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HAIRY ROOT CULTURE THROUGH THE INTERVENTION OF *RHIZOBIUM RHIZOGENES* IN DIFFERENT MEDICINAL PLANTS: A REVIEW

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ABSTRACT
Hairy roots caused by root inducing plasmid explants of *Rhizobium rhizogenes* (formerly *Agrobacterium rhizogenes*), T-DNA transmit in plant cells and express along with plant genome. Hair root culture is a novel source of useful secondary metabolite production due to its rapid growth without hormones, biochemical and genetic stability. Hence the development of large quantities of hairy roots by *in-vitro* conditions to avoid the destruction of root harvesting from several important medicinal plant species. The different strains would be responsible for building hairy roots. This review aims to explain the methods of culture through various strains of *R. Rhizogenes* hairy root propagation of medicinal plants, further secondary development of metabolites by this form of induction and highlights the possibilities provided by hairy roots in terms of viability and perspectives.

KEYWORDS: hairy root culture, *R. rhizogenes*, medicinal plants, medicinal properties.

INTRODUCTION

The word "hairy-root" was first described by Stewart *et al.* in 1900. In 1910, Hedgcock (as quoted by Stapp, 1927) classified the disease into three forms dependent on morphology:

i) *Hairy root*: The name "hairy-root" was derived from the desiccation by a large number of small roots directly formed from the stem without the apparent development of callus-like tissues. The roots were as fine as the hair from this phenomenon.

ii) *Woolly knot*: Warty swellings in the host plant root creating a mass of fresh fibrous roots are known as aerial forms *viz*. burr knot, callus or girdle knot.

iii) *Broom root*: the mass of delicate lateral roots grows from the tip of the stems, often from crown gall tumors. The distinctive sign of a hair-root disorder is the development of a collection of roots (De Cleene and De Ley, 1981).

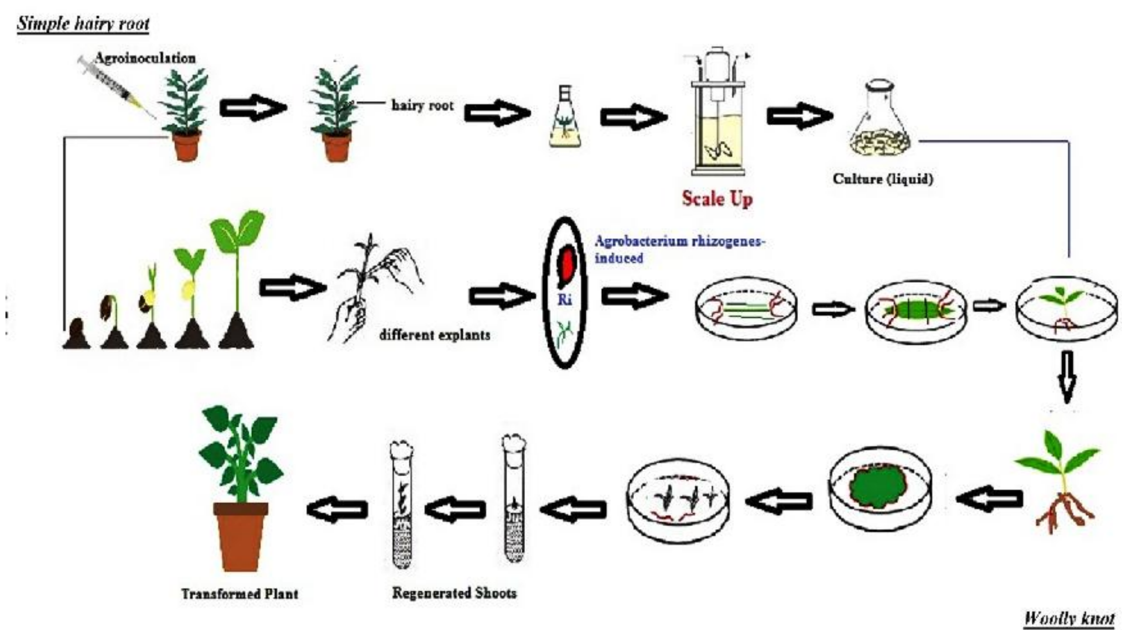

FIGURE 1. *R. rhizogenes* mediated hairy root culture

Hairy root is a plant disease caused by *R. rhizogenes* (formerly *A. rhizogenes*), a Gram-negative soil bacterium that produces hairy root disease in dicotyledonous plants (Young *et al*., 2001). Once the bacterium infects the plant, the T-DNA is passed and inserted into the host plant's nuclear genome between the TR and TL regions of the Ri plasmid within the bacterium. The cycle of transformation creates a valuable by-product, hairy root, which will develop at or near the infection site (Hu and Du, 2006). The root-inducing plasmid, including the transmission of DNA encoding root locus (rol) gene loci (rol - A, B and C), is responsible for the safe insertion of genetic material into host cells. This can trigger the profuse production at the site of infection of highly branched hairy roots, usually the hypocotyl or cotyledon. Auxin signaling is necessary for hairy root development, but not for ethylene signaling (Lima *et al*., 2009). These root systems may be cultivated or host by plants with non-transformed aerial tissue.

Hairy root induction in medicinal plant

From the 1930s -1960s hairy roots were mainly identified as a sign of pathogen invasion in horticultural plants (Gutierrez-Valdes *et al*., 2020; Doran *et al*., 2013). Only until the 1970s - 1980s was *A. rhizogenes* established as the bacterial agent which induces HR syndrome through the gene transfer of the bacterial R*i* (root-inducing) plasmid (Gelvin *et al*., 2009). This significant discovery prompted many studies which helped establish the technology of the hairy root cultures. We now know that hairy root when infected with *A. rhizogenes*, a symbiotic bacterium currently taxonomically renamed *R. rhizogenes*, arises from the wounding site of plantlets. The infection takes place from its root-inducing plasmid (pRi) into plant cells from specific bacterial DNA fragments (T-DNA). While the plant responds to bacterial influenza by triggering several defense related proteins to suppress the pathogen, *R. rhizogenes* developed mechanisms to benefit from those plant protection proteins by counteracting a process which thus disintegrates the pathways of plant protection (Gutierrez-Valdes *et al*., 2020; Mauro *et al*., 2017).

In artificial medias without hormones, the abnormal roots are easy to grow and neoplastic in nature with vapour. *R. rhizogenes* infection derived hairy roots are highly evolved and also have a genetic and biochemical maquillage. Hairy root cultivation is a sort of crop of plant tissue used to study plant metabolism, development of sub metabolites, development of recombinant proteins, phytoremediation, biopharmaceuticals, plant genetic engineering, production of artificial plants, and bio-fortification. The production of hairy root culture has gained growing interest from academic research teams, biotechnology and the pharmaceutical industries due to their technical and economic value. The revolutionary process for voluminous secondary metabolite, phytochemicals growth, has been produced by hairy root culture. This technique is thus of great importance for the production in no time of large quantities of roots and secondary metabolites to continuously supply enhanced value goods (Korde *et al*., 2016). Hairy root is the special application of plants to higher development of valuable items. Various authors' studies found that the laboratory production of hairy roots is cost-effective, yielding much less than conventional production (Table 1). Saito *et al*. (2001) reported that the *A. rhizogenes* 15834 harboring a binary vector pGSGluc1 mediated transformation onto the stems of young aseptic *Ophiorrhizapumila* plants containing 1% sucrose and 1/2 MS salts and results revealed the hairy roots emerged within 80 days. The *Adapathiyan* plant callus was cut into small pieces with a sterile blade. The strains of *A. rhizogenes* were grown on yeast extract mannitol (YEM) medium for 48 h and used for infection. The wounded explants were also co-cultured in liquid MS medium containing bacteria for 24-72h and in MS medium containing bacterial cells for 5-120 min intervals. The inoculated explants were put on 1% agar medium for 24 hours and transferred to a 250mg /ml of MS solid medium. All the cultures were maintained at 25 ±2°C temperatures. One set of cultures was kept in dark and other set in a day/night regime (16/8 h) in each experiment to test the effect of photoperiod on hairy root induction. Seedling hypocotyls induced hairy roots both under continuous dark and in a day/night regime whereas shoot buds induced hairy roots only in day/night regime. Thin whitish roots with numerous hairs emerged from the wounds in a period of 2-4 weeks. These roots grew well in MS liquid medium reported by Karmarkar *et al*. (2001). In initial tests, *Rauvolfia micrantha* plant explants with bacterial strains ATCC 15834 were proven to be the best in 48% of explants for hair-root formation during the 2–3 weeks. Cotyledones and leaf explants infected with injuries and co-cultivation responded very slowly, as opposed to shooting explants for either the formation of a callus or hair induction (Sudha *et al*., 2003). In vitro grown *Centauriumerythraea* seedlings were inoculated with *A. rhizogenes* strains A4M70GUS by puncturing the internodes of stems with a sterile, hollow needle loaded with bacteria. Hairy roots appeared after 14days from inoculation (Subotic *et al*., 2003). For their ability to cause hairy root formation on *Papaver somniferum* hypocotyls, *A. rhizogenes* strains LBA 9402, 15834, and *A. tumefaciens* strain GUS-2 were used. Sonicated hypocotyl is particularly susceptible to transformation with *A. rhizogenes* strain LBA 9402. Hypocotyls infected with *A. rhizogenes* strin (15834) or *A. tumefaciens* strain (GUS-2) and transferred to liquid media that had never produced roots. The growth of the hair root at the wound site of the hypocotyls occurred after 5 weeks of cultivation, only from the hypocotyls infected with the *A. rhizogenes* strain LBA 9402 and then moved to the LS hormone-free liquid media. Under these conditions, roots were shaped by 80 per cent of explants. The formation of six hairy-root cultures reported by Le Flem-Bonhomme *et al*. (2004). So, through the development of genetic engineering of hair root induction have been developed for the efficient pharmaceutical products, enhanced protein content, biofortification of heavy metals, phytoremediation, and processing of artificial seeds in a pharmaceutical manner.

TABLE 1. *A. rhizogenes* mediated hairy root cultures in medicinal plant

Plant name	Explants	Strain use	References
Ophiorrizapumila	Stems	*A. rhizogenes* 15834	Saito *et al*., 2001
(*Holostemmaada-kodien* K. Schum.	hypocotyls and shoot buds	*A. rhizogenes* strains PcA4, 15834 and A4	Karmarkar *et al*., 2001
Rauvolfiamicrantha Hook f.	Cotyledons and leaf	*A. rhizogenes* ATCC 15834	Sudha *et al*., 2003
Centauriumerythraea	internodes of stems	*A. rhizogenes* strains A4M70GUS	Subotic *et al* ., 2003
Papaversomniferum L.	hypocotyls	*A. rhizogenes* LBA 9402	Flem-Bonhomme *et al*., 2004
Plumbagozeylanica L.	Sterile leaves of in vitro-grown plants	*A. rhizogenes* strain A4	Verma *et al*., 2002
Przewalskiatangutica	Leaves	*A. rhizogenes* strain A4	Xiao *et al*. 2010
Salvia miltiorrhiza	plantlets	*A. rhizogenes* bacterium C58C1	Xiao *et al* ., 2009
Picrorhizakurroa	leaf explants	A. rhizogenes LBA9402	Verma *et al*., 2007
Plumbagoindica	Leaf	*A. rhizogenes* strain ATCC 15834	Gangopadhyay *et al*., 2008
Rauvolfiaserpentina	Leaf	*A. rhizogenes* strain A4	Madhusudanan *et al*., 2008
Astragalusmembranaceus, *Gentianamacrophylla* Pall. and*Erucasativa* Mill.	cotyledon or leaf	*A. rhizogenes* R1000	Xue *et al*., 2008
Withaniasominifera	cotyledons and leaf explants	*A. rhizogenes* R1601	Murthy *et al*., 2008
Saussurea involucrata	Explants	*A. rhizogenes* strain R1601	Fu *et al*., 2006
Artemisia annua	derived stems	Agrobacterium rhizogenes ATCC 15834	Putalun *et al*., 2007
Centauriumerythreae	explant	*A. rhizogenes* (strain A4M70GUS)	Subotic ´*et al*., 2009
Maesalanceolata and *Medicagotruncatula*	leaf discs or seedlings	*A. rhizogenes* strain LBA 9402/12	Lambert *et al*., 2009
Glycyrrhizauralensis	cotyledonary nodes	*A .rhizogenes* A4	Zhang *et al*., 2009
Gentianamacrophylla	cotyledon and leaf explants	*A. rhizogenes* R1000	Zhang *et al*., 2010
Salvia miltiorrhiza	plantlets	*A. rhizogenes* ATCC 15834	Zhao *et al*., 2010
Withaniasomnifera (L.)	leaf	R1000	Sivanandhan *et al*., 2013
PogostemonCablin	leaf explants or callus	*A. rhizogenes* ATCC15834	He-Ping *et al*., 2011
Gentiana. scabra	Leaves of invitro plantlets	*A. rhizogenes* strains ATCC15834	Huang *et al*., 2014
Salvia miltiorrhiza	Explants	*A. rhizogenes* bac- terium (C58C1)	Ming *et al*., 2013
Gymnemasylvestre	cotyledon and leaf explants	*A. rhizogenes* strain KCTC 2703	Nagella *et al*., 2013
Papaverbracteatum	excised shoots	LBA9402	Sharafi *et al*., 2013
Dracocephalumkotschyi	Leaf explants	ATCC 15834, A4, LBA9402, MSU440, A13	Sharafi *et al*., 2014
*Polygonummultiflorum*Thunb.	roots, internodals, nodals and leaves	*A. rhizogenes* strain KCTC 2703	Thiruvengadam *et al*., 2014
Nepetapogonosperma	leaves and stems	A4,ATCC15834,LBA9402,MSU440 and A13,	Valimehr *et al*., 2014
Calleryaspeciosa Champ.	cotyledon	LBA9402	Yao *et al*., 2016

Benefits of hairy root

The production of hair roots has been significantly enhanced in various fields: secondary metabolism engineering, rapid aggregation and excretion of metabolites after elicitation, processing of therapeutically recombinant proteins, trapping of medium-produced biomolecules and scaling-up of the culture system. Hairy roots expand and turn quickly. The genetic and biochemical stability of these distinct cultures and their effective efficiency provide major advantages over cell suspensions. The enormous capacity of the hairy root system for metabolite processing and phytoremediation has started to draw private companies (Stephanie Guillon *et al*., 2006). The hairy root system is reliable and extremely active under the conditions of free hormonal cultivation. The fast rise, low cost, simple maintenance and capacity to summarize a variety of hairy root crop chemicals give additional advantages as ongoing sources of useful secondary metabolites. Additionally, hair roots are a good source of phytochemicals which are useful as pharmaceuticals, cosmetics and food additives. In addition, these roots can synthesize more than one metabolite and thus prove cost-effective in commercial development. Transformation can affect the metabolism and create new compounds that cannot normally be produced untransformed (Zhi-Bu Hu and Min Du., 2006).

Phyto-chemical compounds and medicinal properties of different medicinal plants

Plants were used even before ancient periods for medical purposes. For certain instances, conventional treatment methods are also widely followed. The rise in populations, inadequate medication supply, prohibitive prescription rates, adverse effects of some synthesized drugs and susceptibility to commonly available drugs for infectious diseases also given expanded attention on the usage of plant content as a source of medicines for a broad range of human conditions. Medicinal plants are used as rich component options for pharmacopeia, pharmacopoeia or synthetic drugs in drug growth. Throughout fact, these plants play a vital role in the growth of human societies worldwide. Furthermore, certain plants are used as an essential food supply, and as a result, their medicinal qualities are suggested (Table 2). In the natural colour, insect control, diet and scent, tea and so on, plants are used in addition to medicinal uses. Various medicinal plant forms are used in many countries to avoid bees, float, muzzles and escape home and workplace. Now a days medicinal herbs are important sources for pharmaceutical manufacturing. The use of herbal medicine has risen significantly over the last few decades. Medicinal plant therapy is deemed very healthy since no or minimal adverse effects are found. Such treatments are aligned with nature, which is the biggest advantage. More than 90% of conventional medicinal recipes include medicinal plants, but this paper primarily deals with medicinal plants implicated in preventive intervention in disease prevention strategies.

TABLE 2. Medicinal plant and their properties

Plant name	Medicinal properties	Reference
Holostemma ada-kodien K. Schum	ophthalmopathy, orichitis, cough, fever, burning sensation, stomachalgia and also as expectorant, tonic, stimulant and galactagogue	Warrier *et al*., 1995
Rauvolfia micrantha Hook f.	Used for nervous disorders	Sudha *et al*., 2003
Plumbago zeylanica L.,	dys- pepsia, piles, diarrhoea, skin diseases, leprosy and rheuma- tism, anti- bacterial, antifungal, abortifacien	Verma *et al*., 2002
Salvia miltiorrhiza	treatment of menstrual disorders, cardiovascular diseases, and prevention of inflammation	Xiao *et al*., 2009
Rauvolfia serpentina	diarrhea and dysentery and also as an antihelmentic.	Madhusudanan *et al*., 2008
Gentiana macrophylla Pall.	viral-induced, neuro- pathic, respiratory, and cardiovascular diseases	Xue *et al*., 2008
Withania somnifera (L.)	tuberculosis, rheumatism, inflammatory conditions, cardiac diseases	Murthy *et al*., 2008
Saussurea involucrata	arthritis, high-altitude diseases and gynecological diseases	Fu *et al*., 2006
Centaurium erythraea	digestive, hepatic, febrifugal and tonic	Subotic *et al*., 2009
Glycyrrhiza uralensis Fisch	anti-mutagenic activity, anti-ulcer effects, protective action against hepatotoxicity, antitumor promoting activity and antimicrobial effects	Zhang *et al*., 2009
Gentiana macrophylla	abirritation, defervescence, lowering blood pressure, antibacterium, antiinflammation, and antirheumatism effects, curing virusinduced respiration tube and cardiovascular diseases	Zhang *et al*., 2010
Salvia miltiorrhiza	Menstrual disorders and cardiovascular diseases	Zhao *et al*., 2010
Pogostemon Cablin	anti-insecti- cidal, antifungal and bacteriostatic properties	He-Ping *et al*., 2011
Gentiana scabra	Inflammation, anorexia, indigestion, gastric infection	Huang *et al*., 2014
Gymnema sylvestre	dia- bêtes, Antimi- crobial, diuretic, stomachic, antihypercholestremic, hepatoprotective and anti-saccharine activities	Nagella *et al*., 2013
Withanta somnifera (L).	Antiaging, nerve tonic, recovery from neurodegenerative disorder	Praveen *et al*., 2012
Dracocephalum kotschyi	stomach and liver disorders	Sharafi *et al*., 2014
Polygonum multiflorum Thunb.	antibacterial, anti-tumor, anti- oxidative, analgesics, hemo- static, anti-HIV, immunological properties, spasmolytic and lower blood cholesterol	Thiruvengadam *et al*., 2014
Nepeta pogonosperma	anti-cough and asthma, antiseptic and diuretic	Valimehr *et al*., 2014
Callerya speciosa Champ.	cough suppressants and anti-asthmatics	Yao *et al*., 2016

CONCLUSION

We now move away from nature because our lifestyle is technologically knowledgeable. Though we are not able to hide from existence, we are part of it. Because grasses are natural goods, they are non-negative, fairly healthy, environmentally sustainable and readily accessible. Traditionally, several herbs are used for different seasonal conditions. To save human lives, they must be encouraged. Such as the tissue cultures of plants, hair culture is distinguished by rapid growth without the source of exogenous hormones and the strong yield of secondary metabolites. This review summarized systematically research on the hairy roots of medicinal plants including the mechanism, current conditions, applications of medicinal plant hair roots and their uses in human welfare.

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