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REVIEW

Hairy root biotechnology of *Rauwolfia serpentina*: a potent approach for the production of pharmaceutically important terpenoid indole alkaloids

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Abstract Hairy root cultures of *Rauwolfia serpentina* induced by *Agrobacterium rhizogenes* have been investigated extensively for the production of terpenoid indole alkaloids. Various biotechnological developments, such as scaling up in bioreactors, pathway engineering etc., have been explored to improve their metabolite production potential. These hairy roots are competent for regenerating into complete plants and show survival and unaltered biosynthetic potential during storage at low temperature. This review provides a comprehensive account of the hairy root cultures of *R. serpentina*, their biosynthetic potential and various biotechnological methods used to explore the production of pharmaceutically important terpenoid indole alkaloids. The review also indicates how biotechnological endeavors might improve the future progress of research for production of alkaloids using *Rauwolfia* hairy roots.

Keywords Bioreactor · Hairy root cultures · In vitro preservation · Pathway engineering · *Rauwolfia* · Terpenoid indole alkaloids

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Introduction

Plant-based chemicals are a source for a variety of pharmaceuticals, food additives, cosmetics, nutraceuticals, agrochemicals and essential oils. These are secondary metabolites that are produced by the plants in small quantities and are often stored in specialized parts of plant such as roots, trichomes etc. (Hadacek 2002). The major procurement of these 'low volume, high valued' chemicals is based on either direct extraction from plants cultivated for this purpose or by their chemical synthesis. On the whole, organic synthesis is a costly and tedious approach as these compounds usually have complicated structures with regio- and stereo-specific activities. Therefore, the direct extraction of these secondary metabolites from naturally-cultivated plants is the major method used. Nevertheless, this traditional agricultural method

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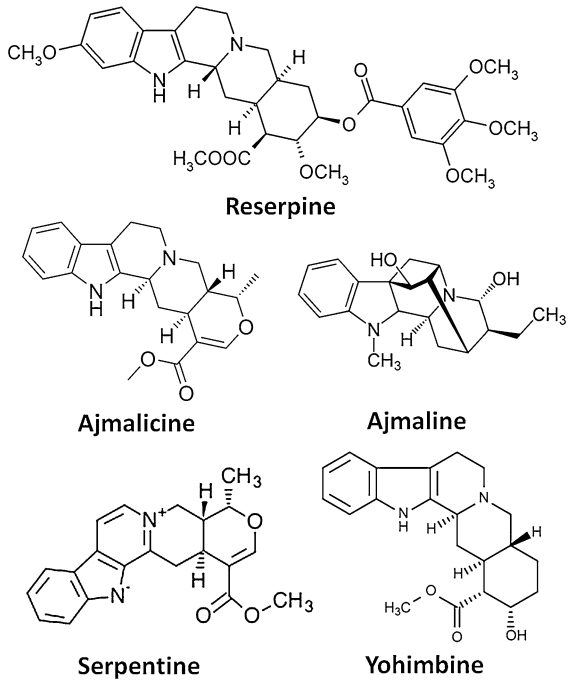

Fig. 1 Major alkaloids in *Rauwolfia serpentina*

often requires months to years to attain a single harvest. In addition, the metabolite synthesis and accumulation may be restricted to a particular species or genus or, in some cases, only become activated in a specific growth and developmental stage, season and/or stress conditions. Another drawback of this method is the continuous destruction of natural flora. Consequently, the alternative method of "in vitro culture technology", which uses plant cell and tissue cultures for the production of important secondary metabolites, has become a matter of environmental and economic concern.

Members of family Apocynaceae produce more than 100 medicinally-important terpenoid indole alkaloids (TIAs). The genus *Rauwolfia*, mainly comprised of small trees and shrubs, is found in tropical regions of Africa, Asia and Latin America. The genus is distinguished by the presence of some important TIAs such as reserpine, ajmalicine, ajmaline, serpentine, vomiline, yohimbine etc. (Fig. 1). These alkaloids are concentrated mostly in the roots which contains about 85–90 % of the total alkaloid content (Pathania et al. 2013). Synthesis and accumulation of these molecules, particularly TIAs, contribute to the medicinal properties of roots. The root extract is used as an important ingredient in drugs for the treatment of hypertension, high blood pressure, mental illness and problems related to central nervous system. Besides having sedative, aphrodisiac and antispasmodic properties, the root extract also possesses hypoglycemic and hypolipidemic activities against animal models (Pathania et al. 2013). Moreover, the root extract of *R. vomitoria*, the African tree species, also contains antidiabetic activity (Campbell-Tofte 2006). Such reports indicate the medicinal value of the roots of different species of this genus. The exploitation of *R. serpentina* for medicinal uses has resulted in its gradual decline in the wild and, consequently, it has been given an endangered status by International Union for the Conservation of Nature and Natural Resources (IUCN) and enlisted in CITES (Convention on International Trade in Endangered Species) Appendix II. The National Medicinal Plant Board, India (NMPB; www.nmpb.nic.in) has placed this plant among 32 plants identified and prioritized for cultivation and conservation through conventional and unconventional methods.

The continuous increase in commercial demand and the restricted supply of TIAs from natural resources has promoted the use of *A. rhizogenes*-mediated hairy root cultures of *R. serpentina* (HRCs) (Goel et al. 2010; Mehrotra et al. 2013a). Hairy root cultures of various species of *Rauwolfia* produce detectable amounts of pharmaceutically-important TIAs (Falkenhagen et al. 1993; Sudha et al. 2003; Goel et al. 2010; Liu et al. 2012). Hairy root cultures of *R. serpentina* have extensively been studied for TIA production. This review provides a comprehensive account of the hairy root cultures of *R. serpentina*, their biosynthetic potential and various biotechnological methods attempted for metabolite production using these roots. The review also specifies how hitherto biotechnological studies might affect the future progress of research focused on TIA production using *Rauwolfia* hairy roots.

Major *Rauwolfia* alkaloids

The genus *Rauwolfia* has been examined in detail for the presence of phytochemicals (Ganpathy et al. 2001). Out of several alkaloids, reserpine is the most potent alkaloid present in *Rauwolfia* plant (Fig. 1).

Reserpine is the 3,4,5-trimethyl benzoic acid ester of reserpic acid, an indole derivative of 18-hydroxyl yohimbine. Aerial parts of the plant also accumulate traces of reserpine which has a depressant action on central nervous system and lowering of blood pressure. Administration of reserpine reduces serotonin and catecholamines in the brain and peripheral vessels which results in sedation followed by antihypertensive action. A variety of commercially-available drugs (brand names of Renese-R; Diuretic Ap-ES; Diutensen-R; Hydropres-25 etc.) contain reserpine as a major component. These drugs are recommended for the treatment of hypertension, strangury, fever, colic, insomnia, giddiness, anxiety, maniacal behavior, psychosis, schizophrenia, dyspepsia, hyperglycemia and hypochondria. Ajmaline, another major alkaloid present in the roots, is a ditertiary indole base and is closely related to quinidine. It stimulates respiratory and intestinal movement, is effective against ventricular extra systoles and exhibits useful adjunctive action in atrial/ventricular fibrillation and Brugada syndrome. It is a class III anti-arrhythmic agent that causes the lowering of cardiac rhythm through blocking sodium channels (Rolf et al. 2003). Ajmaline may be useful in combination with antihypertensive agents for the treatment of hypertension when complicated by a cardiac disorder.

The third major alkaloid is ajmalicine or raubascine which is a central depressant with adrenergic blocking properties. A stereo-isomer of tetrahydro alstonine, almalicine, causes hypotension with renal vasodilation and inhibits the postganglionic functioning of the sympathetic nervous system. Serpentine, another important alkaloid, is a yellow quaternary indolic anhydronium base that causes marked inhibition of succinate dehydrogenase in brain and liver tissues. It produces systemic and pulmonary hypertension due to a decrease in cardiac output. *R. serpentina* roots also contain yohimbine/rauwolscine a cardiovascular depressant with hypnotic activity. These alkaloids contribute to the characteristic properties of roots which are bitter, acrid, laxative, anthelmintic, thermogenic and diuretic in nature. The decoction of the root is also used to stimulate uterine contractions and recommended for use in childbirth in difficult cases. Drugs containing reserpine can be administered by oral and injectable ways for the treatment of hypertension.

Agrobacterium rhizogenes-mediated hairy root cultures

Benjamin et al. (1993) reported hairy roots from the leaf explants of *R. serpentina* through the infection of *A. rhizogenes* strain ATCC 15834. Falkenhagen et al. (1993) also reported hairy root induction in leaf explants infected with *A. rhizogenes* strain A4. The higher alkaloid content and different alkaloid profile of these A4 generated hairy root cultures justified attention. A novel compound, 3-epi-α-yohimbine, was also isolated from these hairy roots. Transformation ability (in terms of number of hairy root lines produced per explant) of different *A. rhizogenes* strains, such as SV4, LBA9402 and SV2, have also been examined where SV2 was found to be more efficient in inducing the hairy roots in *R. serpentina* leaf explants (Sarma et al. 1997). *Agrobacterium* ATCC 15834 was also used to induce hairy roots in *R. micrantha* (Sudha et al. 2003).

To sum up, the reported investigations into the process of *A. rhizogenes*-mediated hairy root induction, the virulence of different strains is represented in Fig. 2a–b. Out of five strains, strain A4 showed the greatest prominence. Nevertheless, in majority of reported hairy root induction experiments, irrespective of bacterial strain, the emergence of roots at infection sites of leaves was observed within 15 days of infection. These reports indicate the high susceptibility of *R. serpentina* for different strains of *A. rhizogenes*.

A. rhizogenes-mediated hairy roots of *R. serpentina* represent a rich repository of a range of terpenoid indole alkaloids (Sheludko et al. 2002; Madhusudanan et al. 2008). The exploitation of *R. serpentina* hairy roots is currently focused on reserpine biosynthesis (Goel et al. 2010; Mehrotra et al. 2013a). *A. rhizogenes* A4-mediated hairy roots show reserpine contents, varying from 0.0064 to 0.088 % dry weight (DW). In comparison to field-grown plants (variety cim-sheel; 0.03–0.034 % DW reserpine) harvested after 18–24 months, the hairy roots had a much higher reserpine content (0.086 % DW) in 10–12 week cultures (Goel et al. 2010; Mehrotra et al. 2013a). Hairy root clones showed a varied morphology (Fig. 2c); however, no relationship between root morphology and reserpine content has been established. The variation in root morphology can be better

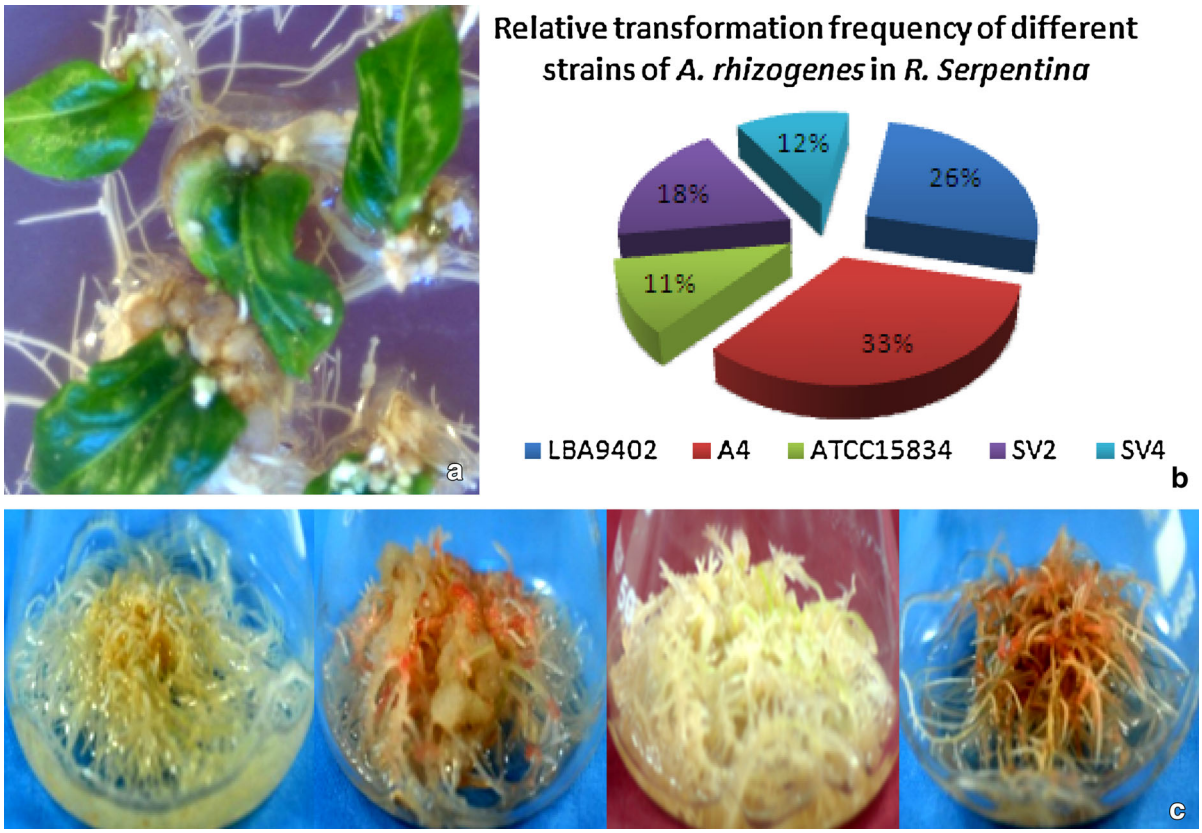

Fig. 2 *A. rhizogenes* mediated transformation in *R. serpentina* leaf explants (**a**). Relative transformation frequency of different *Agrobacterium rhizogenes* strains in *R. serpentina* (**b**). Morphological variation in different hairy root lines (**c**)

explained by studying the number of T-DNA copies inserted in host cell during transformation.

Scale-up of *R. serpentina* hairy root cultures

In the development of techniques for hairy root-based secondary metabolite production at an industrial level, large scale or bioreactor cultivation is the ultimate step (Kim et al. 2002; Srivastava and Srivastava 2007). *R. serpentina* hairy root cultures can be a promising source for the production of reserpine and other valuable alkaloids. In our laboratory, we have examined the feasibility of a scale-up protocol to grow *R. serpentina* hairy roots in a bench top and mechanically-agitated bioreactor. Growth performance and steady reserpine production by hairy roots in bioreactor was also investigated in contrast to the conventional shake-flask culture. The bioreactor used was 5 l with a modified air-lift and was fitted with all necessary probes. It had been earlier used to scale-up hairy root cultures of *Glycyrrhiza glabra* (Mehrotra et al. 2008). To avoid submergence of inoculated tissue and its damage from the impeller an autoclavable nylon mesh (pore size 200 μ; indicated by arrows in Fig. 3) was fabricated that divided the vessel into upper and lower halves. The mesh also provided anchorage to the growing tissue in the upper half of the vessel. A fast growing hairy root clone was selected for the cultivation in bioreactor. In order to compare the growth performance of hairy roots in reactor vessel and culture flasks, the same clone was inoculated in the shake-flasks (150 ml). The initial inoculum was kept constant ($\approx$2 g/l) in both culture vessels.

During growth, the developing nascent rootlets readily made their way through mesh pores and started growing in the lower and upper halves of the vessel. In the ensuing culture duration, the rapid growth of roots resulted in the formation of root clumps (Fig. 3b–c). The eleven week culture resulted in a growth index

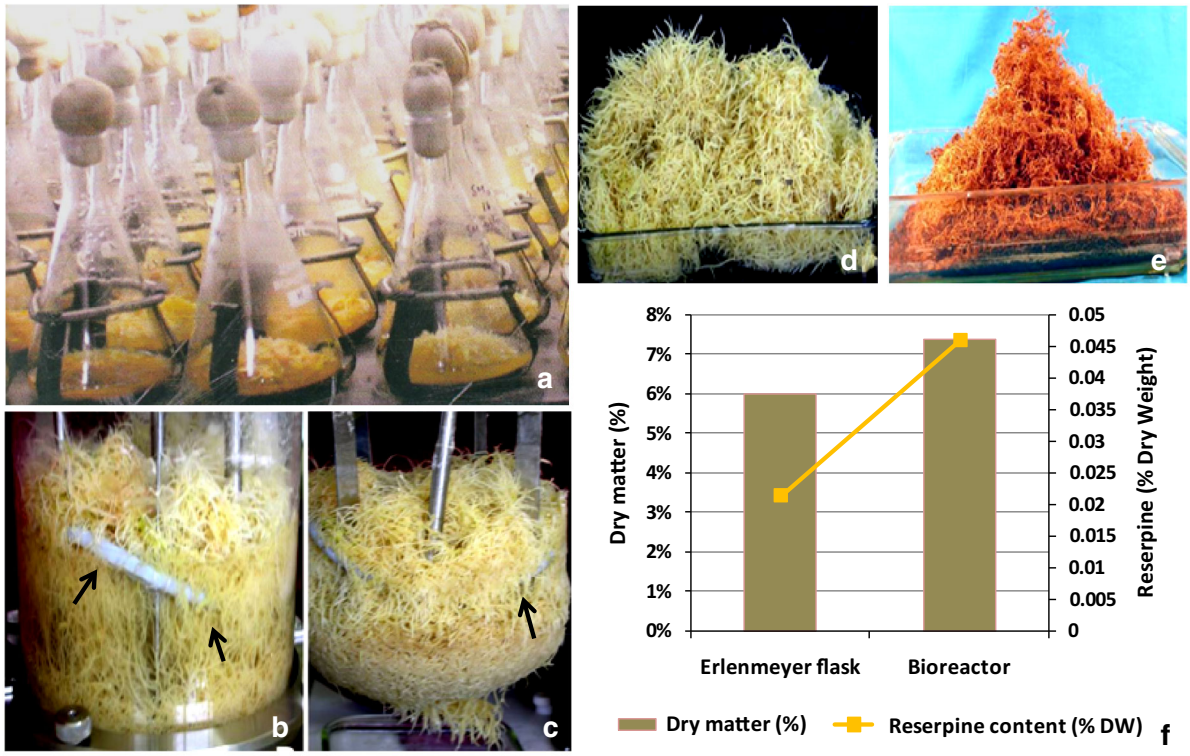

Fig. 3 Hairy root cultures in shake-flasks (**a**) and bioreactor (**b**–**c**; provision of mesh is indicated by *arrows*). Fresh and dry weight biomass obtained from bioreactor culture of 11 weeks (**d**–**e**) and reserpine production on dry matter basis in shake-flasks and bioreactor culture of hairy roots (**f**)

(final DW/initial DW) of 142. This was about twice the growth index of roots grown in shake-flasks (68.5). The dry matter, obtained on fresh weight basis was significantly higher in bioreactor (7.4 %) as compared to shake-flasks (6 %) and was responsible for about 23 % total increment in biomass (Fig. 3d–f). Chemical analysis revealed that bioreactor grown roots had more than double the metabolite content (0.046 % reserpine DW) compared to roots grown in shake-flasks (0.022 % reserpine DW; Fig. 3f).

Similarly, hairy root cultures of various medicinal plants have shown improved growth rates and productivities in bioreactors. This is possibly due to homogenous culture conditions which occur as a result of mechanical agitation (Giri and Narasu 2000; Srivastava and Srivastava 2007). In this context, where a field-grown *R. serpentina* plant produces an average 0.03–0.034 % reserpine DW from roots after 24–36 months of field cultivation, the bioreactor cultivation of hairy roots resulted in a two-fold increase in content in just 11 weeks. This strongly supports the feasibility of scale-up cultivation of *R. serpentina* hairy roots for secondary metabolite production using an air lift modified bioreactor. However, certain issues require particular attention in the development of scale-up protocol for hairy roots. These include the understanding of root growth phenomenon in nutrient and O_2 gradients and, secondly, the technical understanding of designing a larger culture vessel that not only replicate/improves the results obtained from shake-flask culture but also facilitates the process up to an industrial level. In large vessels, the clumping during root growth often leads to local concentration gradients. So was the case with *R. serpentina* HRCs in the bioreactor (Fig. 3b–c).

To enhance the spatial homogeneity and improved supplementation, several modifications in reactor configuration, such as trickle bed, nutrient mist etc., have been proposed. The morphological and physiological properties of root tissue also require specific attention while selecting the reactor configuration. While discussing the second issue, it is relevant to

state that laboratory scale-up should not only intend to show the same yields during industrial scale operation, but also test the feasibility of existing technology at an industrial scale. Though, the generalized concept of bioreactor technology indicates a proportionate production of biomass with that of vessel size, the reality is far behind. This is because with the increase in vessel size, various physical factors begin to affect tissue growth. These factors include gas and liquid flow rates, mass transfer rate, concentration gradients at solid–liquid boundaries etc. which play pivotal roles in tissue growth. Therefore, to make existing scale-up protocols feasible in commercial set ups, the laboratory scale-up should be aimed at acquiring expertise in various phases of process development. Starting from designing laboratory experiments and selection of suitable bioreactor, the laboratory scale-up should include the study of kinetic correlations, fluid dynamics modeling of mass transfer, root growth simulations and mathematical modeling etc. (Curtis 2010). A proper understanding of these issues should lead to a better scale-up system for *Rauwolfia* hairy roots that may resolve the contradiction between investment and process output.

Optimization of culture conditions for a process is another related and relevant aspect that justifies interest. As a rule, experimental optimization involves additional labor, time, cost and space. On the other hand, computational growth simulations not only offer clues about accuracy but also help to overcome the cost and time involved in the actual process. The growth performance of hairy roots of *Glycyrrhiza glabra* and *R. serpentina* has been assessed under different culture conditions with the help of artificial neural networks (ANN). The ANN-based model predicted the optimal culture conditions (pH of liquid growth medium, volume of medium per culture vessel, sucrose and nitrate concentrations in the growth medium and initial inoculum mass per culture vessel) at which maximum hairy root biomass could be obtained (Prakash et al. 2010; Mehrotra et al. 2013b). These studies offer new opportunities to understand and explore a root-based bioprocess from laboratory bench to commercial scale. Computational simulations hypothesize different mathematical algorithms to process and interpret different sets of unpredictable data obtained from the behavior of cultured tissue under variable culture conditions. Since, it also explains the synergistic effect of different physical/chemical culture parameters interacting in a common space, it can easily be used to select the best suitable conditions for scale-up of hairy root cultures.

TIA biosynthetic pathway engineering

Pathway engineering demonstrates the introduction and expression of foreign genes into the host genome for alteration in cell metabolism leading to an increased flux of target compounds in a biosynthetic pathway (Mehrotra et al. 2010; Zhou et al. 2011). This target compound may be the metabolite of interest or any conversion product, the conversion of which is the rate limiting step within the pathway.

The revelation of terpenoid indole alkaloid biosynthetic pathway and cloning of various key genes have promoted pathway engineering in *Rauwolfia* hairy roots. The TIA pathway begins with the combination of tryptamine and monoterpenoid glycoside secologanin. A continuous supply of tryptamine is necessary for operation of the pathway. This tryptamine comes from the conversion of tryptophan through the activity of tryptophan decarboxylase (EC 4.1.1.28). This step is rate-limiting as the overproduction/accumulation of tryptophan results in its feedback inhibition of anthranilate synthase activity. Engineering the TIA pathway engineering in *R. serpentina* hairy roots through heterologous expression of tryptophan decarboxylase (*tdc*) has been attempted (Mehrotra et al. 2013c). Over-expression of *Catharanthus* tryptophan decarboxylase (Cr*tdc*) in six transgenic hairy root lines of *R. serpentina* increased the reserpine and ajmalicine content. Nearly a two-fold increase in total alkaloid content was observed in transgenic hairy root lines. The presence of the transgene was confirmed by Southern blot analysis.

The study promoted the feasibility of pathway engineering and offered striking opportunities for productivity enhancement in *Rauwolfia* hairy roots. Liu et al. (2012) revealed a consistent expression level of *R. verticillata tdc* with the accumulation of ajmalicine in different plant parts as well as in hairy roots. *tdc* expression is thus a crucial event for the accumulation of pathway products in different TIA-harboring or non harboring plants (Goddijn et al. 1994; Geerlings et al. 1999; Hughes et al. 2004). However, in *R. verticillata*, abscisic acid (ABA)-treated hairy root cultures were analyzed for the expression of the five

genes of the MEP (methylerythritol phosphate) pathway and three genes of the ajmalicine (TIA) pathway including *tdc*. While all the MEP pathway genes showed transcriptional up-regulation, all the ajmalicine pathway genes (*tdc*, *str* and *sgd*) did not. The study resulted in increased ajmalicine biosynthesis (Chang et al. 2014). The results show the role of the MEP pathway in ajmalicine biosynthesis in *R. verticillata* hairy roots and also indicated that alkaloid biosynthesis in *Rauwolfia* requires multichannel pathway operation. Therefore, more conceptual inputs and a thorough knowledge base is necessary for overexpression studies in *Rauwolfia* hairy roots for improved alkaloid flux.

The role of various physical, chemical and biological elicitors in affecting the pathway genes directly or by influencing the regulatory factors requires attention. Expression studies of *tdc* against elicitor challenge and its correlation with ORCA3 transcriptional factor have demonstrated the role of elicitor-responsive gene regulation in biosynthetic pathways (Ouwerkerk and Memelink 1999). The role of *C. roseus* WRKY transcription factor (CrWRKY1) in TIA biosynthesis has also been reported and suggests an association of a complex transcriptional control network (Suttipanta et al. 2011). These reports clearly indicate the intricacy of TIA pathway regulation at transcriptional level. A rational analysis of these points is thus worth reviewing for describing the practical viewpoint of this comprehensive study.

Transgenic plant production

Regeneration of transformed plants from *Rauwolfia* hairy roots was initially reported by Benjamin et al. (1993). The transformed plants yielded a high biomass and exhibited similar alkaloid profiles in aerial as well as in underground parts. Spontaneous plant regeneration from *A. rhizogenes*-mediated hairy root cultures of *Rauwolfia* has been reported (Mehrotra et al. 2013a). The hairy root explants produced shoots over 6–8 weeks in Gamborg's B5 liquid culture medium at varying regeneration frequency (39–70 %). The regenerated shoots underwent robust shoot proliferation on phytohormone-supplemented medium (Fig. 4a). Upon transferring to root-induction medium, the regenerated shoots showed 100 % rooting within 3 weeks. An investigation of glasshouse-grown transformed plants revealed that 90 % of them were phenotypically similar to the non-transformed plants while 10 % had stunted growth. All the transformed plants were characterized on molecular and chemical basis. Southern blotting confirmed the presence of single T-DNA in all the transformed plants except those having aberrant and stunted growth where multiple T-DNA copies were observed. Presence of multiple T-DNA copies was proposed as a possible reason for the aberrant phenotypic behavior of these transformed plants which could not survive for more than three months. However, transformed plants with normal phenotypic characters survived after acclimatization, flowered and bore fruits. Roots of regenerated plants had similar levels of reserpine to those of their corresponding hairy roots.

For a commercially-important crop like *R. serpentina*, hairy root cultures and their regeneration into transformed plants can unlock opportunities for biotechnology-based crop improvement. However, the major issue of stability of regenerated transformed plants in ensuing generations cannot be ignored. Thus, despite focusing on solely producing transgenic phenotypes, the major experimentation involving transgenic regeneration should aim at exploring T-DNA integration and the structure of the transgene loci. Each of the two factors alone or in combination may influence gene silencing, *de novo* methylations etc. and, thus, control the expression and stability of foreign gene (Kohli et al. 2010). Hairy root cultures and regenerated transformed plants in *Rauwolfia* can also be used to characterize the transformation event through combined molecular techniques, contemporary gene transfer approaches and cytogenetic analysis. This should lead to the establishment of site-directed integration with the desired T-DNA copy number and orientation. This may consequently result in improved transformation events with desired expression and stability in transgenic tissue and further transgenic plants.

Cryopreservation of *R. serpentina* hairy roots

Hairy root cultures (HRC) can be used as biological matrices for the production of important secondary metabolites in specially designed bioreactors. Therefore, companies such as CBN Biotech, South Korea (maintaining *Panax ginseng* and *Artemisia* hairy root

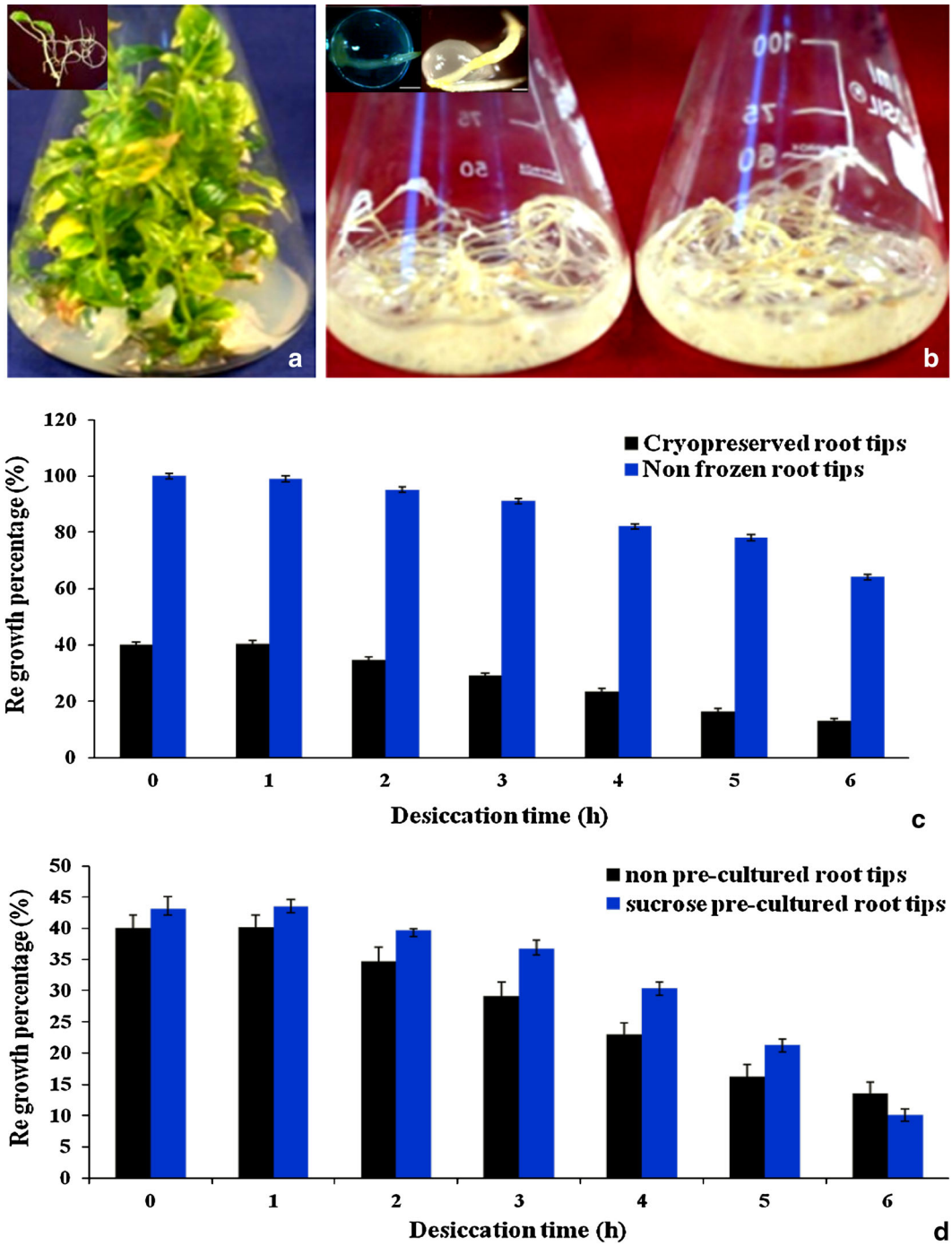

Fig. 4 *In vitro* grown transformed plant of *R. serpentina* (**a**) regeneration of transformed shoots from hairy root explant (*Inset* **a**); 10 weeks old re-grown hairy root cultures after preservation (**b**; re-growth in encapsulated hairy root tips *Inset* **b**; *bar* = 2 mm)

lines for industrial production of ginsenosides and artemisinin, respectively) and ROOTec Bioactives, Switzerland, and others are showing interest in commercial-scale production of root biomass. Thus, the practice of laboratory-scale maintenance of these hairy root cultures is a prerequisite. The laboratory

maintenance of HRCs is done manually and has a serious threat of microbial contamination that increases labor and costs. Moreover, the stress due to regular sub-culturing and prolonged in vitro maintenance may sometimes cause changes in growth and metabolic activities in root tissues which ultimately leads to the loss of the original culture.

This has given a reason to establish a competent cryopreservation protocol that can facilitate the storage and further best revival of *R. serpentina* hairy root germ plasm without affecting their growth and metabolite biosynthetic property. We encapsulated approx. 200 hairy root tips (2–3 mm) in 3 % (w/v) sodium alginate. These root tips were excised from four to six weeks old hairy root cultures maintained in liquid B5 medium under standard in vitro growth conditions. The alginate beads (6–8 mm) formed after encapsulation of root tips were used for sucrose pre-culturing (0.5 M sucrose for 24 h) and dehydration experiments (0–6 h). Subsequently, the beads were sealed in cryovials (12 beads/cryovial) and directly plunged into liquid N_2. After one week's storage, the samples were removed, held at 35 °C for 5 min and cultured on semi-solid B5 medium. About 40 % root tips cryopreserved by this method exhibited re-growth over six weeks of culture. When compared to the non-cryopreserved hairy roots, no morphological and biochemical alterations were observed in re-grown cryopreserved roots even after 8 weeks of culture (Fig. 4b). Sucrose pre-culturing enhanced the re-growth potential; however, no additional dehydration was required as it caused negative effects on re-growth of encapsulated root tips (Fig. 4c–d).

Cryopreservation and revival of hairy root clones of medicinal plants is a known practice (Lambert et al. 2009). The survival potential of *Rauwolfia* hairy root cultures during and after preservation have indicted an easy adaptation to growth medium and restitution of normal growth. For cryopreservation, the need is to optimize the protocol in terms of fast revival and high survival rates of cryopreserved root tips. Such experiments also show the direction needed to find further information about the behavior of the plant or cultured root tissues regarding metabolite accumulation during cold stress. Transcriptomic profiling of root tissues kept in low temperature can provide useful information regarding expression of pathway genes during and after cold treatment of the root tissue.

Conclusion and future aspects

In a plant such as *R. serpentina* where maintenance of natural resources is in demand, exploitation of hairy root cultures can serve as an alternative for the production of medicinally-important TIAs. To utilize these hairy roots for TIA production, different approaches, such as scale-up and productivity enhancement strategies have been tried. Although hairy roots can be grown in laboratory bioreactors, there still there is a need to simplify the process in order to make it feasible at a larger scale with low costs and labor inputs. Computational and mathematical simulations of growth, along with nutrient and sugar consumption, optimization of culture conditions, selection of suitable larger culture vessels and automated operation, are points at which efforts can be made to establish a low cost efficient method for metabolite production using *R. serpentina* hairy roots. Further, due to the lack of knowledge of TIA biosynthesis in roots, there is a lacuna that limits the use of *Rauwolfia* hairy roots for future biotechnological endeavors of enhanced metabolite production. At this stage, a comparative investigation of biochemical, transcriptomic and metabolomic data obtained from hairy root cultures of various related TIA-bearing and non-bearing plant species will facilitate the genetic and functional characterization of TIA biosynthesis in *R. serpentina*. Such studies will also be helpful in identifying the rate-limiting step as well as environmental and molecular factors involved in TIA biosynthetic pathways in these roots.

Taking the advantage of in silico modeling, the use of *R. serpentina* hairy roots as a model system to imitate the metabolic fluxes in a multifaceted biosynthetic network will provide significant information about pathway regulation against genetic and environmental stresses. The effect of various biotic, physical and chemical elicitors on the production potential of hairy root cultures of *Rauwolfia* though, has not yet been studied. Elicitation is not only known as a productivity enhancement strategy for hairy root cultures but also is a method to uncover the physiological and molecular aspects of root-specific responses during stress/pathogen challenge and ultimate metabolite accumulation. Therefore, elicitation studies in *R. serpentina* hairy roots will pave the way for understanding the host–elicitor interactions, *in-planta* functional analysis of receptor mediated recognition, signal processing and accumulation of TIAs.

An improved perceptive of biosynthetic genes and biochemical pathways will definitely enhance the utility of *R. serpentina* hairy root cultures for medicinally important TIA production

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