogenes K599: Many studies have shown that *A. rhizogenes* K599 can efficiently induce hairy root in a wide range of dicotyledonous and monocotyledonous plants such as soybean, carrot, cucumber, impatiens flower, chrysanthemum, corn, petunia, bellflower, *Glycyrrhiza glabra*, cotton, *Artemisia annua*, tomato, and milk vetch etc (Table 2). In particular, K599 is highly infectious on leguminous plants and most of related reports were achieved in soybean and related genera. For example, Cho *et al.* (2000) reported 54 - 95% hairy root induction frequency by K599 in soybean with different genotypes. Xiang *et al.* (2005) used wild-type K599 to infect the cotyledons of soybean, cucumber, and balsam to induce hairy roots at frequencies of 100, 65 and 91%, respectively. K599 also induced hairy root formation in cucumber buds without cutting at a frequency of 10%. The frequency of root formation induced by K599 infection on the engraved aseptic chrysanthemum leaves *in vivo* was 88% (Xiang *et al.* 2011). K599 appeared a suitable progenitor for new *Agrobacterium* strains for plant transformation.

The hairy roots induced by *A. rhizogenes* infection may have different morphology in plant species (Cleene and Ley 1981). Cao (2012) observed the similar phenomenon with K599. The morphology of the adventitious roots induced by K599 on cucumber hypocotyls was divided into two types: type I being branchy and type II being non-branchy with or without woolly roots (Fig. 2A). However, Chrysanthemum (Wang and Xiang 2009) (Fig. 2B) and Petunia (Xu and Xiang 2008; Zhang *et al.* 2011) (Fig. 2C) with the same infection formed typical woolly roots, while soybean only formed non-branched roots (Xiang *et al.* 2005) (Fig. 2D). The morphological variations were seen among the different species in terms of length, thickness and the site of hairy root induction. The results suggest that variations in species, strain and explant type led to different hairy root induction efficiencies (Aarouf *et al.* 2012, Md Setamam *et al.* 2014).

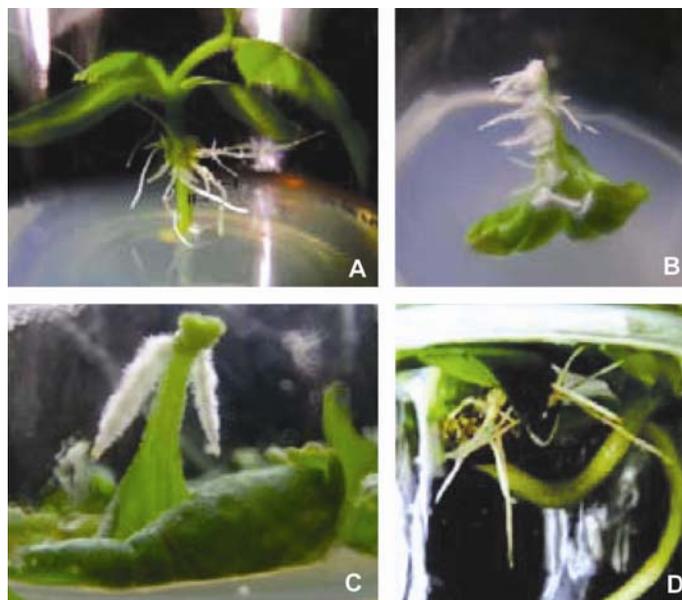


Fig. 2. Different types of hairy roots induced by *Agrobacterium rhizogenes* K599 in different plant species. A: Type I branched hairy root and type II non-branched hairy root on cucumber; B: Woolly hairy roots on the leaf of *Dendranthema morifolium*; C: Woolly hairy roots on the leaf of *Petunia hybrida*; D: Hairy roots without woolly roots on soybean cotyledon.

Table 2. List of plant infected by *A. rhizogenes* K599.

Plant	Infection site	Results	References
Soybean (<i>Glycine max</i>)	cotyledons, apical meristem, hypocotyl	hairy root or composite plantlet with hairy root	Savka <i>et al.</i> (1990), Cho <i>et al.</i> (2000), Cho <i>et al.</i> (2004), Collier <i>et al.</i> (2005), Xiang <i>et al.</i> (2005), Kereszt <i>et al.</i> (2007), Yi <i>et al.</i> (2010a), Yi <i>et al.</i> (2010b), Weber and Bodanese-Zanettini (2011), Indrasumunar and Gresshoff (2011), Cao <i>et al.</i> (2011), Li <i>et al.</i> (2011), Guo <i>et al.</i> (2011), Yi <i>et al.</i> (2011), Liang <i>et al.</i> (2013), Dolatabadian <i>et al.</i> (2013) and Xu <i>et al.</i> (2014)
<i>Glycine canescens</i>	cotyledonary nodes	hairy root	Mohammadi-Deheshmeh <i>et al.</i> (2014)
Carrot (<i>Daucus carota</i>)	carrot disc	hairy root	Serino <i>et al.</i> (1994); Collier <i>et al.</i> (2005)
Sweet potato (<i>Ipomea batata</i>)	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
<i>Nicotiana Benthamiana</i>	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
<i>Nicotiana tabacum</i>	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
Potato (<i>Solanum tuberosum</i>)	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
Beet (<i>Beta vulgaris</i>)	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
<i>Brassica oleracea</i>	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
Pumpkin (<i>Cucurbita sp.</i>)	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
<i>Datisca glomerata</i>	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
<i>Hybiscus esculenta</i>	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
<i>Medicago truncatula</i>	apical stem	composite plantlet with hairy root	Collier <i>et al.</i> (2005)
Tomato (<i>Lycopersicon esculentum</i>)	leaf disc, direct infection, apical stem	plantlet regeneration from wound leaf	Weller and Stead (2002) and Collier <i>et al.</i> (2005)
<i>Petunia hybrida</i>	apical stem, leaf	plantlet regeneration from hairy root	Collier <i>et al.</i> (2005), Xu and Xiang (2008) and Zhang <i>et al.</i> (2011)
<i>Medicago sativa L</i>	leaf disc	plantlet regeneration from calli inducing from infection site	Shabnam <i>et al.</i> (1996)
Cucumber (<i>Cucumis sativus</i>)	cotyledon, epicotyls, hypocotyl	composite plantlet with hairy root	Xiang <i>et al.</i> (2005) and Meng <i>et al.</i> (2010)
<i>Impatiens balsamina L.</i>	cotyledon	hairy root	Xiang <i>et al.</i> (2005)

(Contd.)

Table 2 (contd.)

Plant	Infection site	Results	References
Chrysanthemum	leaf of aseptic seedling	plantlet regeneration from hairy root	Wang and Xiang (2009) and Xiang <i>et al.</i> (2011)
Corn (<i>Zea mays</i>)	wounding plant tissue e.g. leaf, cotyledon, or root hypocotyls	composite plantlet with hairy root	Runo <i>et al.</i> (2012)
<i>Elaeagnus angustifolia</i>	cotyledonary wound	hairy root	Berg <i>et al.</i> (1992)
Liquorice (<i>Glycyrrhiza glabra</i>)	cotyledon, hypocotyl, leaf	hairy root	Chen and Hou (1991) and Mehrotra <i>et al.</i> (2008)
Cotton (<i>Gossypium hirsutum</i>)	cotyledon	hairy root	Zhang (2011)
<i>Podophyllum hexandrum</i>	embryo	hairy root	Giri <i>et al.</i> (2001a)
<i>Artemisia annua</i>	shoot-tip meristem	hairy root	Giri <i>et al.</i> (2001b)
Grapevines (<i>Vitis spp.</i>)	internode, stem-cut, petiole-cut surfaces on in vitro plant, young shoot excised from in vitro plant	hairy root	Jittayasothorn <i>et al.</i> (2011)
Peanut (<i>Arachis pintoi</i>)	hypocotyl	hairy root	Shen <i>et al.</i> (2012)
<i>Astragalus sinicus</i>	hypocotyl	composite plantlet with hairy root	Jia <i>et al.</i> (2012)
<i>Tetrastigma hemsleyanum</i>	leaf	hairy root	Du <i>et al.</i> (2015)
<i>Phaseolus spp</i>	wounding the cotyledonary node with a syringe	hairy root	Estrada-Navarrete <i>et al.</i> (2006)
<i>Lotus corniculatus</i>	stem section with one node and root	plantlet regeneration from hairy root	Jian <i>et al.</i> (2009)
Strawberry	leaf, petiole, and terminal bud	hairy root, transgenic plant	Tavizi <i>et al.</i> (2015)

Application of hairy root induced by A. rhizogenes K599: The hairy roots have important applications. Kereszt *et al.* (2007), Estrada-Navarrete *et al.* (2006) and our group used *A. rhizogenes* K599 to infect soybean, bean, and cucumber to successfully generate composite plantlets with hairy roots (Fig. 3A) and studied root-related genes. Using transformed hairy roots harboring different function genes on the composite transgenic soybeans, Cao *et al.* (2011) found that overexpression of *TaNHX2* improved the plant salt-tolerance; Guo *et al.* (2011) analyzed the correlation between the *GmEXPB2* and soybean root development; Liang *et al.* (2013) discovered that the soybean malic acid transporter *GmALMT1* regulated malate exudation through *GmALMT1* to improve soybean adaptation to acid soils; Dolatabadian *et al.* (2013) investigated the ability of *GmNFR5a* to improve the ability of soybean resistance to salt stress. Using the hairy roots of transgenic soybeans, Yi *et al.* (2010a, 2010b) investigated the functions of the *chs* gene family in soybean; Indrasumunar *et al.* (2011) studied the function of lipo-oligochitin-like receptor. Lin *et al.* (2011) constructed the pHairyRed plasmid containing the red fluorescent protein gene, while Alzohairy *et al.* (2013) constructed the pJan25 plasmid containing the green fluorescent protein. Both plasmids were transferred into the wild-type K599 for the infection of soybean explants to obtain transgenic hairy roots, which verified the effectiveness of the recombinant plasmid. Xu and Xiang (2008) constructed a plasmid pBIN-35S-GFP with green fluorescent protein gene, and obtained highly expressed GFP in transgenic hairy roots of petunia that were infected with K599 containing pBIN-35S-GFP (Fig. 3B). Hairy root is an efficient tool for functional analysis of genes. Moreover, it is well adapted to RNAi-based approaches to study gene function, particularly in case of abiotic and biotic stresses adaptation. Notably, leguminous plant (e.g. soybean) is known to be a recalcitrant species to plantlet regeneration of genetic transformation via *Agrobacterium tumefaciens*, while *A. rhizogene* K599-mediated transformation offers a rapid alternative to study soybean gene functions in hairy root.

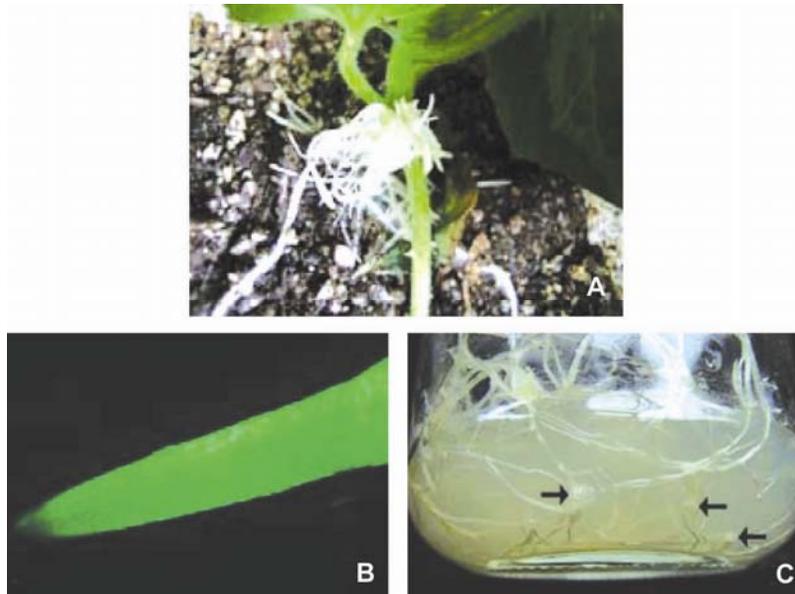


Fig. 3. The application of hairy roots. A: The composite cucumber plantlet with hairy root. B: Hairy root with *gfp* gene emitted strong green fluorescence under fluorescent microscope. C: *Meloidogyne incognita* was propagated in cucumber hairy root and the root knot was observed (arrow).

Both Savka *et al.* (1990) and Cho *et al.* (2000) successfully propagated soybean cyst nematodes using the hairy roots induced by K599 infection of soybean cotyledons. Runo *et al.* (2012) used composite corn plant containing hairy roots to investigate the interaction between corn and its parasitic plant *Striga hermonthica*. We also successfully cultured root knot nematodes using the hairy roots induced by K599 infection of cucumber cotyledons (Fig. 3C). Valuably, the hairy roots that express the gene of interest can be obtained reproducibly and rapidly, and can be used for identification the gene resistance to nematode.

Giri *et al.* (2011a, 2011b) reported that the hairy roots induced by K599 infection were able to effectively produce pureonebio and artemisinin. Yi *et al.* (2010, 2011) discovered that over-expression of *CHS8*, *IFS2* or *CHS8 + IFS2* in K599-induced hairy roots led to increased contents of root isoflavone. Du *et al.* (2015) used K599 to infect the medicinal plant *Tetradigma hemsleyanum*, which resulted in higher contents of total flavonoids in the hairy roots. Especially, the greatest advantage of hairy roots is that they often exhibit similar or greater biosynthetic capacity for secondary metabolite production as compared to their mother plants. Hairy root cultures are also known to produce a spectrum of secondary metabolites that are not present in the parent plant (Srivastava *et al.* 2007, Chandra 2012).

Furthermore, hairy roots are often able to regenerate whole viable plants and maintain their genetic stability during continuous subculturing and plant regeneration. Jian *et al.* (2009) regenerated the whole plants from *Lotus corniculatus* hairy roots induced by K599. Zhang *et al.* (2011) induced hairy roots after K599 infection of petunia leaf explants and regenerated *cyp2e1* transgenic plants. In addition, the hairy roots induced by K599 infection of *chrysanthemum* leaves formed calli, which were then regenerated into the whole plants; the whole plants showed characteristics of dwarf and more roots with normal flowering (Xiang *et al.* 2011).

Perspectives of *A. rhizogenes* K599 in the application

A. rhizogenes K599 has a high infectivity on a variety of plants, especially on early recalcitrant leguminous plants. K599-induced hairy roots have many applications. As mentioned above, transgenic hairy roots can be directly used for studies on genes, plasmid functions and those on the interactions between plants and their root pests; they can be used as bioreactors for the production of active ingredients of medicinal plants, and they may also be used to regenerate complete transgenic plants. In the future, the hairy roots induced by K599 will show the most promising application in root metabolic engineering and producing valuable medicine ingredients.

Ri plasmid pRi2659 in K599 carries a single consecutive T-DNA region containing only 11 genes/coding regions. Because of its simple form, it can serve as the model *A. rhizogenes* to probe process of the insertion and integration of T-DNA (Wang *et al.* 2016). Although the whole sequence of plasmid pRi2659 in K599 has been determined, the functions of the majority of the coding regions on the plasmid, including the *orf2* and *orf4* genes on T-DNA, have not been revealed. Whether *rolE* (*orf13a*), *orf14* and *orf8* are required in hairy root induction and the mechanisms need further investigation. In addition, previous studies on the functions of *orf8*, *rolD*, *rolE* and *orf14* have almost cloned them from agropine type or mannopine *A. rhizogenes*, but their homologous genes on K599 T-DNA are somewhat different. Do the differences lead to its role in higher invasion on plants than other strains? Remarkably, the draft genome sequence of strain K599 has been reported recently (Valdes Franco *et al.* 2016). However, it is poorly understanding which genes in the genome are relation with infectivity to plant cell. Therefore, improved functional studies on related genes should contribute significantly to the in-depth understanding of the infection mechanism of K599.

On the other hand, *A. rhizogenes* K599 has strong infectivity on some dicotyledons, especially cucumber and legumes, and certain infectivity on monocots like corn. However, it is

unknown whether K599 infects rice, wheat, and other important crop plants. Although K599 infection-induced hairy roots of transgenic petunia, *Lotus corniculatus*, and chrysanthemum have been successfully used to regenerate transgenic plants, no success has been reported on the regeneration from hairy roots of soybean and other monocotyledonous crops. Further studies on such topics should help explore the potential applications of K599 in transgenic plants.

Acknowledgements

The work was supported by the Hangzhou Science and Technology Development Plan (Grant No. 20140432B05), the New-shoot Talents Program of Zhejiang Province (Grant No. 2015R423044) and the Zhejiang Provincial Key Laboratory for Genetic Improvement and Quality Control of Medicinal Plants (No. 201302).

References

- Aoki S, Kawaoka A, Sekine M, Ichikawa T, Fujita T, Shinmyo A and Syono K 1994. Sequence of the cellular T-DNA in the untransformed genome of *Nicotiana glauca* that is homologous to ORFs 13 and 14 of the Ri plasmid and analysis of its expression in genetic tumours of *N. glauca* x *N. langsdorffii*. *Mol. Gen. Genet.* **243**: 706-710.
- Alzohairy AM, MacDonald MH and Matthews BF 2013. The pJan25 vector series: an enhancement of the Gateway-compatible vector pGWB533 for broader promoter testing applications. *Plasmid* **69**: 249-256.
- Banerjee S, Singh S and Ur Rahman L 2012. Biotransformation studies using hairy root cultures - A review. *Biotechnol. Adv.* **30**: 461-468.
- Berg RH, Liu L, Dawson JO, Savka MA and Farrand SK 1992. Induction of *Pseudoactinorhizae* by the plant pathogen *Agrobacterium rhizogenes*. *Plant physiol.* **98**: 777-779.
- Cao D, Hou W, Liu W, Yao W, Wu C, Liu X and Han T 2011. Overexpression of *TaNHX2* enhances salt tolerance of 'composite' and whole transgenic soybean. *Plant Cell Tissue. Org. Cult.* **107**: 541-552.
- Cao Q, Xiang T, Meng S and Wang L 2012. Genetic stability analysis of exogenous gene in long-term cultured cucumber hairy roots. *Acta Horti. Sin.* **39**: 1589-1598. (in Chinese)
- Casanova E, Trillas MI, Moysset L and Vainstein A 2005. Influence of *rol* genes in floriculture. *Biotechnol. Adv.* **23**:3-39.
- Chen S and Hou S 1991. *In vitro* transformation of cotyledons and hypocotyls of *glycyrrhiza uralensis* fishch by *Agrobacterium rhizogenes*. *J. Wuhan Bot. Res.* **4**:301-304. (in Chinese)
- Chilton MD, Tepfer DA, Petit A, David C, et al. 1982. *Agrobacterium rhizogenes* insert T-DNA into the genomes of the host plant root cells. *Nature* **295**: 432-434.
- Cho HJ, Brotherton JE and Widholm JM 2004. Use of the tobacco feedback-insensitive anthranilate synthase gene (*ASA2*) as a selectable marker for legume hairy root transformation. *Plant Cell Rep.* **23**: 104-113.
- Cho HJ, Farrand SK, Noel GR and Widholm JM 2000. High-efficiency induction of soybean hairy roots and propagation of the soybean cyst nematode. *Planta* **210**: 195-204.
- Cleene MD and Ley JD 1981. The host range of infectious hairy-root. *Bot. Rev.* **47**: 147-194.
- Collier R, Fuchs B, Walter N, Kevin Lutke W and Taylor CG 2005. *Ex vitro* composite plants: an inexpensive, rapid method for root biology. *Plant J.* **43**: 449-457.
- Combard A, Brevet J, Borowski D, Cam K and Tempé J 1987. Physical map of the T-DNA region of *Agrobacterium rhizogenes* strain NCPPB2659. *Plasmid* **18**: 70-75.
- Dolatabadian A, Modarres Sanavy SA, Ghanati F and Gresshoff PM 2013. *Agrobacterium rhizogenes* transformed soybean roots differ in their nodulation and nitrogen fixation response to genistein and salt stress. *World J. Microbiol. Biotechnol.* **29**: 1327-1339.
- Du S, Xiang T, Song Y, Huang L, Sun Y and Han Y 2015. Transgenic hairy roots of *Tetragonia hemsleyanum*: Induction, propagation, genetic characteristics and medicinal components. *Plant Cell Tissue Org. Cult.* **122**: 373-382.

- Estrada-Navarrete G, Alvarado-Affantranger X, Olivares JE, Díaz-Camino C, Santana O, Murillo E, Guillén G, Sánchez-Guevara N, Acosta J, Quinto C, Li D, Gresshoff PM and Sánchez F. 2006. *Agrobacterium rhizogenes* transformation of the *Phaseolus* spp: a tool for functional genomics. *Mol. Plant Microbe Interact* **19**: 1385-1393.
- Failla MC, Maimone F, De Paolis A, Costantino P and Cardarelli M 1990. The non-conserved region of cucumopine-type *Agrobacterium rhizogenes* T-DNA is responsible for hairy root induction. *Plant Mol. Biol.* **15**: 747-753.
- Giri A, Giri CC, Dhingra V and Narasu ML 2001a. Enhanced podophyllotoxin production from *Agrobacterium rhizogenes* transformed cultures of *Podophyllum hexandrum*. *Nat. Prod. Lett.* **15**: 229-235.
- Giri A and Narasu M 2000. Transgenic hairy roots: recent trends and applications. *Biotechnol. Adv.* **18**: 1-22.
- Giri A, Ravindra ST, Dhingra V and Narasu ML 2001b. Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots and artemisinin production in *Artemisia annua*. *Curr. Sci.* **81**: 378-382.
- Guillon S, Tremouillaux-Guiller J, Pati PK, Rideau M and Gantet P 2006. Hairy root research, Recent scenario and exciting prospects. *Curr. Opin. Plant Biol.* **9**: 341-346.
- Guo W, Zhao J, Li X, Qin L, Yan X, Liao H 2011. A soybean β -expansin gene *GmEXPB2* intrinsically involved in root system architecture responses to abiotic stresses. *Plant J.* **66**: 541-552.
- Hansen G, Vaubert D, Clérot D, Tempé J and Brevet J 1994. A new open reading frame, encoding a putative regulatory protein, in *Agrobacterium rhizogenes* T-DNA. *C R Acad. Sci. III* **317**: 49-53.
- Indrasumunar A and Gresshoff PM 2011. Evolutionary duplication of lipo-oligochitin-like receptor genes in soybean differentiates their function in cell division and cell invasion. *Plant Signal Behav.* **6**: 534-537.
- Jia S, Lin H, Huang W, Liu F and Zhao B 2012. Effect of Zn on co-culture system of *Arbuscular Mycorrhizal* fungus and transformed roots of *Astragalus sinicus*. *Hubei Agric. Sci.* **51**: 685-692. (in Chinese)
- Jian B, Hou W, Wu C, Liu B, Liu W, Song S, Bi Y and Han T 2009. *Agrobacterium rhizogenes*-mediated transformation of Superroot-derived Lotus corniculatus plants: a valuable tool for functional genomics. *BMC Plant Biol.* **9**:78. doi:10.1186/1471-2229-9-78.
- Jittayasothorn Y, Chen S, Wang X and Zhong G 2011. Influences of *Agrobacterium rhizogenes* strains, plant genotypes, and tissue types on the induction of transgenic hairy roots in *Vitis* species. *Vitis* **50**: 107-114.
- Kereszt A, Li D, Indrasumunar A, Nguyen CD, Nontachaiyapoom S, Kinkema M, Gresshoff PM 2007. *Agrobacterium rhizogenes*-mediated transformation of soybean to study root biology. *Nat. Protoc.* **2**: 948-952.
- Kiyokawa S, Kobayashi K, Kikuchi Y, Kamada H and Harada H 1994. Root-inducing region of mikimopine type Ri plasmid pRi1724. *Plant Physiol.* **104**: 801-802.
- Li J, Todd TC and Trick HN 2010. Rapid in planta evaluation of root expressed transgenes in chimeric soybean plants. *Plant Cell Rep.* **29**: 113-123.
- Liang C, Piñeros MA, Tian J, Yao Z, Sun L, Liu J, Shaff J, Coluccio A, Kochian LV, Liao H 2013. Low pH, aluminum, and phosphorus coordinately regulate malate exudation through *GmALMT1* to improve soybean adaptation to acid soils. *Plant Physiol.* **161**:1347-1361.
- Lin MH, Gresshoff PM, Indrasumunar A and Ferguson BJ 2011. pHairyRed: a novel binary vector containing the *DsRed2* reporter gene for visual selection of transgenic hairy roots. *Mol. Plant* **4**: 537-545.
- Mankin SL, Hill DS, Olhoft PM, Toren E, Wenck AR, Nea L, Xing L, Brown JA, Fu H, Ireland L, Jia H, Hillebrand H, Jones T and Song H.-S 2007. Disarming and sequencing of *Agrobacterium rhizogenes* strain K599 (NCPB2659) plasmid pRi2659. *In Vitro Cell Dev. Biol-Plant* **43**: 521-535.
- Mehrotra S, Kumar Kukreja A, Singh Khanuja S and Nath Mishra B 2008. Genetic transformation studies and scale up of hairy root culture of *Glycyrrhiza glabra* in bioreactor. *Electronic J. biotech.* **11**. doi:10.2225/vol11-issue2-fulltext-6.
- Meng S, Xiang T and Wang L 2010. Induction of hairy root on cucumber inbred line NC-46 with high frequency and construction of a hairy root cDNA library. *Acta Horit. Sin.* **37**: 567-574. (in Chinese).

- Mohammadi-Dehcheshmeh M, Ebrahimie E, Tyerman SD and Kaiser BN 2014. A novel method based on combination of semi-in vitro and in vivo conditions in *Agrobacterium rhizogenes*-mediated hairy root transformation of *Glycine* species. *In Vitro Cell Dev. Biol-Plant* **50**: 282-291.
- Moriguchi K, Maeda Y, Satou M, Hardayani NS, Kataoka M, Tanaka N and Yoshida K 2001. The complete nucleotide sequence of a plant root-inducing (Ri) plasmid indicates its chimeric structure and evolutionary relationship between tumor-inducing (Ti) and symbiotic (Sym) plasmids in *Rhizobiaceae*. *J. Mol. Biol.* **307**: 771-774.
- Otten L and Helfer A 2001. Biological activity of the rolB-like 5' end of the A4-orf8 gene from the *Agrobacterium rhizogenes* TL-DNA. *Mol. Plant Microbe Interact* **14**: 405-411.
- Peebles CA, Sander GW, Li M, Shanks JV and San KY 2009. Five year maintenance of the inducible expression of anthranilate synthase in *Catharanthus roseus* hairy roots. *Biotechnol. Bioeng.* **102**: 1521-1525.
- Runo S, Macharia S, Alakonya A, Machuka J, Sinha N and Scholes J 2012. Striga parasitizes transgenic hairy roots of *Zea mays* and provides a tool for studying plant-plant interactions. *Plant Methods* **8**: 20. doi:10.1186/1746-4811-8-20.
- Savka MA, Ravillion B, Noel GR and Farrand SK 1990. Induction of hairy roots on cultivated soybean genotypes and their use to propagate the soybean cyst nematode. *Phytopathol.* **80**: 503-508.
- Serino G, Clerot D, Brever J, Costantino P and Cardarelli M 1994. *rol* genes of *Agrobacterium rhizogenes* cucumopine strain: sequence, effects and pattern of expression. *Plant Mol. Biol.* **26**: 415-422.
- Shabnam S, Zafar Y and Malik KA 1996. Transformation of *Alfalfa* (*Medicago sativa* L.) plants with gus marker containing intron. *Pak. J. Bot.* **28**: 167-172.
- Shen H, Xiong H, Guo X and Zuo Y 2012. A new method of *Agrobacterium*-mediated genetic transformation in peanut plant. *Plant Nutr. Fertilizer Sci.* **18**: 518-522. (in Chinese)
- Slightom JL, Durand-Tardif M, Jouanin L and Tepfer D 1986. Nucleotide sequence analysis of TL-DNA of *Agrobacterium rhizogenes* agropine type plasmid. Identification of open reading frames. *J. Biol. Chem.* **261**: 108-121.
- Tavizi A, Javaran MJ, Moieni A, Mohammadi-Dehcheshmeh M, Mohebodini M, Ebrahimie E 2015. Root and shoot parts of strawberry: factories for production of functional human pro-insulin. *Mol. Biol. Rep.* **42**: 1013-1023.
- Umber M, Clément B, Otten L. 2005. The T-DNA oncogene A4-orf8 from *Agrobacterium rhizogenes* A4 induces abnormal growth in tobacco. *Mol. Plant Microbe Interact* **18**: 205-211.
- Valdes Franco JA, Collier R, Wang Y, Huo N, Gu Y, Thilmony R and Thomson JG 2016. Draft Genome Sequence of *Agrobacterium rhizogenes* Strain NCPPB2659. *Genome Announc.* **4**: e00746-16
- Wang L and Xiang T 2009. Cloning of flavonoid-3',5'-hydroxylase gene (*F3',5'H*) of petunia hybrida and construction of a novel vector expression F3', 5'H driven by flower-specific promoter. *J. Trop. Subtrop. Bot.* **17**: 358-364. (in Chinese)
- Wang S, Song Y, Xiang T, Wu P, Zhang T, Wu D, Zhou S and Li Y 2016. Transgenesis of *Agrobacterium rhizogenes* K599 *orf3* into plant alters plant phenotype to dwarf and branch. *Plant Cell Tissue Org. Cult.* **127**: 207-215.
- Weber RLM and Bodanese-Zanettini MH 2011. Induction of transgenic hairy roots in soybean genotypes by *Agrobacterium rhizogenes*-mediated transformation. *Pesq. Agropec. Bras.* **46**: 1070-1075.
- Weller SA and Stead DE 2002. Detection of root mat associated *Agrobacterium* strains from plant material and other sample types by post-enrichment TaqMan PCR. *J. Appl. Micro.* **92**: 118-126.
- White FF and Nester EW 1980. Hairy Root: plasmid encodes virulence traits in *Agrobacterium rhizogenes*. *J. Bacteriol.* **141**: 1134-1141.
- Xiang T, Wang L, Jiang H and Tian J 2011. Hairy roots induced by *Agrobacterium rhizogenes* K599 in chrysanthemum *in vivo* and plant regeneration from hairy roots. *Acta Horti. Sin.* **38**: 1365-1370. (in Chinese)

- Xiang TH, Wang LL, Pang JL, Chen M and Xu C 2005. Hairy root induced by wild-type *Agrobacterium rhizogenes* K599 in soybean, cucumber and garden balsam *in vivo*. *Yi Chuan* **27**: 783-786. (in Chinese)
- Xu JM, Yang Q, Li S, Liu Q and Jian H 2014. Improvement of soybean hairy-root system mediated by *Agrobacterium rhizogenes*. *Soybean Science* **33**: 161-167. (in Chinese)
- Xu JM and Xiang TH 2008. Construction of a novel vector harboring green fluorescence protein gene (*gfp*) and high expression of *gfp* in transformed roots of *Petunia hybrida*. *Yi Chuan* **30**: 1069-1074. (in Chinese)
- Yi JX, Derynck MR, Li X, Telmer P, Marsolais F and Dhaubhadel S 2010a. A single-repeat MYB transcription factor, *GmMYB176*, regulates *CHS8* gene expression and affects isoflavonoid biosynthesis in soybean. *Plant J.* **62**: 1019-1034.
- Yi JX, Michael RD, Ling C and Sangeeta D 2010b. Differential expression of *CHS7* and *CHS8* genes in soybean. *Planta* **231**: 741-753.
- Yi JX, Xu ZL, Wang JF, Zhang DY, He XL, Zulfiqar A, Zhu HR, Ma HX, Sangeeta D 2011. *GmCHS8* and *GmIFS2* gene co-determine accumulation of isoflavonoid in soybean. *Acta Agron. Sin.* **37**: 571-578. (in Chinese)
- Zhang D, Xiang T, Li P and Bao L 2011. Transgenic plants of *Petunia hybrida* harboring the *CYP2E1* gene efficiently remove benzene and toluene pollutants and improve resistance to formaldehyde. *Genet. Mol. Biol.* **34**: 634-639
- Zhang C 2011. Research on *Agrobacterium rhizogenes*-mediated genetic transformation in cotton. Dissertation, Chinese Academy of Agricultural Sciences. (in Chinese)

(Manuscript received on 3 October, 2016 ; revised on 20 October, 2016)