Chapter 2
The Effects of *rol* Genes of *Agrobacterium rhizogenes* on Morphogenesis and Secondary Metabolite Accumulation in Medicinal Plants

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Abstract Induction of hairy roots by *Agrobacterium rhizogenes* and regeneration of Ri-transformed plants from such transgenic roots are reported in a large number of taxonomically diverse plant species. Ri-transformed cultures (roots/calli/plants) have altered characteristics of their own compared to non-transformed ones. Four *rol* genes (*rolA*, *rolB*, *rolC*, *rolD*) of T-DNA of Ri-plasmid are known to be responsible for these phenomena. However, few attempts have been made to elucidate the role of individual *rol* genes on morphogenic ability. In addition, the effect of wild-type *A. rhizogenes* on the production of secondary metabolites is well studied in wide number of plant species. The popularity of this research has never declined through time which explains its immense value and provides a hope for a promising future. Based on such studies, several reviews have been written from time to time, explaining the '*rol* effect' on secondary metabolite accumulation in medicinal plants and to discuss the advances in this field of research. However, investigations dealing with the effect of individual *rol* genes are comparatively less and need further attention. Therefore, in this chapter, we have discussed in detail the effects of each of the four *rol* genes individually or in combination on in vitro morphogenesis and secondary metabolite accumulation in medicinal plants.

Keywords *Agrobacterium rhizogenes* · Medicinal plants · Morphogenesis · *rol* genes · Secondary metabolites

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2.1 Introduction

Plants are known for their ability to regenerate new tissues and organs to whole plants (morphogenesis) from damaged cells for survival due to their high cellular totipotency (de Almeida et al. 2015; Ikeuchi et al. 2016). It is well known that morphogenesis in vitro is affected by a variety of endogenous and exogenous factors with cumulative effects in order to acquire organogenic competence and organ initiation followed by its development (Hicks 1994; Das et al. 1996; Kumar and Reddy 2011; Sarkar and Jha 2017). It is clear from the pioneering work of Skoog and Miller (1957) that the type and concentration of auxin and cytokinin in the culture medium have a great influence on morphogenesis as they determine the developmental fate of regenerating organs: high ratios of auxin to cytokinin generally led to root regeneration, and high ratios of cytokinin to auxin tended to promote shoot regeneration.

Agrobacterium rhizogenes, a soil-borne gram-negative bacterium, is well known to have a unique capability to induce 'hairy root' formation at the site of infection in higher plants. Different strains of *A. rhizogenes* are known to induce such roots from the host plant cells by transferring its T-DNA (transfer DNA) from root-inducing (Ri) plasmid to the host genome (Tepfer 2017). Several studies have revealed that only four open reading frames (ORFs) of the T-DNA are critical for induction, growth and morphology of hairy roots in infected plants. These loci were thus called *rol* (root-inducing locus) oncogenes and named as *rolA* (ORF 10), *rolB* (ORF 11), *rolC* (ORF 12) and *rolD* (ORF 15) (White et al. 1985, Slightom et al. 1986). Such transgenic roots can be excised from the wound site and cultured indefinitely on hormone-free medium. The hairy roots exhibit fast, plagiotropic growth characterized by profuse lateral branching and rapid root tip elongation in growth regulator-free medium in contrast to non-transformed roots (Tepfer and Tempé 1981; Chilton et al. 1982; Tepfer 1984).

While in some plant species the Ri-transformed roots exhibit this typical 'Hairy root syndrome' (Tepfer and Tempé 1981; Chilton et al. 1982; Tepfer 1984), in other plants, the Ri- roots lack the presence of extensive root hairs (Chaudhuri et al. 2005; Roychowdhury et al. 2015a; Halder and Jha 2016). In addition to the variation of the hairy roots between the species, variation in growth and morphology was also noted among the different rhizoclones of a single species (Batra et al. 2004; Chaudhuri et al. 2005; Alpizar et al. 2008; Roychowdhury et al. 2015a; Bandhyopadhyay et al. 2007; Majumdar et al. 2011; Ray et al. 2014; Basu and Jha 2014; Halder and Jha 2016; Basu et al. 2015). The variation in hairy root morphology includes variation in thickness of primary root, lateral density of the roots (i.e. number of laterals per cm), presence of rooty callus, etc. These variations among the transformed rhizoclones were attributed due to the variation in nature, site and number of T-DNA integration into the host genome (Jouanin et al. 1987; Amselem and Tepfer 1992; Batra et al. 2004; Alpizar et al. 2008). Molecular variation among the rhizoclones in terms of T-DNA insertion and effect of T_L-DNA and T_R-DNA on root morphology has been investigated by different groups (Batra et al. 2004; Bandhyopadhyay et al.

Fig. 2.1 Differences in in vitro responses of five different strains of *Agrobacterium rhizogenes* in axenic shoots of *Tylophora indica* after 6 weeks of infection. Infection was done at two different sites, i.e., nodal wound site (NWS) and internodal wound site (INWS). (**a**, **b**) Control NWS (bar = 0.2 cm) and INWS (bar = 0.13 cm) showing no response; (**c**, **d**, **e**) root induction from NWS of explants infected with *A. rhizogenes* strain LBA 9402 (bar = 0.28 cm), A4 (bar = 0.25 cm) and HRI (bar = 0.2 cm); (**f**) wound callus induction in 100% of NWS and INWS of explants infected with *A. rhizogenes* strain 15,834 (bar = 0.4 cm); (**g**) wound sites showing swelling and necrosis in explants infected with *A. rhizogenes* strain R1000 (bar = 0.4 cm)

2007; Alpizar et al. 2008; Taneja et al. 2010; Roychowdhury et al. 2015a). While the morphology of hairy roots has been well characterized in many species, their anatomy has not been fully explored. The anatomy of *A. rhizogenes*-transformed roots is more or less similar to wild-type roots with some notable exceptions (Kim and Soh 1996; Odegaard et al. 1997; Park and Facchini 2000; Peres et al. 2001; Halder and Jha 2016). In addition to the above-mentioned variations, morphogenetic ability of *A. rhizogenes* varies with different strains within a single species (Vanhala et al. 1995; Kim et al. 2008; Ionkova et al. 2009; Thwe et al. 2016) (Fig. 2.1).

The morphogenic capability of the hairy roots is widely reported in a number of plant species. Regeneration of Ri-transformed plants from the hairy root cultures have been reviewed thoroughly from time to time (Christey 1997, 2001; Roychowdhury et al. 2013b). Ri-transformed roots have been observed to show spontaneous as well as induced, direct and/ or indirect, organogenesis and/or somatic embryogenesis forming complete transgenic plants as reviewed earlier in details (Fig. 2.2) (Roychowdhury et al. 2013b). Such plants showed altered phenotypes when compared with wild-type plants, some of which are wrinkled leaf, shortened internodes, decreased apical dominance, altered flower morphology, increase in the number of branches, reduced pollen and seed production and abundant

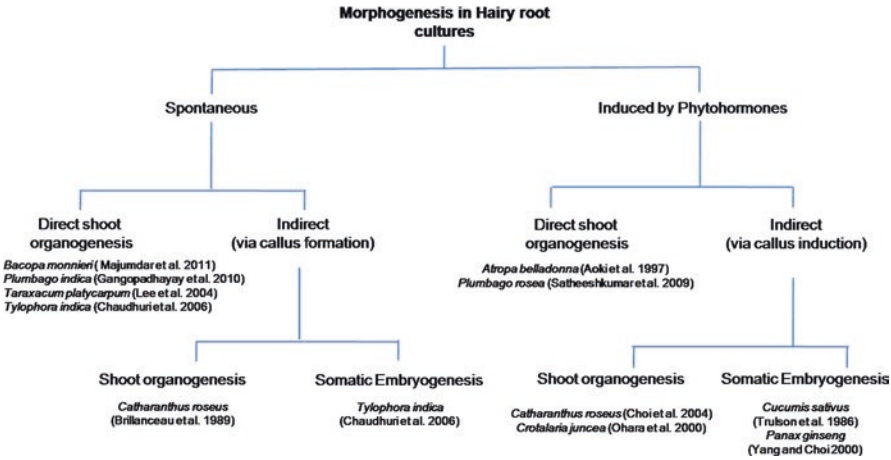

Fig. 2.2 Regeneration in hairy root cultures of different plant species

production of highly branched plagiotropic roots (Tepfer 1984). It has been shown that these abnormal morphological traits or 'hairy root syndrome' is due to the combined actions of *rolA*, *rolB*, *rolC* and *rolD* genes since each of the *rol* genes are associated with specific phenotypic alterations (Nilsson and Olsson 1997). Majority of the hairy roots and Ri-transformed plants are known to be stable in long-term culture, although some instability was also noted (Roychowdhury et al. 2013b, 2015a, b, 2017). Additionally, it has been reported that these Ri-transformed plants were able to transmit the traits to their offspring in a Mendelian manner (Tepfer 1984).

In general, it has been amply demonstrated that the morphogenic response in hairy roots transformed with wild-type *A. rhizogenes* is due to the presence of *rol* genes (Fig. 2.3). However, the role of individual *rol* genes on morphogenesis is not yet well documented. Therefore, in this chapter, we have summarized the effects of each of the four *rol* genes individually or in combination on in vitro morphogenesis of medicinal plants.

2.2 Effect of *rolA* Gene on Morphogenesis

Different explants infected with *Agrobacterium tumefaciens* strains harbouring *rolA* gene showed induction of shoot buds from the wound sites on hormone-supplemented media, either directly (Zhu et al. 2001a; Zia et al. 2010; Amanullah et al. 2016; Bettini et al. 2016b) or indirectly through callus formation (van Altvorst et al. 1992; Holefors et al. 1998). In all the reports, such hormone-supplemented media could trigger shoot bud formation in non-transformed explants as well. The developing shoots from the shoot buds were rooted in hormone-free (Bettini et al. 2016b) or

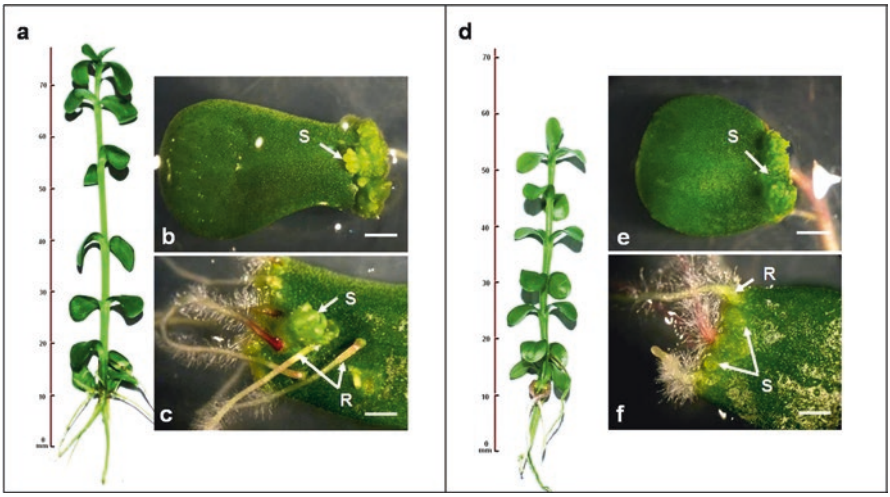

Fig. 2.3 Morphogenic potential of leaf explants excised from Ri-transformed plants of *Bacopa monnieri*. (**a**) Ri-transformed plant obtained following transformation with *A. rhizogenes* strain 9402, (**b**, **c**) morphogenesis in excised leaf explants excised from Ar 9402 Ri-transformed plants harbouring *rol* genes of TL-T-DNA on basal medium (R-root organogenesis, S-shoot organogenesis) (bar = 1.5 mm and 1.0 mm). (**d**) Ri-transformed plant obtained following transformation with *A. rhizogenes* strain A4 (**e**, **f**) morphogenesis in excised leaf explants excised from Ar A4-Ri-transformed plants harbouring *rol* genes of TL-T-DNA on basal medium (R root organogenesis, S shoot organogenesis) (bar = 1.0 mm and 0.9 mm)

hormone-supplemented media (van Altvorst et al. 1992; Holefors et al. 1998; Zhu et al. 2001a; Zia et al. 2010; Amanullah et al. 2016) to generate complete transgenic plants.

Alteration of phenotype of *rolA*-transgenic plants such as wrinkled leaves, lower percentage of rooting, early flowering, reduced flower bud length, hyperstyly, small fruits often lacking seeds and decreased pollen viability have been reported (Schmülling et al. 1988; Sinkar et al. 1988; van Altvorst et al. 1992; Carneiro and Vilaine 1993; Dehio et al. 1993; Holefors et al. 1998; Zhu et al. 2001a; Zia et al. 2010; Amanullah et al. 2016; Bettini et al. 2016b). However, these phenotypic alterations were found to be dependent on the level of transgene expression. When expressed under its own promoter, this gene caused dwarfism, severe wrinkling of leaves, shortened internodes, small leaves and condensed inflorescence (Schmülling et al. 1988; Sinkar et al. 1988). These phenotypes were even more exaggerated in 35S::*rolA*-transformed plants which showed stunted growth with small, dark-green, severely wrinkled leaves and were late-flowering, with a reduced number of flowers (Dehio et al. 1993). On the other hand, soybean *rolA* transformants showed enhanced rooting in presence of auxin and early flowering as compared to the control plants (Zia et al. 2010), while lower rooting percentage in *rolA*-transformed apple rootstock A2 in auxin-supplemented medium and delayed flowering in *rolA*-transformed *Artemisia dubia* plants are reported (Zhu et al. 2001a; Amanullah et al. 2016).

Therefore, the effect of *rolA* gene was also found to be species specific. However, phenotypic variations were also observed among different *rolA*-transformed clones regenerated from the same plant species. Several authors have suggested that this may be due to the copy number of transgene (Bettini et al. 2016b) or the position effect where transgene is integrated into the host genome (Holefors et al. 1998).

2.3 Effect of *rolB* Gene on Morphogenesis

White et al. (1985) first identified that *rolB* gene plays a critical role in the formation of adventitious roots from different explants after infection. Later on, several authors agreed and showed that plant vector constructions carrying only *rolB* gene were capable, to different extents, of triggering root differentiation on different plant tissues (Cardarelli et al. 1987; Spena et al. 1987; Altamura et al. 1994; Schmülling et al. 1988). However, the frequency of root formation differed when *rolB* gene was allowed to express under different promoters (Spena et al. 1987). More intense and earlier root formation was observed with pPCV002-B1100 (where *rolB* is under the control of its own 5′ flanking sequences) than with pPCV002-B300 (containing only 300 bp of *rolB* 5′ flanking sequences). It is interesting to note that the CaMVBT chimeric gene (where *rolB* is under the control of the cauliflower mosaic virus 35S promoter) showed a weaker response than pPCV002-B1100 probably due to overexpression of *rolB* transcript which is not conducive to root induction. On the contrary, this gene was found incapable of inducing roots on carrot discs when inoculated alone (Cardarelli et al. 1987). But hairy root symptoms almost comparable to those induced by wild-type *A. rhizogenes* were elicited when the discs were inoculated with *rolB* gene with 1200-bp-long 5′ upstream region along with the T_R-DNA (Capone et al. 1989). The roots induced by *rolB* were fast growing, highly branched and plagiotropic (Capone et al. 1989). For several years, *rolB* has then been regarded as a 'root-inducing gene' which is capable of turning on a specific morphogenetic programme in higher plants. Apart from direct root formation, indirect root formation was also reported in tobacco protoplast (Spena et al. 1987) and leaf explants of *Rubia cordifolia* (Bulgakov et al. 2002). Unlike the control calli which did not produce roots, pPCV002-B300-transformed calli showed spontaneous root formation on media containing either low or high auxin concentrations (Spena et al. 1987; Bulgakov et al. 2002).

The morphology of *rolB*-transformed roots was very similar to hairy roots transformed with wild-type *A. rhizogenes*. Compared to non-transformed roots, *rolB*-induced roots were fast growing, highly branched and plagiotropic as a result of increased sensitivity of auxin in plant cells transformed by this oncogene (Spena et al. 1987; Schmülling et al. 1988; Capone et al. 1989).

Shoot organogenesis and establishment of *rolB*-transformed plants from infected explants are reported on hormone-supplemented media, either directly (Koltunow et al. 2001; Carmi et al. 2003; Zhu et al. 2003; Zia et al. 2010; Arshad et al. 2014; Dilshad et al. 2015a; Bettini et al. 2016a, Kodahl et al. 2016) or indirectly via callus

induction (van Altvorst et al. 1992; Welander et al. 1998; Sedira et al. 2001; Zhu et al. 2001b). In all the reports, such hormone-supplemented media could induce shoot bud formation in non-transformed explants as well. However, when compared with the non-transformed plants, the *rolB*-transgenic plants showed numerous altered phenotypes such as profuse rooting with altered root morphology; reduced stem length, node number and apical dominance; shortened internodes; smaller, wider leaves with altered shape; increased trichome density; early necrosis of rosette leaves; altered floral morphology; more inflorescence; early flowering; high flower production; infertile flowers; decreased pollen viability; abnormal ovary and ovule development; early maturing of fruits; parthenocarpic fruits; and small size and less number of fruits (Schmülling et al. 1988; van Altvorst et al. 1992; Welander et al. 1998; Sedira et al. 2001; Koltunow et al. 2001; Zhu et al. 2001b; Carmi et al. 2003; Zhu et al. 2003; Zia et al. 2010; Arshad et al. 2014; Dilshad et al. 2015a; Bettini et al. 2016a; Kodahl et al. 2016).

rolB-transformed shoots showed enhanced rooting percentage and number of roots per shoot in absence of auxin suggesting that the endogenous auxin level in *rolB* transformants is sufficient for rooting (Welander et al. 1998; Sedira et al. 2001; Zhu et al. 2001a, 2003). In presence of auxin in the medium, transformed shoots produced profuse callus at the base of the stem and reduced both rooting percentage and number of roots significantly which was probably due to increased auxin sensitivity (Welander et al. 1998; Sedira et al. 2001). The morphology of the roots was even altered where the roots became shorter and thicker in auxin-supplemented medium (Sedira et al. 2001). However, multiple copies of *rolB* gene insertion into the plant genome also imposed a negative impact on rooting (Sedira et al. 2001; Zhu et al. 2003). Zhu et al. (2001b) suggested that this reduction was more associated with the position of the transgene on the plant genome.

rolB gene has been shown to significantly affect the phenotype of transformed calli as compared to non-transformed calli in different species. The non-transformed or empty vector transformed callus culture of *Maackia amurensis* was friable, aqueous and vigorously growing with light yellow or brown colour (Grishchenko et al. 2016). But the *rolB*-transformed calli displayed morphological variation and could be correlated with the level of *rolB* expression. The calli with low level of transcription were friable, globular and yellow-whitish to light-brown colour. In contrast, compact, non-watery, yellow-brown to brown callus with active growth was obtained in high level of *rolB* gene-expressing callus lines (Grishchenko et al. 2016). Similarly, inoculation of *Vitis amurensis* callus culture with *rolB* gene also produced *rolB*-transformed calli lines of friable and compact type (Kiselev et al. 2007). The colour of leaf-derived-*rolB*-transformed calli of *R. cordifolia* was reported to depend on the level of *rolB* gene expression – yellow in low-expressing callus, orange in moderately expressing callus and orange-red in highly expressing callus due to maximum accumulation of anthraquinones (Bulgakov et al. 2002; Shkryl et al. 2007). Some of the *R. cordifolia* calli transformed with *rolB* gene spontaneously formed small roots (Bulgakov et al. 2002).

The level of *rolB* gene expression was also found to affect greatly the growth of transformed callus (Kiselev et al. 2007; Shkryl et al. 2007). Compared to the

non-transformed callus culture which grew vigorously, the growth of *rolB*-transformed calli was reported to depend on the level of its expression. *rolB* gene when expressed at a low level supported the growth of callus (fast growing), but excessive expression of *rolB* gene inhibited callus growth and was associated with necrosis in callus tissues (Kiselev et al. 2007; Shkryl et al. 2007). This negative effect of *rolB* gene on the growth was found to be completely abolished when *rolB*-transformed callus was treated with a tyrosine phosphatase inhibitor (Kiselev et al. 2007; Shkryl et al. 2007). This result indicated that the growth of *rolB*-transformed cells is mediated by tyrosine dephosphorylation.

2.4 Effect of *rolC* Gene on Morphogenesis

The *rolC* gene was able to induce root formation directly from leaf explants in tobacco (Spena et al. 1987; Schmülling et al. 1988; Palazón et al. 1998) under 35S CaMV promoter and in *Atropa belladonna* under its own promoter (Bonhomme et al. 2000). However, in *Kalanchoe* leaves, this gene could not stimulate root formation when driven by its own or 35S CaMV promoter but induced roots when expressed along with *rolB* gene (Spena et al. 1987; Schmülling et al. 1988). On the other hand, hairy root induction has been reported from *rolC*-transgenic calli in absence or presence of auxin when expressed under strong CMV35S promoter in *Panax ginseng* and *R. cordifolia* (Bulgakov et al. 1998, 2002).

Apart from root induction, *rolC* gene also affected the growth and morphology of transgenic roots that were induced directly or indirectly from the explants (White et al. 1985; Schmülling et al. 1988). The morphology of *rolC*-transgenic roots also varied depending on the plant species as well as type of media used (Bulgakov et al. 1998, 2005; Palazón et al. 1998; Bonhomme et al. 2000). By analysing insertional mutants of *rolC* locus in the A4 Ri-plasmid, White et al. (1985) reported that the growth of roots induced from *Kalanchoe* leaves was attenuated. When combined with *rolB* gene, *rolBC* transgenic roots grew straight from these explants (Schmülling et al. 1988). In hormone-free medium, *rolC*-induced transgenic roots of *A. belladonna* and tobacco showed fast and plagiotropic growth and were highly branched (Schmülling et al. 1988; Palazón et al. 1998; Bonhomme et al. 2000). In contrast, Bulgakov et al. (1998) reported that *P. ginseng* transgenic roots derived from *rolC*-transformed calli were slow growing with reduced lateral branching in absence of hormone. These transgenic roots grew better in the medium supplemented with auxins (Bulgakov et al. 1998, 2005).

In *P. ginseng*, non-transformed callus obtained from the stem did not show any morphogenesis even after long-term culture in different combinations of hormone-supplemented media (Gorpenchenko et al. 2006). However, introduction of *rolC* gene into this callus resulted in morphological differentiation to form shoot buds in absence of hormones. The majority of the shoots regenerated displayed fasciated shoot apical meristems and fused leaf primordia.

Formation of *rolC*-transformed adventitious shoot buds has been reported from different explants on hormone-supplemented media either directly (Fladung 1990; Kurioka et al. 1992; Oono et al. 1993; Bell et al. 1999; Kaneyoshi and Kobayashi 1999; Zuker et al. 2001; Koshita et al. 2002; Kubo et al. 2006; Bettini et al. 2010; Zia et al. 2010; Dilshad et al. 2015a; Ismail et al. 2016) or indirectly via callus induction (Palazón et al. 1998; Zhang et al. 2006). *rolC* gene is known to cause substantial morphological and biochemical alterations in transgenic plants which were related to the degree of its expression (Schmülling et al. 1988; Kurioka et al. 1992; Kaneyoshi and Kobayashi 1999). Transgenic plants expressing *rolC* gene from its endogenous promoter had reduced apical dominance, plant height, internodal distance, node number and leaf area, enhanced branching, altered leaf morphology, small flowers, small fruits, more number of fruits and reduced seed production compared to wild-type plants (Schmülling et al. 1988; Bell et al. 1999; Kaneyoshi and Kobayashi 1999; Bettini et al. 2010; Kubo et al. 2006; Landi et al. 2009). However, *rolC*-transformed *A. belladonna* plants when expressed under native promoter did not exhibit any morphological alteration and resembled with wild-type plants (Kurioka et al. 1992).

When *rolC* is expressed under strong 35S CaMV promoter, these characteristics were exaggerated, with drastically reduced apical dominance and internodal length; highly dwarf, very small leaves with altered shape; more lateral branching; higher rooting capacity; increased axillary budbreak; dramatic promotion of flowering; reduced inflorescence; smaller flowers; and male sterile flowers (Schmülling et al. 1988; Fladung 1990; Kurioka et al. 1992; Oono et al. 1993; Palazón et al. 1998; Kaneyoshi and Kobayashi 1999; Zuker et al. 2001; Koshita et al. 2002; Dilshad et al. 2015a; Ismail et al. 2016). In *A. belladonna*, although majority of *rolC*-transformed plants showed typical altered phenotypes, only two unusual regenerants showed unexpected morphology of leaves where leaf periphery was severely wrinkled and darker than central region (Oono et al. 1993). Under the control of 70S CaMV promoter, *rolC*-transformed soybean plants were dwarf and altered leaf morphology, early flowering and lower number of flowers (Zia et al. 2010). Phenotypic alteration such as shortened internodes and increased branching suggested that the expression of *rolC* gene might be linked to an increase in cytokinin activity (Schmülling et al. 1988).

It has been hypothesized that the extreme dwarf phenotype and early flowering in *rolC*-transformed plants were due to the reduction in gibberellic acid. Bettini et al. (2010) reported that higher ratio of abscisic acid to indole-3-acetic acid (ABA/IAA) may be responsible for the stunted aspect of these plants. Furthermore, *rolC* leads to better rooting ability in transformed fruit trees (Kaneyoshi and Kobayashi 1999; Koshita et al. 2002), soybean (Zia et al. 2010) and carnation plants (Zuker et al. 2001) which indicates that the expression of this gene could exert auxin-like activity.

When callus culture of *P. ginseng* was transformed with *rolC* gene under strong promoter (35S CaMV), induction of somatic embryogenesis was observed (Gorpenchenko et al. 2006). Non-transformed calli of *P. ginseng* did not show any morphogenesis; however, introduction of *rolC* gene into the non-transformed calli

resulted in morphological differentiation to form proembryos and somatic embryos even in absence of hormone which indicated that *rolC* gene is also able to induce somatic embryogenesis (Gorpenchenko et al. 2006). But the proembryos and somatic embryos that were formed had enlarged and fasciated meristems and terminated at different stages of their development to form secondary adventitious meristems. According to them, overexpression or ectopic expression of WUSCHEL (WUS) gene and reduced CLAVATA (CLV) activities caused similar such developmental abnormalities. Whether *rolC* gene affects embryogenesis in *P. ginseng* callus through WUS/CLV signalling pathway is not clearly understood.

Establishment of *rolC*-transformed callus cultures has been reported in some species using *A. tumefaciens* harbouring 35S *rolC* gene in hormone-supplemented media (Bulgakov et al. 1998, 2002; Grishchenko et al. 2013). It was found that integration of *rolC* gene resulted in significant alteration of phenotype, growth and biomass accumulation when compared with non-transformed callus culture. The empty vector transformed callus of *M. amurensis* and *R. cordifolia* was friable and watery, while *rolC*-transformed callus was compact and non-watery (Bulgakov et al. 2002; Grishchenko et al. 2013). But in *P. ginseng*, the same *rolC* construct produced friable and almost watery-type callus in auxin-containing medium (Bulgakov et al. 1998). *rolC* gene also affects the growth and biomass accumulation of transformed callus cultures depending upon the plant species used. Both empty vector and *rolC*-transformed callus cultures of *M. amurensis* demonstrated active growth, but the latter accumulated twice the amount of dry biomass compared to the former (Grishchenko et al. 2013). The growth of *rolC*-transformed *P. ginseng* callus was also rapid in auxin-containing medium (Bulgakov et al. 1998). However, Bulgakov et al. (2002) reported that the growth of one of the *rolC*-transformed *R. cordifolia* callus lines was reduced (almost twofold) compared to the control culture.

2.5 Effect of *rolD* Gene on Morphogenesis

To date, unlike other *rol* genes, *rolD* gene has not been thoroughly investigated although it has been identified as a root locus (White et al. 1985). The mutants of *rolD* gene in *Kalanchoe* leaves produced roots of attenuated growth along with increased amount of callus (White et al. 1985). However, when *rolD* gene was introduced in tobacco stems under the control of long version of promoter, root formation was achieved on hormone-free medium (Mauro et al. 1996). But no difference was observed in adventitious root production, root morphology and its growth pattern between *rolD* and mock-infected plants.

rol-transformed plants (*rolD* gene under the control of 578 bp of its 5′ upstream non-coding region) have been established in tobacco (Mauro et al. 1996), tomato (Bettini et al. 2003) and *Arabidopsis* (Falasca et al. 2010). In tobacco, control as well as *rolD*-transformed leaf explants was able to show shoot induction in hormone-free MS medium (Mauro et al. 1996). In tomato, *rolD*-transformed plants were established in hormone-supplemented medium from cotyledons after infection;

however, non-transformed plants were also obtained when explants were cultured on non-selective medium (Bettini et al. 2003). Falasca et al. (2010) established seed derived non-transformed and *rolD*-transformed *Arabidopsis* plants in hormone-free medium.

In transgenic plants, the *rolD* gene does not seem to induce significant morphological modifications during vegetative growth except early bolting of the stem, smaller leaves with characteristically curved pointed tips in tobacco (Mauro et al. 1996), increased branching in tomato (Bettini et al. 2003) and an increased production of axillary buds and adventitious root meristems along with frequent occurrence of wrinkled leaves in *Arabidopsis* (Falasca et al. 2010). The most conspicuous alteration of *rolD* transgenic plants was precocity in floral transition leading to early flowering and increased number of inflorescences (Mauro et al. 1996; Bettini et al. 2003; Falasca et al. 2010). Since *rolD* gene product is assumed to catalyse the conversion of ornithine to proline, effect of *rolD* on flowering may be therefore due to the accumulation of proline or depletion of ornithine (Trovato et al. 2001). However, Falasca et al. (2010) suggested that proliferation of axillary meristems in *rolD* plants could be due to modification in cytokinin/auxin ratio in *rolD*-transformed plants.

2.6 Effect of *rolABC* Gene on Morphogenesis

When explants were transformed with *A. tumefaciens* harbouring *rolABC* together (expressed under its own promoter), root induction occurred in absence of exogenous hormone directly (Spena et al. 1987; Palazón et al. 1998; Bonhomme et al. 2000) or indirectly through callus induction (Spena et al. 1987; Rugini et al. 1991). Nearly half of *rolABC*-transformed calli obtained from tobacco leaf protoplasts were found to develop roots in the absence of exogenous auxin, whereas no root induction occurred in calli transformed with the binary vector (pPCV002) (Spena et al. 1987). The same construct when used to transform leaf explants of tobacco and *Kalanchoe* was also able to induce roots in hormone-free medium (Spena et al. 1987; Palazón et al. 1998; Bonhomme et al. 2000). However, in *Kalanchoe*, co-inoculation with *A. tumefaciens* strain pGV3297 harbouring a Ti-plasmid with auxin-producing genes was needed for root formation although *A. tumefaciens* strain pGV3297 itself did not form roots (Spena et al. 1987). In kiwi, the emergence of some roots from leaf-derived *rolABC*-transformed callus was noticed when cultured in callus induction medium containing auxin (Rugini et al. 1991). On the other hand, high concentration of auxin in this medium prohibited root formation from control calli which indicates that root morphogenesis was directed by *rol* genes in transformed cells.

Compared to non-transformed roots, *rolABC*-transformed roots could grow well in hormone-unsupplemented medium and showed the typical hairy root phenotype as observed in roots transformed with wild-type *A. rhizogenes* (Palazón et al. 1998; Bonhomme et al. 2000). Non-transformed roots obtained from in vitro grown plants grew slowly with no lateral branching when cultured on MS basal medium (Palazón

et al. 1998). In contrast, transformed roots expressing *rolABC* together grew more vigorously, were highly branched with a plagiotropic growth and were thick (more than 3 mm diameter) in the medium without phytohormone (Palazón et al. 1998; Bonhomme et al. 2000). Schmülling et al. (1988) reported that *rol ABC*-transformed tobacco roots grew better in hormone-free medium than non-transformed roots. The growth rate of transformed root lines was also significantly higher than non-transformed roots (Palazón et al. 1998; Bonhomme et al. 2000).

The morphology of *rolABC*-transformed plants was more or less similar to that of Ri-transformed plants and showed typical hairy root syndrome. Tobacco plants transgenic for *rolABC* exhibited high growth rate of plagiotropic roots, reduced apical dominance in roots and stems, wrinkled and epinastic leaves with altered morphology, shorter internodal length, small flowers and reduced seed production (Schmülling et al. 1988; Palazón et al. 1998). van Altvorst et al. (1992) reported that *rolABC*-transformed tomato plants showed similar morphology with control plants with respect to leaf shape, leaf wrinkling, apical dominance and pollen production. However, these transformed plants produced small, thin roots, low pollen viability and reduced flower bud length compared to control plants (van Altvorst et al. 1992). Rugini et al. (1991) reported that in vitro grown *rolABC*-transformed kiwi plants had shorter internodes, dark-green wrinkled leaves and high rooting ability. In general, three T-DNA genetic loci indicated as *rolA*, *rolB and rolC* act synergistically in the induction and morphology of hairy roots as well as hairy root phenotype of regenerated plants.

2.7 The '*rol* Effect' on Secondary Metabolites in Plants

The application of plant tissue culture and plant genetic transformation for successful production of highly valuable secondary metabolites is not a new trend and can be traced back to the early works of Flores and Filner (1985), Kamada et al. (1986), Mano et al. (1989) and Robins et al. (1991). It has been found that hairy roots, Ri-transformed plants and Ri-transformed callus cultures showed activation of secondary metabolites in more than a hundred, taxonomically diverse medicinal plant species (Ray et al. 1996, 2014, Ray and Jha 1999; Christey 1997, 2001; Chaudhuri et al. 2005, 2006; Bulgakov 2008; Bulgakov et al. 2005, 2011; Majumdar et al. 2011; Roychowdhury et al. 2013a, b, 2015a, b; Basu and Jha 2014; Basu et al. 2015; Paul et al. 2015; Halder and Jha 2016). In some cases decreased content of some of the target metabolites has also been observed (Bulgakov et al. 2005). These changes could be attributed to the variation in the pattern of T-DNA integration within the genome of the host plant which caused the differential expression of key regulators of biosynthetic pathways (Jouanin et al. 1987; Amselem and Tepfer 1992; Bulgakov 2008). The preference for 'Ri-transformed plant or hairy roots or calli' system for production of secondary metabolites was due to their high growth rates acting as a factory for continued production of high amounts of important compounds and the

stability in metabolite accumulation in long-term cultures (Häkkinen et al. 2016, Roychowdhury et al. 2017). Though there are various other factors affecting the accumulation of secondary metabolites, which include media composition and pH, effect of hormones, bacterial strain used for inoculation, temperature, light and effect of elicitors (Chaudhuri et al. 2009; Majumdar et al. 2011; Simic et al. 2014; Khalili et al. 2015; Paul et al. 2015; Sivanandhan et al. 2016; Basu et al. 2017); in this review, we intended to confine our attention to the influence of *rol* genes on the increase or decrease of secondary metabolites produced in medicinal plants. The effect of wild-type *A. rhizogenes* on the production of secondary metabolites is well studied in plants. Based on such studies, several reviews have been written from time to time, explaining the '*rol* effect' on secondary metabolite accumulation in medicinal plants and to discuss the advances in this field of research (Hamill et al. 1987; Tepfer 1990; Toivonen 1993; Constantino et al. 1994; Bourgaud et al. 2001; Rao and Ravishankar 2002; Verpoorte et al. 2002; Guillon et al. 2006; Srivastava and Srivastava 2007; Bulgakov 2008; Bulgakov et al. 2013; Karuppusamy 2009; Pistelli et al. 2010; Chandra 2012; Sharma et al. 2013; Roychowdhury et al. 2013a, 2017; Matveeva et al. 2015; Parr 2017; Mitra et al. 2017). The popularity of this research has never declined through time which explains its immense value and provides a hope for a promising future.

However, the knowledge regarding the respective roles of *rolA*, *rolB*, *rolC* and *rolD* genes or when integrated in combinations is still insufficient. Very few attempts have been reported, some of the pioneers being Palazón et al. (1997) and Bulgakov et al. (1998). Years later, for the first time, Bulgakov (2008) took an initiative to review how the individual *rol* genes impacted secondary metabolism. In the following section of this chapter, the effects of individual and combined *rol* genes on secondary metabolites of medicinal plants have been discussed in details based on updated list of reports. During the compilation it was observed that *rolC* gene has been the most popular choice for this study followed by *rolB* and then *rolA* (Fig. 2.4). Surprisingly, the role of *rolD* in the accumulation of secondary metabolites has remained unexplored.

2.8 Effect of *rolA* Gene on Plant Secondary Metabolite Accumulation

There are very few reports on the effect of *rolA* gene on secondary metabolite production (Palazón et al. 1997; Shkryl et al. 2007; Amanullah et al. 2016). The earliest report where *rolA* was seen to stimulate nicotine production in transformed root lines of *Nicotiana tabacum* was that of Palazón et al. (1997). Later, Shkryl et al. (2007) transformed *R. cordifolia* plant with *A. tumefaciens* (strain GV3101) harbouring *rolA* construct pPCV002-A controlled by its own native promoter. In this study, the effect of *rolA* gene on anthraquinone accumulation was investigated. There was a 2.8-fold increase in the content of anthraquinones in the

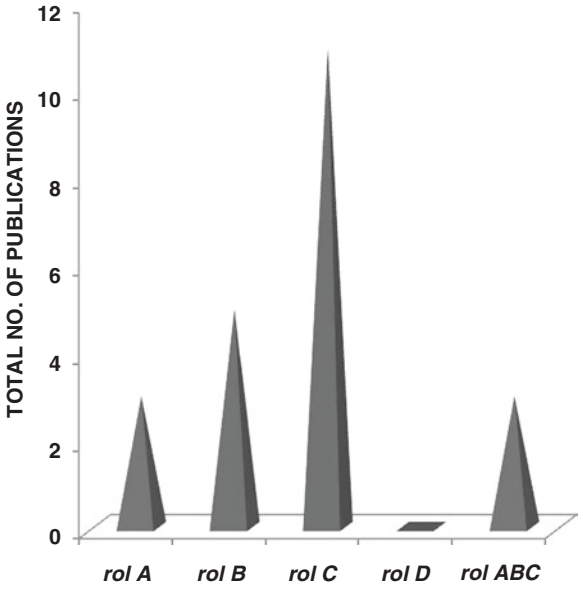

Fig. 2.4 Graphical representation of relative number of publications available on the effect of individual *rol* genes on secondary metabolism in medicinal plants

rolA-transformed calli compared to wild-type non-transformed ones. In *rolA*-transformed cultures of *A. dubia* plants, artemisinin and its derived compounds were found to be comparable to that of the non-transformed plant (Amanullah et al. 2016). Therefore, on one hand, *rolA* showed enhancement of secondary metabolites in transformed *N. tabacum* plants and *R. cordifolia* calli (Palazón et al. 1997; Shkryl et al. 2007) and, on the other hand, maintained the levels of secondary metabolite production comparable to non-transformed *A. dubia* plants.

2.9 Effect of *rolB* Gene on Plant Secondary Metabolite Accumulation

rolB gene has been a master regulator in secondary metabolite accumulation in majority of the studies (Shkryl et al. 2007; Kiselev et al. 2007; Arshad et al. 2014; Dilshad et al. 2016; Grishchenko et al. 2016). Presence of *rolB* gene has been co-related to the enhanced secondary metabolite content in transformed plants with respect to the non-transformed plants and plants transformed with *rolA*, *rolC* and *rolABC* genes (Shkryl et al. 2007).

Transformed callus culture of *R. cordifolia* showed a 15-fold increase in anthraquinone levels when compared to the non-transformed callus culture (Shkryl et al. 2007). In this study, stimulation of isochorismate synthase gene (ICS) was positively correlated with the enhancement in anthraquinone content since ICS is a key gene involved in biosynthesis of anthraquinones. Transformed callus lines express-

ing low levels of *rolB* produced twofold higher anthraquinones, whereas transformants expressing medium and higher levels of *rolB* gene produced 2.8-fold and 4.3-fold anthraquinones, respectively. The effect of *rolB* on production of another target metabolite, resveratrol, was studied by Kiselev et al. (2007) in transformed calli lines of *V. amurensis* Rupr. Compared to non-transformed callus, a striking 100-fold increase was seen in the *rolB*-transformed calli. It was further shown that tyrosine phosphatase inhibitors played antagonistic role with the stimulatory effects of *rolB* gene, suggesting the involvement of tyrosine phosphorylation in plant secondary metabolism. Grishchenko et al. (2016) established *rolB*-transformed callus cultures of *M. amurensis* Rupr., and the yield of isoflavonoids was studied in the transformed calli. Isoflavonoid accumulation in *rolB*-transformed calli ranged from 1.4 to 2.1% DW (dry weight) compared to 1.22% DW in empty vector control.

A. carvifolia Buch. plants transformed by *rolB* gene showed an increase in flavonoid levels (Dilshad et al. 2016). Caffeic acid, quercetin, isoquercetin, rutin, catechin, apigenin, gallic acid and kaempferol were some of the flavonoids which were compared among the transformed and non-transformed plants. Of these, apigenin and catechin were absent in wild-type plants but present (75 mg/g DW) in the transformed shoots. The transgenics showed an increased content of quercetin (sixfold), rutin (2.4-fold) and isoquercetin (1.9-fold) in the transformed plants. *Solanum lycopersicum* L. transformed with *A. tumefaciens* harbouring *rolB* gene of *A. rhizogenes* (Arshad et al. 2014) showed up to 62% increase in the lycopene content in *rolB*-expressing tomato fruit lines compared to the control non-transformed fruits.

Hence, *rolB* gene can be considered having a positive effect on enhanced secondary metabolite production in medicinal plants because transformation with *rolB* gene has resulted into higher metabolite content in most cases (Kiselev et al. 2007; Shkryl et al. 2007; Dilshad et al. 2016).

2.10 Effect of *rolC* Gene on Plant Secondary Metabolite Accumulation

Several reports are available which explained the role of *rolC* as a modulator of secondary metabolite production among diverse group of medicinal plants (Bulgakov et al. 1998, 2005; Palazón et al. 1998; Shkryl et al. 2007; Dubrovina et al. 2010; Grishchenko et al. 2013; Vereschagina et al. 2014; Dilshad et al. 2015a, b, 2016; Ismail et al. 2016).

Palazón et al. (1998) examined levels of nicotine production in *rolC*-transformed plants of *N. tabacum*. In comparison with the non-transformed control, the roots of *rolC*-transformed plants accumulated twice the amount of nicotine, and the transformed leaves showed a threefold increase. Similarly, transformation of *P. ginseng* with *rolC* oncogene resulted into production of threefold higher levels of ginsenoside (Bulgakov et al. 1998). *rolC*-transformed callus cultures of

R. cordifolia showed a 4.3-fold higher levels of anthraquinone compared to the control calli (Shkryl et al. 2007). Dubrovina et al. (2010) in their study with *V. amurensis* showed that *rolC*-transformed callus lines produced 3.7- to 11.9-fold increase in resveratrol content compared to non-transformed calli. A stable two- to fourfold increase (stable over a period of 2 years) in the polyphenol levels was recorded in *rolC*-transformed calli of *Cynara cardunculus* var. *altilis* (Vereshchagina et al. 2014).

Artemisinin content in *rolC*-transgenic plant of *A. annua* showed a 4- to 4.6-fold increment (Dilshad et al. 2015b). In the same study, artesunate and dihydroartemisin also increased up to 9.1-fold and 2-fold, respectively. A similar investigation was conducted with another species of *Artemisia*, *A. carvifolia* (Dilshad et al. 2015a), where the artemisinin content recorded in transgenic plants was up to six-fold higher than determined in non-transformed plants. The increase in contents of artesunate, dihydroartemisinin and artemether was measured to be up to 8.9-, 3.2- and 5-fold, respectively. Dilshad et al. (2016) reported twofold increase of caffeic acid in *rolC*-transformed plants of *A. carvifolia* compared to non-transformed controls. In addition, such *rolC*-transformed plants showed increased levels of quercetin (fourfold), isoquercetin (1.6-fold) and rutin (1.6-fold) compared to control. *rolC*-transformed plants of *Lactuca sativa* showed enhancement of flavonoid content in the range of 7.5–8.2 μg/ml in contrast to 5.1 μg/ml in control (Ismail et al. 2016).

The stimulatory effect of *rolC* on secondary metabolite accumulation was quite evident in all of the above examples; however, it was interesting to note that *rolC* gene has shown a reverse effect on production of certain metabolites in transformed cultures of *Eritrichium sericeum* and *Lithospermum erythrorhizon* (Bulgakov et al. 2005). *rolC*-transformed cultures of *E. sericeum* (root and calli) and *L. erythrorhizon* (calli) showed reduced rhabdosin and rosmarinic acid content than the respective controls. Grischenko et al. (2013) reported *rolC*-transformed callus cultures of *M. amurensis* with slightly higher isoflavonoid productivity compared to control. Interestingly, on one hand, in the *rolC* callus cultures, increased contents of six isoflavonoids were obtained; on the other hand, genistin production decreased compared to control. This effect of *rolC* on isoflavonoid production was stable for 4 years.

Therefore, *rolC* gene showed both stimulatory effect (Bulgakov et al. 1998; Palazón et al. 1998; Shkryl et al. 2007; Dubrovina et al. 2010; Vereshchagina et al. 2014; Dilshad et al. 2015a, b, 2016; Ismail et al. 2016) and inhibitory effect (Bulgakov et al. 2005) on the accumulated levels of target secondary metabolites. *rolC* gene might be considered responsible for differential regulation of different secondary metabolites within the same transformed plant causing increase in level of one compound and reduction in level of others simultaneously (Grishchenko et al. 2013).

2.11 Synergistic Effect of *rol ABC* Gene on Plant Secondary Metabolite Accumulation

The *rol* genes have individually shown to exert a neutral or a stimulatory as well as negative effect on secondary metabolites in various medicinal plants (Bulgakov et al. 2005; Kiselev et al. 2007; Shkryl et al. 2007; Dilshad et al. 2015a,b; Amanullah et al. 2016; Grishchenko et al. 2016). To study the combinatorial effect of *rol* genes (*rolA*, *rolB*, *rolC*), transformed cultures have been reported to be established with *A. tumefaciens* harbouring *rolABC* genes (Palazón et al. 1998; Bonhomme et al. 2000; Shkryl et al. 2007).

Palazón et al. (1998) established *rolABC* transgenic root lines of *N. tabacum* $_{CV}$. Xanthi, where the mean nicotine level showed a drastic enhancement (86 mg) in comparison to that of the non-transformed root lines (0.8 mg). Shkryl et al. (2007) performed an experiment with *rolABC* transformed callus cultures of *R. cordifolia* to monitor the effect on anthraquinone accumulation. Total anthraquinone content in *rolABC* transformed calli was measured to be almost 2 times the anthraquinone levels recorded in non-transformed callus line and 1.4 times the anthraquinone levels in wild-type (A4) transformed calli. Bonhomme et al. (2000) reported similar increase in accumulation of total alkaloid contents in Ri-transformed and *rolABC* transformed root lines of *A. belladonna* compared to non-transformed roots, suggesting *rolABC* genes to be enough for increasing the tropane alkaloid content in this plant.

Hence, the *rolABC* showed a considerably stronger effect on the enhancement of secondary metabolite productivity of transformed plants of *Nicotiana* (Palazón et al. 1998). However, the stimulatory effect of *rolABC* gene on transformed *R. cordifolia* calli (Shkryl et al. 2007) was relatively weaker than on *Nicotiana* as measured in terms of fold increment of their respective target metabolites. Differential regulation of two different compounds within the transformed hairy root lines of the same plant is yet another remarkable aspect of *rolABC* gene effect (Bonhomme et al. 2000).

2.12 Conclusion

Transformation of plants with wild-type *Agrobacterium rhizogenes* has been subject of many studies. However the functions of individual oncogenes of the Ri-plasmid are not well known. Rhizogenic property of *A. rhizogenes* is a well-known phenomenon in higher plants, as well as morphogenesis from such hairy roots. Four *rol* genes (*rolA*, *rolB*, *rolC*, *rolD*) are known to be responsible for such ability for a long time. However, few attempts have been made to elucidate the morphogenic ability of individual *rol* genes. Transformation with individual *rol* genes results in transformed cultures (roots/calli/plants) having altered characteristics, extent of which varies with plant species, choice of promoter and number of

transcripts of the respective *rol* gene. In contrast, direct evidence of rhizogenic ability (direct and indirect) has been found in case of *rolABC* genes together and the morphology of such transformed cultures is comparable to typical Ri-transformed ones.

While numerous reports are available on production of secondary metabolites from Ri-transformed plants or callus or hairy root cultures, investigations dealing with the effect of individual *rol* genes on secondary metabolite accumulation are comparatively less and need further attention. Majority of the reports have highlighted the role of *rolC* on secondary metabolite accumulation in medicinal plants. We observed that phenylpropanoids were the most common group of target secondary metabolites studied for the effects of individual *rol* genes followed by terpenoids, alkaloids, quinones and steroids, respectively. While *rolA* caused a stimulatory effect in the accumulation of secondary metabolites, the *rolB* and *rolC* genes were found to play dynamic roles leading to a differential regulation of the target metabolites even in the same species. It was interesting to note that the *rolABC* genes exerted a greater effect on secondary metabolite synthesis than individual *rol* genes. From the overall study, the lack of reports for effect of individual *rol* genes suggests that there is ample scope of research in this field in spite of being in the business for more than three decades.

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References

Alpizar, E., Dechamp, E., Lapeyre-Montes, F., et al. (2008). *Agrobacterium rhizogenes*-transformed roots of coffee (*Coffea arabica*): Conditions for long-term proliferation, and morphological and molecular characterization. *Annals of Botany, 101*(7), 929–940.

Altamura, M. M., Capitani, F., Gazza, L., et al. (1994). The plant oncogene *rolB* stimulates the formation of flower and root meristemoids in tobacco thin cell layers. *The New Phytologist, 126*(2), 283–293.

Amanullah, B. M., Rizvi, Z. F., & Zia, M. (2016). Production of artemisinin and its derivatives in hairy roots of *Artemisia dubia* induced by *rolA* gene transformation. *Pakistan Journal of Botany, 48*(2), 699–706.

Amselem, J., & Tepfer, M. (1992). Molecular basis of novel root phenotypes induced by *Agrobacterium rhizogenes* A4 on cucumber. *Plant Molecular Biology, 19*(3), 421–432.

Aoki, T., Matsumoto, H., Asako, Y., Matsunaga, Y., et al. (1997). Variation of alkaloid productivity among several clones of hairy roots and regenerated plants of *Atropa belladonna* transformed with *Agrobacterium rhizogenes* 15834. *Plant Cell Reports, 16*, 282–286.

Arshad, W., Haq, I. U., Waheed, M. T., et al. (2014). *Agrobacterium*-mediated transformation of tomato with *rolB* gene results in enhancement of fruit quality and foliar resistance against fungal pathogens. *PLoS One, 9*(5), e96979. https://doi.org/10.1371/journal.pone.0096979.

Bandyopadhyay, M., Jha, S., & Tepfer, D. (2007). Changes in morphological phenotypes and withanolide composition of Ri-transformed roots of *Withania somnifera*. *Plant Cell Reports, 26*(5), 599–609.

Basu, A., & Jha, S. (2014). Genetic transformation of *Digitalis purpurea* L. by *Agrobacterium rhizogenes*. *Journal of the Botanical Society of Bengal, 68*, 89–93.

Basu, A., Joshi, R. K., & Jha, S. (2015). Genetic transformation of *Plumbago zeylanica* with *Agrobacterium rhizogenes* strain LBA 9402 and characterization of transformed root lines. *Plant Tissue Culture Biotechnology, 25*, 21–35.

Basu, A., Roychowdhury, D., Joshi, R. K., et al. (2017). Effects of *cryptogein* gene on growth, phenotype and secondary metabolite accumulation in co-transformed roots and plants of *Tylophora indica*. *Acta Physiologiae Plantarum, 39*(1), 3.

Batra, J., Dutta, A., Singh, D., et al. (2004). Growth and terpenoid indole alkaloid production in *Catharanthus roseus* hairy root clones in relation to left- and right-termini linked Ri T-DNA gene integration. *Plant Cell Reports, 23*(3), 148–154.

Bell, R. L., Scorza, R., Srinivasan, C., et al. (1999). Transformation of "Beurre Bosc" pear with the *rolC* gene. *Journal of the American Society for Horticultural Science, 124*(6), 570–574.

Bettini, P., Michelotti, S., Bindi, D., et al. (2003). Pleiotropic effect of the insertion of the *Agrobacterium rhizogenes rolD* gene in tomato (*Lycopersicum esculentum* Mill.). *Theoretical and Applied Genetics, 107*(5), 831–836.

Bettini, P., Baraldi, R., Rapparini, F., et al. (2010). The insertion of the *Agrobacterium rhizogenes rolC* gene in tomato (*Solanum lycopersicum* L.) affects plant architecture and endogenous auxin and abscisic acid levels. *Scientia Horticulturae, 123*(3), 323–328.

Bettini, P. P., Marvasi, M., Fani, F., et al. (2016a). *Agrobacterium rhizogenes rolB* gene affects photosynthesis and chlorophyll content in transgenic tomato (*Solanum lycopersicum* L.) plants. *Journal of Plant Physiology, 204*, 27–35.

Bettini, P. P., Santangelo, E., Baraldi, R., et al. (2016b). *Agrobacterium rhizogenes rolA* gene promotes tolerance to *Fusarium oxysporum* f. sp. *lycopersiciin* transgenic tomato plants (*Solanum lycopersicum* L.). *Journal of Plant Biochemistry and Biotechnology, 25*(3), 225–233.

Bonhomme, V., Laurain-Mattar, D., & Fliniaux, M. A. (2000). Effects of the *rolC* gene on hairy root: Induction development and tropane alkaloid production by *Atropa belladonna*. *Journal of Natural Products, 63*(9), 1249–1252.

Bourgaud, F., Gravot, A., Milesi, S., et al. (2001). Production of plant secondary metabolites: A historical perspective. *Plant Science, 161*(5), 839–851.

Brillanceau, M. H., David, C., & Tempé, J. (1989). Genetic transformation of Catharanthus roseus G. Don by Agrobacterium rhizogenes. *Plant Cell Reports, 8*, 63–66.

Bulgakov, V. P. (2008). Functions of *rol* genes in plant secondary metabolism. *Biotechnology Advances, 26*(4), 318–324.

Bulgakov, V. P., Khodakovskaya, M. V., Labetskaya, N. V., et al. (1998). The impact of plant *rolC* oncogene on ginsenoside production by ginseng hairy root cultures. *Phytochemistry, 49*(7), 1929–1934.

Bulgakov, V. P., Tchernoded, G. K., Mischenko, N. P., et al. (2002). Effect of salicylic acid, methyl jasmonate, ethephon and cantharidin on anthraquinone production by *Rubia cordifolia* callus cultures transformed with the *rolB* and *rolC* genes. *Journal of Biotechnology, 97*(3), 213–221.

Bulgakov, V. P., Veselova, M. V., Tchernoded, G. K., et al. (2005). Inhibitory effect of the *Agrobacterium rhizogenes rolC* gene on rabdosiin and rosmarinic acid production in *Eritrichium sericeum* and *Lithospermum erythrorhizon* transformed cell cultures. *Planta, 221*(4), 471–478.

Bulgakov, V. P., Shkryl, Y. N., Veremeichik, G. N., et al. (2011). Application of *Agrobacterium rol* genes in plant biotechnology: A natural phenomenon of secondary metabolism regulation. In M. Alvarez (Ed.), *Genetic transformation* (pp. 261–271). Rijeka: InTech.

Bulgakov, V. P., Shkryl, Y. N., Veremeichik, G. N., et al. (2013). Recent advances in the understanding of *Agrobacterium rhizogenes*-derived genes and their effects on stress resistance and plant metabolism. In P. Doran (Ed.), *Biotechnology of hairy root systems. Advances in biochemical engineering/biotechnology* (Vol. 134, pp. 1–22). Berlin/Heidelberg: Springer.

Capone, I., Spanò, L., Cardarelli, M., et al. (1989). Induction and growth properties of carrot roots with different complements of *Agrobacterium rhizogenes* T-DNA. *Plant Molecular Biology, 13*(1), 43–52.

Cardarelli, M., Mariotti, D., Pomponi, M., et al. (1987). *Agrobacterium rhizogenes* T-DNA genes capable of inducing hairy root phenotype. *Molecular & General Genetics, 209*(3), 475–480.

Carmi, N., Salts, Y., Dedicova, B., et al. (2003). Induction of parthenocarpy in tomato via specific expression of the *rolB* gene in the ovary. *Planta, 217*(5), 726–735.

Carneiro, M., & Vilaine, F. (1993). Differential expression of the *rolA* plant oncogene and its effect on tobacco development. *The Plant Journal, 3*(6), 785–792.

Chandra, S. (2012). Natural plant genetic engineer *Agrobacterium rhizogenes*: Role of T-DNA in plant secondary metabolism. *Biotechnology Letters, 34*(3), 407–415.

Chaudhuri, K. N., Ghosh, B., Tepfer, D., et al. (2005). Genetic transformation of *Tylophora indica* with *Agrobacterium rhizogenes* A4: Growth and tylophorine productivity in different transformed root clones. *Plant Cell Reports, 24*, 25–35.

Chaudhuri, K. N., Ghosh, B., Tepfer, D., et al. (2006). Spontaneous plant regeneration in transformed roots and calli from *Tylophora indica*: Changes in morphological phenotype and tylophorine accumulation associated with transformation by *Agrobacterium rhizogenes*. *Plant Cell Reports, 25*(10), 1059–1066.

Chaudhuri, K. N., Das, S., Bandyopadhyay, M., et al. (2009). Transgenic mimicry of pathogen attack stimulates growth and secondary metabolite accumulation. *Transgenic Research, 18*(1), 121–134.

Chilton, M. D., Tepfer, D. A., Petit, A., et al. (1982). *Agrobacterium rhizogenes* inserts T-DNA into the genomes of the host-plant root cells. *Nature, 295*, 432–434.

Choi, P. S., Kim, Y. D., Choi, K. M., et al. (2004). Plant regeneration from hairy-root cultures transformed by infection with *Agrobacterium rhizogenes* in *Catharanthus roseus*. *Plant Cell Reports, 22*, 828–831.

Christey, M. C. (1997). Transgenic crop plants using *Agrobacterium rhizogenes* mediated transformation. In P. M. Doran (Ed.), *Hairy roots: Culture and applications* (pp. 99–111). Amsterdam: Harwood Academic Publishers.

Christey, M. C. (2001). Use of Ri-mediated transformation for production of transgenic plants. *In Vitro Cellular & Developmental Biology. Plant, 37*(6), 687–700.

Costantino, P., Capone, I., Cardarelli, M., et al. (1994). Bacterial plant oncogenes: The *rol* genes' saga. *Genetica, 94*(2), 203–211.

Das, S., Jha, T. B., & Jha, S. (1996). Organogenesis and regeneration from pigmented callus in Camellia sinensis (L.) O. Kuntze cv. Nandadevi, an elite Darjeeling tea clone. *Plant Science, 121*, 207–212.

de Almeida, M., Graner, E. M., Brondani, G. E., et al. (2015). Plant morphogenesis: Theorical bases. *Advances in Forestry Science, 2*, 13–22.

Dehio, C., Grossmann, K., Schell, J., et al. (1993). Phenotype and hormonal status of transgenic tobacco plants overexpressing the *rolA* gene of *Agrobacterium rhizogenes* T-DNA. *Plant Molecular Biology, 23*(6), 1199–1210.

Dilshad, E., Cusido, R. M., Estrada, K. R., et al. (2015a). Genetic transformation of *Artemisia carvifolia* Buch with *rol* genes enhances artemisinin accumulation. *PLoS One, 10*, e0140266.

Dilshad, E., Cusido, R. M., Palazon, J., et al. (2015b). Enhanced artemisinin yield by expression of *rol* genes in *Artemisia annua*. *Malaria Journal, 14*(1), 424.

Dilshad, E., Ismail, H., Cusido, R. M., et al. (2016). *Rol* genes enhance the biosynthesis of antioxidants in *Artemisia carvifolia* Buch. *BMC Plant Biology, 16*(1), 125.

Dubrovina, A. S., Manyakhin, A. Y., Zhuravlev, Y. N., et al. (2010). Resveratrol content and expression of phenylalanine ammonia-lyase and stilbene synthase genes in *rolC* transgenic cell cultures of *Vitis amurensis*. *Applied Microbiology and Biotechnology, 88*(3), 727–736.

Falasca, G., Altamura, M. M., D'Angeli, S., et al. (2010). The *rolD* oncogene promotes axillary bud and adventitious root meristems in *Arabidopsis*. *Plant Physiology and Biochemistry, 48*(9), 797–804.

Fladung, M. (1990). Transformation of diploid and tetraploid potato clones with the *rolC* gene of *Agrobacterium rhizogenes* and characterization of transgenic plants. *Plant Breeding, 104*(4), 295–304.

Flores, H. E., & Filner, P. (1985). Metabolic relationships of putrescine, GABA and alkaloids in cell and root cultures of Solanaceae. In K.-H. Neumann, W. Barz, & E. Reinhard (Eds.), *Primary and secondary metabolism of plant cell cultures* (pp. 174–185). Berlin/Heidelberg: Springer.

Gangopadhyay, M., Chakraborty, D., Bhattacharyya, S., et al. (2010). Regeneration of transformed plants from hairy roots of *Plumbago indica*. *Plant Cell Tissue and Organ Culture, 102*, 109–114.

Gorpenchenko, T. Y., Kiselev, K. V., Bulgakov, V. P., et al. (2006). The *Agrobacterium rhizogenes rolC*-gene-induced somatic embryogenesis and shoot organogenesis in *Panax ginseng* transformed calluses. *Planta, 223*(3), 457–467.

Grishchenko, O. V., Kiselev, K. V., Tchernoded, G. K., et al. (2013). The influence of the *rolC* gene on isoflavonoid production in callus cultures of *Maackia amurensis*. *Plant Cell Tissue and Organ Culture, 113*(3), 429–435.

Grishchenko, O. V., Kiselev, K. V., Tchernoded, G. K., et al. (2016). *RolB* gene-induced production of isoflavonoids in transformed *Maackia amurensis* cells. *Applied Microbiology and Biotechnology, 100*(17), 7479–7489.

Guillon, S., Trémouillaux-Guiller, J., Pati, P. K., et al. (2006). Hairy root research: Recent scenario and exciting prospects. *Current Opinion in Plant Biology, 9*(3), 341–346.

Häkkinen, S. T., Moyano, E., Cusidó, R. M., et al. (2016). Exploring the metabolic stability of engineered hairy roots after 16 years maintenance. *Frontiers in Plant Science, 7*, 1486. https://doi.org/10.3389/fpls.2016.01486.

Halder, M., & Jha, S. (2016). Enhanced trans-resveratrol production in genetically transformed root cultures of Peanut (*Arachis hypogaea* L.). *Plant Cell Tissue and Organ Culture, 124*(3), 555–572.

Hamill, J. D., Parr, A. J., Rhodes, M. J., et al. (1987). New routes to plant secondary products. *Nature Biotechnology, 5*(8), 800–804.

Hicks, G. S. (1994). Shoot induction and organogenesis in vitro: A developmental perspective. *In Vitro Cellular & Developmental Biology, 30*(1), 10–15.

Holefors, A., Xue, Z. T., Welander, M., et al. (1998). Transformation of the apple rootstock M26 with the *rolA* gene and its influence on growth. *Plant Science, 136*(1), 69–78.

Ikeuchi, M., Ogawa, Y., Iwase, A., et al. (2016). Plant regeneration: Cellular origins and molecular mechanisms. *Development, 143*, 1442–1451.

Ionkova, I., & Fuss, E. (2009). Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots and lignan production in *Linum tauricum* ssp. *tauricum*. *Pharmacognosy Magazine, 5*(17), 14.

Ismail, H., Dilshad, E., Waheed, M. T., et al. (2016). Transformation of *Lactuca sativa* L. with *rolC* gene results in increased antioxidant potential and enhanced analgesic, anti-inflammatory and antidepressant activities in vivo. *3 Biotechnology, 6*(2), 215.

Jouanin, L., Guerche, D., Pamboukdjian, N., et al. (1987). Structure of T-DNA in plants regenerated from roots transformed by *Agrobacterium rhizogenes* strain A4. *Molecular & General Genetics, 206*(3), 387–392.

Kamada, H., Okamura, N., Satake, M., et al. (1986). Alkaloid production by hairy root cultures in *Atropa belladonna*. *Plant Cell Reports, 5*(4), 239–242.

Kaneyoshi, J., & Kobayashi, S. (1999). Characteristics of transgenic trifoliate orange (*Poncirus trifoliate* Raf.) possessing the *rolC* gene of *Agrobacterium rhizogenes* Ri plasmid. *Journal of the Japanese Society for Horticultural Science, 68*(4), 734–738.

Karuppusamy, S. (2009). A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. *Journal of Medicinal Plant Research: Planta Medica, 3*(13), 1222–1239.

Khalili, S., Moieni, A., & Abdoli, M. (2015). Influence of different strains of *Agrobacterium rhizogenes*, culture medium, age and type of explant on hairy root induction in *Echinacea angustifolia*. *IJGPB, 3*(1), 56–49.

Kim, Y. S., & Soh, W. Y. (1996). Amyloplast distribution in hairy roots induced by infection with *Agrobacterium rhizogenes*. *Biological Sciences in Space, 10*(2), 102–104.

Kim, J. S., Lee, S. Y., & Park, S. U. (2008). Resveratol production in hairy root culture of peanut, *Arachis hypogaea* L. transformed with different *Agrobacterium rhizogenes* strains. *African Journal of Biotechnology, 7*, 3788–3790.

Kiselev, K. V., Dubrovina, A. S., Veselova, M. V., et al. (2007). The *rolB* gene-induced overproduction of resveratrol in Vitis amurensis transformed cells. *Journal of Biotechnology, 128*(3), 681–692.

Kodahl, N., Müller, R., & Lütken, H. (2016). The *Agrobacterium rhizogenes* oncogenes *rolB* and ORF13 increase formation of generative shoots and induce dwarfism in *Arabidopsis thaliana* (L.) Heynh. *Plant Science, 252*, 22–29.

Koltunow, A. M., Johnson, S. D., Lynch, M., et al. (2001). Expression of *rolB* in apomictic *Hieracium piloselloides* Vill. Causes ectopic meristems in planta and changes in ovule formation, where apomixis initiates at higher frequency. *Planta, 214*(2), 196–205.

Koshita, Y., Nakamura, Y., Kobayashi, S., et al. (2002). Introduction of the *rolC* gene into the genome of the Japanese persimmon causes dwarfism. *Journal of the Japanese Society for Horticultural Science, 71*(4), 529–531.

Kubo, T., Tsuro, M., Tsukimori, A., et al. (2006). Morphological and physiological changes in transgenic *Chrysanthemum morifolium* Ramat. 'Ogura-nishiki' with *rolC*. *Journal of the Japanese Society for Horticultural Science, 75*, 312–317.

Kumar, N., & Reddy, M. P. (2011). *In vitro* plant propagation: A review. *Journal of Forest Science, 27*, 61–72.

Kurioka, Y., Suzuki, Y., Kamada, H., et al. (1992). Promotion of flowering and morphological alterations in *Atropa belladonna* transformed with a CaMV 35S-*rolC* chimeric gene of the Ri plasmid. *Plant Cell Reports, 12*(1), 1–6.

Landi, L., Capocasa, F., & Costantini, E. (2009). *ROLC* strawberry plant adaptability, productivity, and tolerance to soil-borne disease and mycorrhizal interactions. *Transgenic Research, 18*(6), 933–942.

Lee, M. H., Yoon, E. S., Jeong, J. H., et al. (2004). *Agrobacterium rhizogenes*-mediated transformation of *Taraxacum platycarpum* and changes of morphological characters. *Plant Cell Reports, 22*, 822–827.

Majumdar, S., Garai, S., & Jha, S. (2011). Genetic transformation of *Bacopa monnieri* by wild type strains of *Agrobacterium rhizogenes* stimulates production of bacopa saponins in transformed calli and plants. *Plant Cell Reports, 30*, 941–954.

Mano, Y., Ohkawa, H., & Yamada, Y. (1989). Production of tropane alkaloids by hairy root cultures of *Duboisia leichhardtii* transformed by *Agrobacterium rhizogenes*. *Plant Science, 59*(2), 191–201.

Matveeva, T. V., Sokornova, S. V., & Lutova, L. A. (2015). Influence of *Agrobacterium* oncogenes on secondary metabolism of plants. *Phytochemistry Reviews, 14*(3), 541–554.

Mauro, M. L., Trovato, M., De Paolis, A., et al. (1996). The plant oncogene *rolD* stimulates flowering in transgenic tobacco plants. *Developmental Biology, 180*(2), 693–700.

Mitra, A., Mukherjee, C., & Sircar, D. (2017). Metabolic phytochemistry-based approaches for studying secondary metabolism using transformed root culture systems. In S. Jha (Ed.), *Transgenesis and secondary metabolism*, *Reference series in phytochemistry* (pp. 513–537). Cham: Springer.

Nilsson, O., & Olsson, O. (1997). Getting to the root: The role of the *Agrobacterium rhizogenes rol* genes in the formation of hairy roots. *Physiologia Plantarum, 100*(3), 463–473.

Odegaard, E., Nielsen, K. M., Beisvag, T., et al. (1997). Agravitropic behaviour of roots of rapeseed (*Brassica napus* L.) transformed by *Agrobacterium rhizogenes*. *Journal of Gravitational Physiology, 4*(3), 5–14.

Ohara, A., Akasaka, Y., Daimon, H., et al. (2000). Plant regeneration from hairy roots induced by infection with *Agrobacterium rhizogenes* in *Crotalaria juncea* L. *Plant Cell Reports, 19*, 563–568.

Oono, Y., Suzuki, T., Toki, S., et al. (1993). Effects of the over-expression of the *rolC* gene on leaf development in transgenic periclinal chimeric plants. *Plant & Cell Physiology, 34*(5), 745–752.

Palazón, J., Cusidó, R. M., Roig, C., et al. (1997). Effect of *rol* genes from *Agrobacterium rhizogenes* TL-DNA on nicotine production in tobacco root cultures. *Plant Physiology and Biochemistry, 35*(2), 155–162.

Palazón, J., Cusidó, R. M., Roig, C., et al. (1998). Expression of the *rolC* gene and nicotine production in transgenic roots and their regenerated plants. *Plant Cell Reports, 17*(5), 384–390.

Park, S. U., & Facchini, P. J. (2000). *Agrobacterium rhizogenes* -mediated transformation of opium poppy, *Papaver somniferum* L., and California poppy, *Eschscholzia californica* Cham., root cultures. *Journal of Experimental Botany, 51*(347), 1005–1016.

Parr, A. J. (2017). Secondary products from plant cell cultures–early experiences with *Agrobacterium rhizogenes*-transformed hairy roots. In S. Jha (Ed.), *Transgenesis and secondary metabolism*, *Reference series in phytochemistry* (pp. 1–13). Cham: Springer.

Paul, P., Sarkar, S., & Jha, S. (2015). Effects associated with insertion of *cryptogein* gene utilizing Ri and Ti plasmids on morphology and secondary metabolites are stable in *Bacopa monnieri*-transformed plants grown *in vitro* and *ex vitro*. *Plant Biotechnology Reports, 9*(4), 231–245.

Peres, L. E. P., Morgante, P. G., Vecchi, C., et al. (2001). Shoot regeneration capacity from roots and transgenic hairy roots of tomato cultivars and wild related species. *Plant Cell Tissue and Organ Culture, 65*(1), 37–44.

Pistelli, L., Giovannini, A., Ruffoni, B., et al. (2010). Hairy root cultures for secondary metabolites production. In M. T. Giardi, G. Rea, & B. Berra (Eds.), *Bio-farms for nutraceuticals*, *Advances in experimental medicine and biology* (Vol. 698, pp. 167–184). Boston: Springer.

Rao, S. R., & Ravishankar, G. A. (2002). Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances, 20*(2), 101–153.

Ray, S., & Jha, S. (1999). Withanolide synthesis in cultures of *Withania somnifera* transformed with *Agrobacterium tumefaciens*. *Plant Science, 146*(1), 1–7.

Ray, S., Ghosh, B., Sen, S., et al. (1996). Withanolide production by root cultures of *Withania somnifera* transformed with *Agrobacterium rhizogenes*. *Planta Medica, 62*(06), 571–573.

Ray, S., Majumder, A., Bandyopadhyay, M., et al. (2014). Genetic transformation of sarpagandha (*Rauvolfia serpentina*) with *Agrobacterium rhizogenes* for identification of high alkaloid yielding lines. *Acta Physiologiae Plantarum, 36*(6), 1599–1605.

Robins, R. J., Parr, A. J., & Walton, N. J. (1991). Studies on the biosynthesis of tropane alkaloids in *Datura stramonium* L. transformed root cultures. *Planta, 183*(2), 196–201.

Roychowdhury, D., Ghosh, B., Chaubey, B., et al. (2013a). Genetic and morphological stability of six-year-old transgenic *Tylophora indica* plants. *The Nucleus, 56*(2), 81–89.

Roychowdhury, D., Majumder, A., & Jha, S. (2013b). *Agrobacterium rhizogenes*-mediated transformation in medicinal plants: Prospects and challenges. In S. Chandra, H. Lata, & A. Varma (Eds.), *Biotechnology for medicinal plants: Micropropagation and improvement* (pp. 29–68). Berlin/Heidelberg: Springer.

Roychowdhury, D., Basu, A., & Jha, S. (2015a). Morphological and molecular variation in Ri-transformed root lines are stable in long term cultures of *Tylophora indica*. *Plant Growth Regulation, 75*(2), 443–453.

Roychowdhury, D., Chaubey, B., & Jha, S. (2015b). The fate of integrated Ri T-DNA *rol* genes during regeneration via somatic embryogenesis in *Tylophora indica*. *Journal of Botany, 2015*, 1–16.

Roychowdhury, D., Halder, M., & Jha, S. (2017). *Agrobacterium rhizogenes*-mediated transformation in medicinal plants: Genetic stability in long-term culture. In S. Jha (Ed.), *Transgenesis and secondary metabolism*, *Reference series in phytochemistry* (pp. 323–345). Cham: Springer.

Rugini, E., Pellegrineschi, A., Mencuccini, M., et al. (1991). Increase of rooting ability in the woody species kiwi (*Actinidia deliciosa* A. Chev.) by transformation with *Agrobacterium rhizogenes rol* genes. *Plant Cell Reports, 10*(6–7), 291–295.

Sarkar, S., & Jha, S. (2017). Morpho-histological characterization and direct shoot organogenesis in two types of explants from *Bacopa monnieri* on unsupplemented basal medium. *Plant Cell Tissue and Organ culture, 130*(2), 435–441.

Satheeshkumar, K., Jose, B., Soniya, E. V., et al. (2009). Isolation of morphovariants through plant regeneration in *Agrobacterium rhizogenes* induced hairy root cultures of *Plumbago rosea* L. *Indian Journal of Biotechnology, 8*(4), 435–441.

Schmülling, T., Schell, J., & Spena, A. (1988). Single genes from *Agrobacterium rhizogenes* influence plant development. *The EMBO Journal, 7*(9), 2621–2629.

Sedira, M., Holefors, A., & Welander, M. (2001). Protocol for transformation of the apple rootstock Jork 9 with the *rolB* gene and its influence on rooting. *Plant Cell Reports, 20*(6), 517–524.

Sharma, P., Padh, H., & Shrivastava, N. (2013). Hairy root cultures: A suitable biological system for studying secondary metabolic pathways in plants. *Engineering in Life Sciences, 13*(1), 62–75.

Shkryl, Y. N., Veremeichik, G. N., Bulgakov, V. P., et al. (2007). Individual and combined effects of the *rolA*, *B* and *C* genes on anthraquinone production in *Rubia cordifolia* transformed calli. *Biotechnology and Bioengineering, 100*(1), 118–125.

Simic, S. G., Tusevski, O., Maury, S., et al. (2014). Effects of polysaccharide elicitors on secondary metabolite production and antioxidant response in *Hypericum perforatum* L. shoot cultures. *The Scientific World Journal*. https://doi.org/10.1155/2014/609649.

Sinkar, P. V., Pythoud, F., White, F. F., et al. (1988). *rolA* locus of the Ri plasmid directs developmental abnormalities in transgenic tobacco plants. *Genes and Development, 2*(6), 688–697.

Sivanandhan, G., Selvaraj, N., Ganapathi, A., et al. (2016). Elicitation approaches for withanolide production in hairy root culture of *Withania somnifera* (L.) Dunal. In A. Fett-Neto (Ed.), *Biotechnology of plant secondary metabolism*, *Methods in molecular biology* (Vol. 1405, pp. 1–18). New York: Humana Press.

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. *Symposia of the Society for Experimental Biology, 11*, 118–131.

Slightom, J. L., Durand-Tardif, M., Jouanin, L., et al. (1986). Nucleotide sequence analysis of TL-DNA of *Agrobacterium rhizogenes* agropine type plasmid. Identification of open reading frames. *The Journal of Biological Chemistry, 261*, 108–121.

Spena, A., Schmülling, T., Koncz, C., et al. (1987). Independent and synergistic activity of *rolA*, *B* and *C* loci in stimulating abnormal growth in plants. *The EMBO Journal, 6*(13), 3891–3899.

Srivastava, S., & Srivastava, A. K. (2007). Hairy root culture for mass-production of high-value secondary metabolites. *Critical Reviews in Biotechnology, 27*(1), 29–43.

Taneja, J., Jaggi, M., Wankhede, D. P., et al. (2010). Effect of loss of T-DNA genes on MIA biosynthetic pathway gene regulation and alkaloid accumulation in *Catharanthus roseus* hairy roots. *Plant Cell Reports, 29*(10), 1119–1129.

Tepfer, D. (1984). Genetic transformation of several species of higher plants by *Agrobacterium rhizogenes*: Phenotypic consequences and sexual transmission of the transformed genotype and phenotype. *Cell, 37*, 959–967.

Tepfer, D. (1990). Genetic transformation using *Agrobacterium rhizogenes*. *Physiologia Plantarum, 79*(1), 140–146.

Tepfer, D. (2017). DNA transfer to plants by *Agrobacterium rhizogenes*: A model for genetic communication between species and biospheres. In S. Jha (Ed.), *Transgenesis and secondary metabolism*, *Reference series in phytochemistry* (pp. 3–43). Cham: Springer.

Tepfer, D., & Tempé, J. (1981). Production of d'agropine par des racines transformes sous I'action d'*Agrobacterium rhizogenes* souche A4. *Comptes Rendus Académie des Sciences, 292*, 153–156.

Thwe, A., Valan Arasu, M., Li, X., et al. (2016). Effect of different *Agrobacterium rhizogenes* strains on hairy root induction and phenylpropanoid biosynthesis in tartary buckwheat (*Fagopyrum tataricum* Gaertn). *Frontiers in Microbiology, 7*, 318.

Toivonen, L. (1993). Utilization of hairy root cultures for production of secondary metabolites. *Biotechnology Progress, 9*(1), 12–20.

Trovato, M., Maras, B., Linhares, F., et al. (2001). The plant oncogene *rolD* encodes a functional ornithine cyclodeaminase. *Proceedings of the National Academy of Sciences of the United States of America, 98*(23), 13449–13453.

Trulson, A. J., Simpson, R. B., & Shahin, E. A. (1986). Transformation of cucumber (*Cucumis sativus* L.) plants with *Agrobacterium rhizogenes*. *Theoretical and Applied Genetics, 73*, 11–15.

van Altvorst, A. C., Bino, R. J., van Dijk, A. J., et al. (1992). Effects of the introduction of *Agrobacterium rhizogenes rol* genes on tomato plant and flower development. *Plant Science, 83*(1), 77–85.

Vanhala, L., Hiltunen, R., & Oksman-Caldentey, K. M. (1995). Virulence of different *Agrobacterium* strains on hairy root formation of *Hyoscyamus muticus*. *Plant Cell Reports, 14*(4), 236–240.

Vereshchagina, Y. V., Bulgakov, V. P., Grigorchuk, V. P., et al. (2014). The *rolC* gene increases caffeoylquinic acid production in transformed artichoke cells. *Applied Microbiology and Biotechnology, 98*(18), 7773–7780.

Verpoorte, R., Contin, A., & Memelink, J. (2002). Biotechnology for the production of plant secondary metabolites. *Phytochemistry Reviews, 1*(1), 13–25.

Welander, M., Pawlicki, N., Holefors, A., et al. (1998). Genetic transformation of the apple rootstock M26 with the *rolB* gene and its influence on rooting. *Journal of Plant Physiology, 153*(3–4), 371–380.

White, F. F., Taylor, B. H., Huffman, G. A., et al. (1985). Molecular and genetic analysis of the transferred DNA regions of the root-inducing plasmid of *Agrobacterium rhizogenes*. *Journal of Bacteriology, 164*, 33–44.

Yang, D. C., & Choi, Y. E. (2000). Production of transgenic plants via *Agrobacterium rhizogenes* mediated transformation of *Panax ginseng*. *Plant Cell Reports, 19*, 491–496.

Zhang, Z., Sun, A., Cong, Y., et al. (2006). *Agrobacterium*-mediated transformation of the apple rootstock *Malus micromalus* Makino with the *RolC* gene. *In Vitro Cellular & Developmental Biology Plant, 42*(6), 491–497.

Zhu, L. H., Ahlman, A., Li, X. Y., et al. (2001a). Integration of the *rolA* gene into the genome of the vigorous apple rootstock A2 reduced plant height and shortened internodes. *The Journal of Horticultural Science and Biotechnology, 76*(6), 758–763.

Zhu, L. H., Holefors, A., Ahlman, A., et al. (2001b). Transformation of the apple rootstock M.9/29 with the *rolB* gene and its influence on rooting and growth. *Plant Science, 160*(3), 433–439.

Zhu, L. H., Li, X. Y., Ahlman, A., et al. (2003). The rooting ability of the dwarfing pear rootstock BP10030 (*Pyrus communis*) was significantly increased by introduction of the *rolB* gene. *Plant Science, 165*(4), 829–835.

Zia, M., Mirza, B., Malik, S. A., et al. (2010). Expression of *rol* genes in transgenic soybean (*Glycine max* L.) leads to changes in plant phenotype, leaf morphology, and flowering time. *Plant Cell Tissue and Organ Culture, 103*(2), 227–236.

Zuker, A., Tzfira, T., Scovel, G., et al. (2001). *rolC*-transgenic carnation with improved agronomic traits: Quantitative and qualitative analyses of greenhouse-grown plants. *Journal of the American Society for Horticultural Science, 126*(1), 13–18.