Journal of Applied Biology & Biotechnology Vol. 11(2), pp. 1-10, Mar-Apr, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.110201

Agrobacterium rhizogenes as molecular tool for the production of hairy roots in *Withania somnifera*

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ARTICLE INFO

Article history:
Received on: June 28, 2022
Accepted on: October 10, 2022
Available online: January 22, 2023

Key words:
Agrobacterium rhizogenes,
Transformation,
Hairy roots,
Enhanced secondary metabolites,
Sustainable agriculture

ABSTRACT

India is the world's richest country, with a vast array of plants and genetic resources for medicinal plants. When it comes to the introduction of new plant species, environmental factors are crucial. When plants are subjected to various environmental conditions, they produce tissue-specific secondary metabolites. The main metabolites are found in varying amounts throughout the medicinal plant's tissues. With regard to location and environmental conditions, production of bioactive metabolites plays a critical impact. As a result, the agro-climatic conditions are favorable for the introduction and domestication of new imported plant types with improved and consistent contents. Keeping in mind the pharmacological importance of bioactive components, the current chapter focuses on the hairy root production used to increase their production through the use of new technologies. Keeping in mind the pharmacological importance of bioactive substances, the current chapter focuses on hairy root formation, which is aided by rhizosphere modeling through *Agrobacterium rhizogenes*. In some dicotyledonous plants, soil bacteria called *A. rhizogenes* causes hairy root disease. *A. rhizogenes*-mediated transformation aids in a better understanding of the rhizosphere's host-plant association system, as well as the use, transformation, and formation of new upgrade transgenic crops hairy root culture, which is beneficial for improved growth and continuous production of pharmacologically bioactive ingredients in elite germplasm.

1. INTRODUCTION

In the time of 1900s, the symphonic microbiological analysis discovered by Lorenz Hiltner finds out that the most diverse or microbial density was found very close to soil [1]. The biotic and abiotic elicitors have a major role in constituting the soil microbiome [2]. The carbon energy source is the primary constituent of microbial enrichment which is given by the plant. Furthermore, plant releases10–15% of their photosynthetic and absorbs into the rhizosphere [3]. Subsequently, the composition of root microbial networks is impacted by the plant species [4-6]. Indeed, there has been coevolution between the rhizosphere and plants occupied by communities of microbial. In different plant species, the composition of rhizo-deposit varies including species-species and unique rhizo-deposit, which require further studies on various plant species to recognize and learn their impact between microbes and root interaction.

Plants have been considered as a significant medicinal herb in the arrangement of Ayurvedic medicine [2,6]. Plants possess immense pharmacological significance thus are a great subject of research interest. According to the researchers, the Ashwagandha plant is helpful to treat neurological disorders and has types of properties such as anti-cancerous, antioxidative, and immunomodulatory [7-9]. The extraction of Ashwagandha was described to diminish the two-stage skin carcinogenesis decrease with the influence of croton oil and dimethylbenzanthracene [10]. It is likewise observed to be efficient in treating arthritis, behavioral, and problems related to stress [11]. Ashwagandha is an ancient medicinal herb with multiple health benefits in the reproductive or nervous system. Ashwagandha is helpful in early recovery after an illness. In India, the consumption of Ashwagandha

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is similar to the consumption of ginseng in China [12] and is likewise stated to create a positive cause onto the endocrine system, inflammation of joints, neural disorder, asthma, ulcers, insomnia, epilepsy female disorders, and osteoarthritic inflammation. The decoction of plant dried roots is utilized as a tonic for the medication of hiccup, cold, cough, and is viewed as exceptionally powerful in controlling diabetes and cholesterol level, overall rejuvenating [13]. Naturopathy gains positive impact worldwide for the medication of these infections [14-16]. As per the report of the WHO, the world's 80% of the total population relies on various kinds of drugs and APIs for their health issues [17].

These chapters give the summary of interaction between microbes and plants and increment the creation of derivatives from hairy roots. The research has been empowered by the utilization of bushy root composite plant systems, discussing the future potential, and featuring important applications. The transformation-based studies have been an advantage for research purposes including stable transgenic plant production [18, 19], secondary metabolite production, plant-pathogen interactions, and gene analysis**.** At present, technologies used in genetic engineering employing transformation through *Agrobacterium rhizogenes* are rapidly improving for cloning of plants, gene transfer which plays a key role in agriculture and plant biotechnology.

A. rhizogenes also called *Rhizobium rhizogenes* [20] is a strain of Gram-negative soil bacterium. It causes disease of hairy root in infected monocot and dicotyledon plants species. Moreover, its Ri (root inducing) plasmid consists of transferrable DNA encrypt root locus (rol) gene loci (rolA, rolC, and rolB) and also volunteers for the demonstration of hereditary material into the host cell. They also activate the adequate amount of growth of extremely branched hairy roots at the place of inflammation usually cotyledon or hypocotyl. It has been accounted that for the hairy root production, a high concentration of auxin signaling is required not ethylene signaling [21]. With untransformed aerial tissue, these roots systems can be maintained by culturing and hosted by plants. Over the most recent 30 years, a variety of plants for different purposes is widely used for metabolic engineering and production of recombinant protein to analyses of rhizosphere physiology and organic chemistry [22]. More recently, a biotechnology tool has been used for *A. rhizogenes* mediated hairy root production in various types of plant species to find a new biological vision like rhizosphere and its modeling to know the biochemical pathways involved, transformation and development of novel transgenic crops that can be utilized as a food product [23,24]. Metabolic enzyme functions can be managed by RNA interference or gene overexpression using root with a hair transformation, for example, a particular protein clarification whose main function is depicted in the species *Nicotiana glauca* for the biosynthesis of pyridine alkaloid [25]. For further studies of stable and reliable isotopes, hairy roots are used for a better understanding and explanation of various reactions which take place inside a biosynthetic pathway. For example, many researches on hairy roots of *Ophiorrhiza pumila* show that camptothecin is obtained from shikimate pathways and 2C-methyl-Derythritol 4-phosphate [26].

2. SECONDARY METABOLITES IN PLANT DEFENSE MECHANISM

Plants produced chemical constituents in the natural form for the strategy of survival called as secondary metabolites. In the plant kingdom, these secondary metabolites are regulated under different taxonomic or chemotypic group. Unlike primary metabolites such as nucleotides, amino acids, phytosterols, lipids, and organic acids, the biosynthesis of these secondary metabolites was initially related with inessentiality which was restricted to particular plant groups. These secondary metabolites as such do not participate primarily in the metabolic function in the plants such as growth, development, or reproduction. Moreover, their absence not resulting in death, but after a specific timeframe can cause danger to the survivability of the plant.

3. PHARMACOLOGICAL SIGNIFICANCE OF THE BIOACTIVE METABOLITES

Due to its tremendous pharmacological significance, the plants are being explored worldwide as a subject of considerable modern scientific research. Thus, the evaluation of their phytopharmacological activity is of great importance [27]. Various pharmacological activities are taken from the natural products, including antitumor, antiangiogenic, anti-inflammatory, cardioprotective, and immunomodulatory effects [28, 29]. Various herbal medicines are prepared for the medication of stress and anxiety, osteoarthritis, immunomodulatory, conjunctivitis, and tuberculosis in which the secondary metabolite used a constituent [30-34]. Various effects possess by these metabolites are antioxidative, immunomodulatory, anti-convulsant, adaptogenic, anticancer, and neurological effects.In the treatment of osteoarthritis, geriatric, behavioral, and anxiety Ashwagandha has been found to be very efficient [2, 35-41].

4. PLANTS SERVE AS RICH SOURCE OF BIOACTIVE METABOLITES

The direct relationship between biomedical and local use exists for the advancement of many different medicines by the utilization plants [42]. Worldwide for health and medicinal purpose on an average around 35,000–45,000 plant species are used for the treatment of various health ailments. [43]. Herbal medicine is utilized in wide forms (infusions, decoctions, ointments, powder, and syrup) worldwide [44-46] for the treatment of varied health ailments in different age groups of patients with no or fewer side effects [47,48]. Steroidal lactones, alkaloids, flavonoids, tannin, etc., are the several groups of chemical components that have been detected, extracted, and isolated from different plant sources. However, lower yield, genotypic variation, and variation in the substance of the pharmacologically active metabolite, long incubation period which is about 4–5 years between sowing, and uneconomical chemical synthesis and harvesting are the bottleneck in industrial creation of the pharmacological bioactive metabolites.

5. ENHANCEMENT OF SECONDARY METABOLITE FROM *IN VITRO*

This is a novel approach for producing bioactive substances with the utilization of cell or tissue culture techniques. With the utilization of this technique, we produce therapeutically important compounds from many medicinal plants. Under suitable conditions, *in vitro* culture of plants techniques gives a good deal with concentrating the production, regulation, and enhancement of secondary metabolites. In few cases, the cultures have been taken advantage for commercial production [49]. *In vitro* culturing of plant tissues gives an outstanding experimental and ethical framework to study growth, enhancement, and regulation of subsidiary products by providing them favorable conditions.

6. HAIRY ROOT COMPOSITE PLANTS AS A COMPLEMENTARY SOLUTION TO STABLE PLANT TRANSFORMATION

Colonization of rhizosphere by microorganisms results changes in plant development and growth. In different plant species, there is an absence

of effective transformation method which is significant for research. However, this is present in parts by the usage of *A. rhizogenes,* a direct relation of *A. tumefaciens* and a normally developing microbe of plants [50]. Most recent 100 years have revolutionized plant molecular hereditary thus dedication gives to the genetic modification to give birth to a new industry. Both organisms are responsible of transferring T-DNA into the plant. Roots with hair are evolved out of a wide variety of different dicotyledons plant families and also few gymnosperms. Hairy roots have the capacity to promote their growth in the absence of external plant chemicals (unlike organ culture system) which are considered as an advantage of to produce hairy root cultures, at that point, the roots are highly developed and branched under sterilized *in vitro* conditions. For a stable genetic engineered plant generation, studies of root microbe interaction and other secondary metabolite productions hairy root cultures are used [51]. These strategies are transformative particularly for root-microorganism interaction studies because of decreased time required to create transgenic plant tissues of the recalcitrant plants and the capacity to maintained independent of tissue culture. Working *Agrobacterium* and different framework recommend that discharge system may give a method for *Agrobacterium* to accomplish the benefit along with different microbes in the acidic region of the rhizosphere [52,53].

7. MECHANISM OF AGROBACTERIUM AND PLANT CELL INTERACTION

For the gene transfer from the bacterial cell, it is critical to study the bacterium host relationship and to optimize its DNA transfer systems [54]. The bacterium attached to the plant cell surface is helpful for *Agrobacterium* to interact with a plant cell. A plant is infected by root inducing plasmid to transform the cell gene of a plant due to which unorganized growth of plant cells occurs. Auxin and cytokinin help stimulate plant development hormones bring about in the arrangement of profusely branched hairy roots transferring the hairy root inducing plasmid to the plant cell which is encoded by protein. Vir area of the bacterial plasmid is activated by phenolic compounds like acetosyringone that is delivered by injured cells of the host plant.

8. *AGROBACTERIUM* PLASMIDS CHARACTERISTICS

Agrobacterium plasmid is large in size greater than 800 kb, it contains virulence region and T-DNA which is mobile DNA component that is integrated into plant cells useful for rhizogenesis. According to opine synthesized by hairy root, the Ri plasmid is categorized into two principal classes that are agropine type (more virulent) and other types of opine [55]; Rhodes *et al*. (1990). Plasmid size is between 180 and 250 kbp. As indicated by the development of opine by transformed plant tissue, the plasmid is arranged into agropine, mannopine, and cucumopine. For agropine, the T region is divided into TR-DNA and TL-DNA has the size 8–30 kbp and 15–20 kbp, respectively. Others two plasmid contains a single T-DNA [55,54]. Different *Agrobacterium* strains plasmid show varying degrees of similarity and share a large region of similarity. Ri plasmid has origin of replication, catabolism, opine synthesis, and virulence [44]. Ends of the T-DNA have 25 bp repeats. The right end of the T-DNA is utilized for the DNA transfer but not by the left sequence [56]. The wounded area of the host plant release phenolic signal compounds to this response vir region gene product induces transmit of T-DNA [44]. *Agrobacterium* is treated with acetosyringone which is a plant signal compound as an outcome single-stranded linear T-DNA molecule is induced [57]. Virulence areas contain vir genes that do not enter the plant cell but cause communication of T-DNA when joining the chromosomal DNA. Border sequence also helps in the cell of plant transformation for direct T-DNA processing [58]. From the vir region, six transcripts are integrated subsequently two consecutively expressed operons (virA and virG), signals are recognized and other four operons are activated (virE, virB, virD, and virC) and this leads to T-DNA transfer [59].

9. ROLE OF T-DNA IN HAIRY ROOT INDUCTION

In the transformed plant cells, T-DNA genes are expressed [54,56]. In oncogenes, there is unlimited proliferation in the changed cell occur without the presence of externally added phytochromes [60]. Tobacco has strong impact on the differentiation with having high degree of auxins which results in the formation of roots, although increased levels of cytokinins lead to shoot induction [61-63]. The transformed cells can be grown without the trace of phytohormones due to the presence of these hormones [55,64]. Plant cells cannot catabolize opine, its gene is present in T-DNA. Opine can be used as a significant source of C and N (carbon and nitrogen) by *Agrobacterium* [63].

10. MECHANISM OF TRANSFER T-DNA FROM BACTERIUM TO HOST PLANT

Mechanism of transmit of transfer DNA into plant cell is a multistep process involves repair, replication, and recombination activity but its complete integration is unknown [64]. For the transmit of transfer DNA from plasmid to plant cell, certain genetic elements are essential like three chromosomal *vir* gene, T-DNA right border sequences, and *vir* genes. The three different chromosomal genes are responsible for the switch in the bacterial cell surface composition and this promotes the attachment of T-DNA to the plant cell wall [65]. Border sequences are used to delimitate the regions of Ri plasmid that is transmitted to plant cell [66]. Endonuclease enzyme is encoded by virD operon which makes the gaps in the T-DNA on both the sides of same strand. As a conclusion, free ssDNA (single-stranded DNA) is formed. By cutting RB region, the 5'-3' single-stranded T-DNA is created. Then, this strand is transferred to plant cells [67]. When these border sequences are cut by VirD2 and VirD1, then T-DNA transfer initiates [68]. VirD2 helps to transfer to the nucleus by a nuclear localization sequence through the host importin alpha protein interaction and for efficient transfer of T-strands, this sequence is required [69]. The different effector proteins that are important for gene transfer are encoded by root inducing plasmid such as VirE, VirH, VirF, and VirD5 [Table 1]. Ri plasmid that induces adventitious hairy roots is formed closer to the site of infection. The transformed plant tissue produces unusual metabolites called opines by T-DNA gene [70,71]. TL-DNA of agropine plasmid and T-regions of cucumopine and mannopine plasmid induce the root formation [72]. TR-DNA agropine plasmid induces the roots which are phenotypically like to ordinary roots [61]. It was seen that alone TL and TR regions do not respond as strongly as when these both transformation regions are expressed together [73]. Alteration of auxin metabolism plays an important role for the outflow of root having hair phenotype in transformed cells [74]. T-DNA expression plays a crucial part in the induction of hairy root in transformed plant cell but it seems like auxin does not play a part for this expression. Transformed cells are more sensitive for auxin formation because the gene responsible for this is located on TL-DNA, yet it is restricted in certain plant species [75].

11. HAIRY ROOTS CULTURES CHARACTERISTICS

Culture of root having hair is able to grow in hormone-free medium and have fast-growing tendency with laterally highly branched [Figure 1]. The hairy roots are generally white to brown in color, soft, adventitious, and fast growing. The root having hair has growth rate

ranging between 0.1 and 2.0 g dry weight/liter per day. The capacity to form many new growing points of hairy roots making them more advantageous as compared to the conventional roots [76]. Between different species, the increment rate of hairy roots varies but difference also viewed in same species with different root clones [77].

12. HAIRY ROOTS CULTURE ESTABLISHMENT IN *IN VITRO*

Transformation is done aseptically by inoculating thick viable *A. rhizogenes* suspension cells with wounded plant parts. Emergence of roots takes place later the succeeding of 1–4 weeks at the particular place of transformation which is being cut off for further transfer it inside the growth medium which is hormone free but contains antibiotic to prevent contamination [78].

13. THE SENSITIVITY OF PLANT SPECIES TO *AGROBACTERIUM*

Different strains have different transformation ability that varies with other strains [79]. The chance for successful transformation is afflicted by plant tissue age and its differentiation status. The capacity to give rise to transformed cell also varies with the elevation of tissue differentiated after *A. rhizogenes* inoculation [80-83]. By assaying opine productions, the genetic modification can be confirmed. However, in hairy roots, opine production is unstable or may disappear after some time. Hence, to confirm the genetic modification, Southern blot hybridization techniques are performed to detect T-DNA [81].

Figure 1: *Agrobacterium rhizogenes*-induced profuse production of highly branched hairy roots on nutrient media using aseptic tissue culture technique.

14. *A. RHIZOGENES*: A MOLECULAR TOOL FOR RHIZOSPHERE MODELING

Hairy root production mediated by *A. rhizogenes* is known as a remarkable aid for the biosynthesis of subsidiary intermediates and other metabolic studies. It can also be used for various biotechnological formations of compounds which are derived from roots [82]. Roots with hairs consist of a better framework for persistent production of these intermediates in a sterile condition without using the cost-effective plant hormones in the culture medium. These organs are genetically fixed, and in host plant, they produce high content of secondary metabolites. By culture of root having hair, the alkaloid production is stable for years but its production decreases when callus is formed due to the induced roots. When roots redifferentiation is allowed, then the formation of alkaloid reappears [83,84]. Secondary metabolites are excreted into the growth medium by some hairy roots but the secondary product release varies in species. Secondary metabolite growth pattern and production also vary of root having hair culture. For commercial production, the secondary metabolites are dissociated from the growth [85] [Figure 2].

15. *A. RHIZOGENES*: FOR TRANSGENIC CROPS PRODUCTION

The crops which were obtained using transformation mediated by *Agrobacterium* are broccoli, pepper, sugarcane, carrot, barley, alfalfa, soybean, cotton, maize, wheat, rice, potato, and tomato [86,87]. *Agrobacterium* species has ability to transform a wide range of different neoplastic diseases, including cane gall from *A. rubi*, hairy root from *A. rhizogenes,* and crown gall from *A. vitis* and *A. tumefaciens* [88]. In the 1930s, *A. rhizogenes* was first identified, it belongs to Rhizobiaceae family in the alpha-2 subclass of Proteobacteria [50]. This assay is

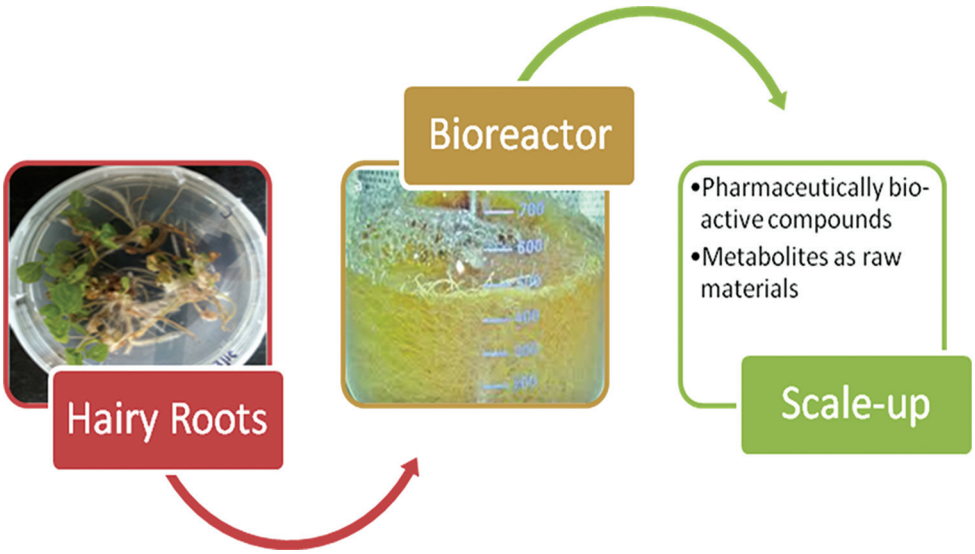

Figure 2: Hairy root culture for mass production of bioactive compounds.

rapid and inexpensive, required simple media for rapid growth and genetic manipulation can easily be grown in *Escherichia coli.*

16. MOLECULAR CHARACTERIZATION OF *A. RHIZOGENES*-MEDIATED HAIRY ROOT INDUCTION

Effective implementation of many crop yield improvement plans through molecular rearing includes isolation of gene regulatory sequences and useful genes by exploring different plant hereditary resources. For this means, the genomic DNA arrangement should be fit for restriction cleavage, polymerase chain reactions (PCR), and construction of complete and partial gene library. Besides, the genomic DNA samples are regularly use in restriction fragment length polymorphism, random amplified polymorphic DNA, Southern blot analyses, genome fingerprinting and genome mapping, screening of transgenic lines, or in other important molecular method [2]. Literature overview throughout the previous two decades clearly uncovers that the cetyltrimethyl ammonium bromide, cationic detergent-based DNA extraction conventions were significantly more time utilized for various plant materials as compared to different protocols.

Recognition of T-DNA in the apparently changed lines was recognized and displayed by PCR [29]. Cross primers such as *rolB* and *rolA* are utilized to recognize the TL T-DNA (5-ATGGAATTAGCCGACTAAACG-3 and 5-ATGGATCCCAAATTGCTATTCC-3), which is known as the normal fragment size of around 1440 base pairs. Primers used for the TRDNA (5-AATCGTTCAGAGAGCGTCCGAAGTT-3 and 5-CGGAAATTGTGGCTCGTTGTGGAC-3) (Slightom *et al*., 1986) created a large fragment of 1672 base pairs. Agropine synthase gene (*ags*) was identified using a primer (5-AGGTCTGGCGATCGCGAGGA-3 and 5-GCGCATCCCGAGGCGATG-3) [89,90], by producing a 512 base pair fragment as an complementary indicator for the TR T-DNA. Primers used for detecting the virD1 gene (5-ATGTCGCAAGGACGTAAGCCCA-3 and 5-GGAGTCTTTCAGCATGGAGCAA-3), which is present at the external region of the T-DNA of the root inducing plasmid and are not allowed to enter inside the plant genome, were utilized to remove the chances of error of polymerase chain reaction due to *A. rhizogenes* contamination caused by the root lines [91].

17. ADVANTAGES OF TRANSFORMATION

Transformation through *A. rhizogenes* has low copy number with few rearrangements as comparison to other technologies. *A. tumefaciens* method is preferred to transfer bacterial virulence protein to plant which will help to target the T-DNA into the nucleus, and hence at the time of integration inside the genome, it maintains the integrity. *Agrobacterium* is fast growing with genetic stability and can be cultured in huge scale ferment or for the secondary metabolite production. The transformation study helps in knowing better the rhizosphere microbiome outside and inside of a susceptible plant, to know the mechanisms which maintain *Agrobacterium* niche in the rhizosphere and what plant signal and environmental cues are coopted to promote its function, how the signals are recognized, does any signal given from *Agrobacterium* to plant that can promote signaling and become beneficial to it, the molecules excreted by the bacterium affect which gene of a plant does it show any host defense, should know the outcome of *Agrobacterium* to protoplasm attachment to

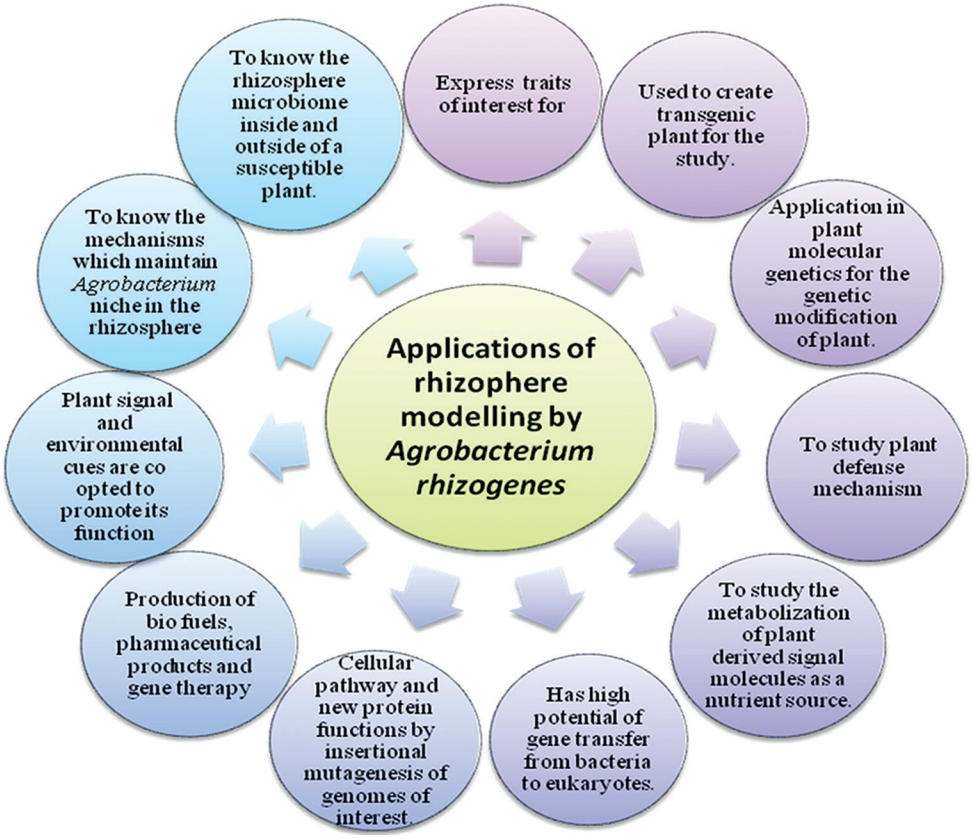

Figure 3: Applications of rhizosphere modeling by *Agrobacterium rhizogenes*-mediated transformation.

Table 1: Genes and other factors responsible in inducing transformation by *Agrobacterium rhizogenes.*

Gene	Function	References
VirA	For the sensing of phenolic compounds and acidity	[76]
VirG	Induction of the vir regulon by phenolic and monosaccharide inducers.	[77]
Dimethoxyphenol [acetosyringone and hydroxyacetosyringone or phenolic inducer	Induce the vir genes	[78]
Monosaccharides	Cell walls act in concert with phenolic inducers to increase the level of induction	[76]
Acidic conditions (pH 5.5)	Vir gene induction	[79]
VirD; VirD2	VirD2 is an endonuclease that nicks one of the two strands of the Ti plasmid at two sites which flank and delineate the T-DNA contains nuclear localization signals (NLSs) that help in direct transport of the T-DNA into the nucleus of the plant cell	[78]
VirB; VirB1–11	VirB1 encodes a transglycosylase which is not included and cleaves beta-1,4 glycosidic bonds, VirB2 encodes the synthesis of pilin, the subunit of the T-pilus	[78]
VirC; C1 and C2	VirC1 enhances the site-specific nicking by the VirD endonuclease and binds to the overdrive sequences, VirC1 helps to increase the no. of T-DNA copies per cell.	[80]
VirE; E1, E2, and E3	VieE1 helps to protects the T-DNA against nuclease degradation and maintains the integrity of the 3' end of the T-DNA prior to integration by the help of DNA transfer VirE1 encodes a chap erone which keeps the VirE2 protein from aggregating with itself inside *Agrobacterium*, virE3 encodes a host range locus	[81]
VirH	For a variety of compounds, these genes are usually associated with detoxification.	[81,82]
VirF	Mediates ubiquitination of proteins targeted for degradation by the proteasome, may be involved in proteolysis of proteins such as VirE2	[80]
VirD5	To overcome the instability of VirF, *Agrobacterium* transfers VirD5 into the plant where it binds to VirF and prevents its rapid turnover.	[80]

know whether this attachment affect bacterium physiology, does opine, phytohormones, and transformation products cause any changes to *Agrobacterium* physiology [Figure 3].

The advancement of a quick developing root culture framework would vary unique chances for construct root drugs in the research center crude turning on Pasteur developing [2,70]. In contrast with interrupted cell culture, modified roots having hair are exceptionally changed and can produce plant materials almost rich in derivatives. This raised the quantity of secondary products and, accordingly, the quick growth of transfect roots having hair is only the features of a successful production model for helpful phytochemicals.

Transgenic root cultures transfigured the job of plant tissue culture in the formation of subsidiary intermediate. They are interesting for biosynthetic and genetic conditions, proliferate, and more simply support. Utilizing this system, a broad of chemical components has been modified [55,92,93]. The promotive benefits of roots with hairs are that their cultures are frequently manifest approximately on an equivalent or larger synthesized limit for the production of secondary metabolite contrast to their mother plants [94].

In the middle of organ culture systems or different plant cell, culture of bushy root is one of the significant tools for the formation of root-derived compounds, biosynthesis of optional metabolites, and metabolic engineering studies [90]. Preceding reports recorded nearly low yields of withanolides from cultures of furry root [95].

A. rhizogenes moderate bristly root culture is a significant aid for the biosynthesis of valuable subsidiary products such as withanolides. Hairy roots are viewed a great technology for union of beneficial intermediatary components in a purified surroundings within the shortfall of cost effective development controllers inside the medium [86,74]. For some reason, *A. rhizogenes* moderate culture of root with hairs hairy root is consumed in various prime pharmaceutical plants for the proffering of intermediates of secondary production [95]. However, *A. rhizogenes* intervened hairy root inductive in *Withania somnifera* is restricted due to shortage of obtainable and proficient root with hairs initiation method [86]. Another methodology for a proficient root with hairs initiation is varying for extensive production of withanolides. Exertion is made to beat issues related to host/tissue to extend the amount of contaminated sites, such as utilization of highly toxic *Agrobacterium* strains and inclusion of few components to the crop productive medium. Laterally, sonication-assisted *Agrobacterium*-mediated transformation attracted high consideration in some species of plant [48,59]. It has been profitably put in for hairy root construction in *opium poppy* [70] and *Verbascum xanthophoeniceum* [52]. It gives high assurance for the increment of root with hairs creation. The lead of this technique is that the cavities achieved by sound effect create as many of microwounds on the outer layer of the explants. These microwounds allow *Agrobacterium* to tint prick and more absolutely all-round the explant than standard wounding, extend the probability of contamination to host cells (productivity in *Agrobacterium* was likewise refined in various species of plant by the use of heat care) [74].

The changed cultured of hairy roots in LB medium did not show any bacterial development demonstrating the without the presence of live *A. rhizogenes*, in accordance with perceptions by Hayta *et al*. (2011) in the culture of roots with hairs of *Gentiana cruciata*. In this review, a high rate of transformation (90%) was acquired in leaf explants which are infected with R1000 strain, with the development of 28.2 hairy roots (2–3 cm root length) per explants after the culturing of 12 days. The roots with hairs are delivered seen to be highly branched, fine, and soft. This broad branching, because numerous meristems are present, represented greater development rates of roots with hairs in culture as this occurrence was normal for the components of Solanaceae [78]. The novel framed roots with hairs were at first

white and eventually became brown with the exception of the growth in root tips and they exhibited the typical highlights of the crown ball disorder, that is, extensive lateral branching, hormone independence, and plagiotropic growth. Plagiotropism of furry roots was trademark as the consequence of *A. rhizogenes* intervened change [56]. In the current review, the furry roots got risen up out of the profound injured locales of the midrib of leaf explants as seen by Tiwari *et al*. (2007) in *Gentiana macrophylla*. Nilsson and Olsson (1997) guessed that cells that contain undeniable degree of sucrose and auxin are ideal focuses for bristly root enlistment. Tiwari *et al*. (2007) seen that the phloem cells, situated somewhere down in plant organs, could be the target of *A. rhizogenes*.

Transformation mediated by *Agrobacterium* is one of the techniques for genetic modification in different species of plant. The biosynthetic pathway of complicated compounds of *Agrobacterium* is still not revealed. The pharmaceutical products that are derived by hairy roots are not been profit oriented. The main cause for this is too low content production as compare to conventional extraction. The compound produce through *Agrobacterium* is re-evaluated by authorities for safety, efficiency, and quality which are hard for an industry to use.

The work on the results of molecular and genetic method toward creation of genetic plants with desired characteristics in integrated methodology under various national programs will provide a basis and opportunity to develop reliable biotechnology for functional purpose and create designer plants. There is an acute need to identify and characterize medicinal plants, that is, the chemistry of the active components, as they are the reservoir of the "medicine of the future." Further development is essential to protect wild populations of different plant species with their inherent interspecific diversity. Biotechnological approaches, obviously, have found potential as an upgrade to conventional agriculture in the culture of plant tissue, the advanced manufacture of plant bioactive metabolites, in search of alternative production of useful medicinal compounds from plants. *A. rhizogenes* are stable and show high productivity in hormone-free culture conditions so can be used for the manufacture of derivatives. Certain modifications in culture conditions can induce growth and increased alkaloid production. Components of signal acceptance by plant cells, and guidelines for the differential expression of enzymes and genes are attractive areas in the investigation of the biosynthesis of routine products of nature, and plant cell culture would be a very suitable model framework for these examinations. It is necessary to know the pivotal biosynthetic enzymes that affect its regulation or its expression. Misuse of varietal cell strains for such examinations will serve up the possibilities of using cell suspension cultures for the bioproduction of metabolites by bioreactor cultivation, biotransformation, and immobilization, which needs further consideration. Furthermore, rhizosphere modeling requires the development of efficient lineage transformation protocols, which can be used to recover plants containing transgenes encoding enzyme(s) for the rate-limiting step(s) of biosynthetic pathways. This may guide for enhanced enzymatic(s) activity *in vivo* and higher bioproduction of pharmaceutically active secondary metabolites.

18. FUTURE PERSPECTIVES

With the increasing demands of the pharmaceutical industries, natural products are being utilized at large scale for the manufacture of drugs. The best about the natural formulations is that the patients develop no to very less side effects. Thus, enhancement of pharmaceutically significant plant secondary metabolite through *Agrobacterium* can serve as very efficient and promising tool for the plant tissue culturists. Thus, we intend to transform the elite varieties of Ashwagandha with *A. rhizogenes* and develop new tissue culture of plant developed elite plantlets for commercial farming. Later, these plants can be utilized by pharma companies for manufacturing of natural drugs for the treatment of ailments with very less or no side effects.

19. AUTHORS' CONTRIBUTIONS

There are no conflicts of interest of the authorship.

20. FUNDING

No funding applicable in this work.

21. CONFLICTS OF INTEREST

There are no conflicts of interest of the authorship. The idea of manuscript and main manuscript was written by Dr. Manali Singh; while Kuldeep Jayant, Shruti Bhasin, Deep Chandra Suyal, and Dr. Sanjeev Agrawal helped in final editing.

22. ACKNOWLEDGMENT

We acknowledge G.B. Pant University of Agriculture and Technology, Pantnagar, and Invertis University, Bareilly, for providing the platform and necessary support and cooperation in preparing the manuscript.

23. ETHICAL APPROVALS

The work does not need any ethical appoval.

24. DATA AVAILABILITY

All the data pertaining to the manuscript has been provided in the manuscript.

25. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Singh M, Suyal DC, Dinkar N, Joshi S, Srivastava N, Maurya VK, Agnihotri A, Agrawal S. *Agrobacterium rhizogenes* as molecular tool for the production of hairy roots in *Withania somnifera*. J App Biol Biotech. 2023;11(2):1-10. DOI: 10.7324/JABB.2023.110201