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Hairy Root Culture: A Promising Approach in Biotransformation

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ABSTRACT

The production of hairy root culture by soil bacterium Agrobacterium rhizogene mediated gene transformation using plant tissue culture. Hairy roots induced when explants of plant infected with root inducing plasmid of Agrobacterium rhizogenes, T- DNA transmit in plant cell and express along with plant genome. Hairy root culture is novel source for valuable secondary metabolite production because of its fast growth without hormones, biochemical and genetic stability. To prevent destruction of root harvesting from several important plant species hence production of large amount hairy roots through in-vitro condition. The different strains are responsible for construction of hairy roots. This has been demoralized to make use of hairy roots as a convenient and superior option to yield constructive metabolites from the plants. Numerous compensation from using the hairy roots as a supply for plant products as well as facility for modification of metabolic pathways by integration of related genes have been demonstrated in recent times in plants of diverse origin. Integration of desirable genes to the plants using Agrobacterium rhizogenes mediated genetic transformation for plant improvement has also been successfully accomplished in several agriculture horticulture crops and in woody plants.

Keywords: Hairy roots, *Agrobacterium rhizogenes*, secondary metabolites.

INTRODUCTION

Plants are requisite source for the production of number of chemical substances like secondary metabolites and phytochemicals which find relevance in the pharmaceutical, food and flavor industries [1]. Transgenic plant produced by genetic engineering made it possible to commence the genes of interest to plants. This has cemented the way for plant upgrading as well as to achieve and amalgamate desirable products front the transgenic plant plants. *Agrobacterium rhizogenes* bacteria has the capability to introduce a part of its DNA called T-DNA into the plant cells resulting into production of neo plastic roots called hairy roots.

Hairy Root Culture was developed as the innovative path for bulky production of secondary metabolite [2], phytochemicals production [3]. Thus this technique is of massive significance to develop large amount of roots and secondary metabolites in short time to continuous supply of improved value products [4].

Hairy roots

Hairy roots production is carried out through the plant tissue culture technique in study plant metabolic processes or to manufacture precious secondary metabolites or recombinant proteins, often with plant genetic engineering. Hairy root culture is also called as transformed root culture from naturally occurring Gram negative soil bacterium *Agrobacterium rhizogenes* that contains root inducing plasmids (Ri plasmids). It infect roots of dicot and some monocot plants cause them to produce the opines which is a type of unusual amino acids (octopine, agropine,

nopaline, mannopine, and cucumopine). Such opines are used by the bacterium as a carbon, nitrogen and energy source and to grow abnormally. Transformed roots are morphologically different from normal roots in that they much more branched and have much lateral meristematic growth, which will lead to higher biomass.

The abnormal roots are easy to culture in artificial media without hormone, and they are neoplastic in nature, with hazy growth. The hairy roots produced by *Agrobacterium rhizogenes* infection have a high growth rate as well as genetic and biochemical makeup. Hairy root culture is a kind of plant tissue culture that is use to study metabolic processes of plants, secondary metabolites production, production of recombinant proteins, plant genetic engineering, phytoremediation, artificial seed production, biofortification, biopharmaceuticals.

Perspectives of Hairy root culture :

1] More genotype and phenotype stability :

Hairy root exhibits a high degree of chromosomal stability over prolonged culture period. The stability of hairy roots is an important advantage for both research and large scale industrial application. Stability demonstrated in terms of growth characteristics, DNA analysis, gene expression and secondary metabolites production.

2] High levels of secondary metabolite production

The common role of secondary metabolites in plants is defense mechanisms against their predators. Secondary metabolites are used as pharmaceuticals, agrochemicals, flavor, faineances, pesticides etc. The extraction of secondary metabolite from plants can not be economically synthesized due to its complex structure. Hence the use of hairy roots for the synthesis of secondary metabolites in larger quantity. Hairy roots shows high genetic stability as well fast growth rate on hormone free medium. Hairy root culture is unique tool for synthesis of high value secondary metabolite production and also valuable in studying secondary metabolite pathways such as follows :

- L-DOPA: A precursor of catecholamines, an important neurotransmitter used in the treatment of Parkinson's disease
- Shikonin: Use as an anti-ulcer agent and anti-bacterial
- Anthraquinone: used for dyes and medicinal purpose
- Opiate alkaloids: mostly codeine and morphine alkaloid use in medical purposes
- Berberine: an alkaloid with medicinal uses for cholera and bacterial dysentery
- Valepotriates: used as a sedative
- Ginsenosides: for medicinal purposes
- Rosmarinic acid: Use for medicinal purposes, antiviral and other suppression of endotoxin shock
- Quinine: for malaria
- Cardioactive or Cardenolides glycosides: To cure of heart disease

3] Metabolic studies using phytoremediation:

Environmental pollution is a global problem that is aggressive to all life forms including humans, animals and environment. The cost of cleaning up contaminated sites is very high. Genetically transformed hairy roots offer many practical advantages in experimental studies, such as ease of initiation, culture, and maintenance, indefinite propagation of material derived from the same parent plant, and genotypic and phenotypic stability. In phytoremediation use of plants for removal of environmental pollutants due to its low cost and safety of implementation. Transgenic plant roots make direct contact with pollutants in contaminated water or soil, for remediation of toxic substances and phytomining research [5].The hairy roots technology is an excellent platform for studying numerous aspects encompassing phytoremediation, xenobiotic biotransformation and degradation in plants, and for determining the responses of plant tissues to toxic heavy metals because hairy roots can be grown in large mass in culture media in a controlled environment and can therefore be subjected to various physiological assays. Also, these transformed roots are amenable to genetic operation and may make easy the categorization of genes that influence the phytoremediation capacity of plants. Thus, hairy roots offer a good prospect for the primary evaluation of transgenic efficacy in phytoremediation.

4] Production of artificial seed :

Production of synthetic seeds is helpful *in vitro* plant propagation technology, because it having many useful advantages over a commercial scale for the propagation of a variety of crop plants. These tools provide important technique for production of synthetic seeds for conversion of plantlets under *in vitro* and *in vivo* circumstances. This

technology is valuable for multiplication and conservation of best agricultural and endangered medicinal plant species, which are difficult to regenerate from natural seeds conventional methods [6].This technology developed in diverse cost-effectively significant plant species such as forage legumes, vegetable crops, industrially important crops, cereals, spices, fruit crops, plantation crops, medicinal plants, ornamental plants, orchids and wood yielding forest trees etc.

In artificial seed production experiments, the effect of concentrations of both gel matrix and time of exposure to calcium chloride on encapsulation of *in vitro* regenerated hairy roots with sodium alginate must be determined. Hairy roots in uniform size were taken and blot dried using filter paper and then mixed properly with the sodium alginate which prepared in distilled water. To absorb phenols and other compounds activated charcoal (0.2%) was added to the matrix to encapsulated hairy roots. The percentage of survival and conversion to plantlets were recorded after 60 days of storing [7].

5] Biofortification :
Biotransformation is a process of breeding crops with higher levels of proteins, minerals, vitamins, and fat content micro-nutrients in crops. In such a way rising the dietary substance of the edible portion of plant foods are to levels that consistently exceed the average content. This can be done either through conventional selective breeding or through genetic engineering. For example Wheat variety Atlas 66, this has high protein content. Other examples of plants are rice, carrot, spinach etc.

Deficiencies of iron, zinc, and vitamin A influence in excess of one-half of the world's population. Progress has been made to control micronutrient deficiencies throughout food fortification and supplementation, but new approaches are needed, especially to reach the rural poor. Scientific evidence shows this is technically feasible without compromising agronomic productivity. Predictive cost-benefit analyses too maintain biofortification as individual important in the armamentarium for controlling micronutrient deficiencies [8].

6] Green factories for Biopharmaceuticals:
Biopharmaceuticals are high value therapeutic proteins that propose immense consequence in the treatment of various diseases like heart attack, cancer, diabetes, strokes, hemophilia and anemia. Commercial production of therapeutic proteins was done by using bacteria and mammalian cell cultures. There are some disadvantages related to the cost, scalability, safety and authenticity of protein related to the maintaining aseptic conditions. Plants are one of the most powerful alternatives for the bioproduction platforms because of their economic and safety advantages over the traditional method or commercial methods.

7] Bioaccumulation of heavy metals :
Hairy roots are a convenient experimental tool for investigating the interactions between plant cells and metal ions. Hairy roots are proficient of collect heavy metals and we can investigate heavy metal tolerance in plants. Hairy roots also have potential for biological synthesis of quantum dot nanocrystals [9].Metal accumulation play important role in to understand plant biology, physiology and metabolism. Accumulation of heavy metals also shows some detrimental effect on cellular functions of plants.

Metals such as zinc, cadmium, nickel, manganese, cobalt, copper, are released into the environment from a range of sources of industries and agricultural field such as industrial effluent, sewage sludge, mining, military operations, fertilizers, and fossil fuel combustion. Soils and water metal contamination represents a risk to humans health, animals, ecosystems and also reduce soil fertility and crop yield in agricultural. Low-cost strategies are required for removing or. Dropping the bioavailability of metals from soil, environment, rivers and lakes, groundwater and sediments is very costly. Hence eco-friendly and low cost strategies are employed by production of hairy roots. The ability of plants to uptake and to detoxify metals can be supplementary exploited for the production of nanocrystals in living plant tissues [10].The nanoparticles of Au and Ag with dimensions 1 to 200 nm have been produced using a wide range of plant species [11]; the isolated nanoparticles play important role in medicine, chemical analyses, catalysis, biosensors, DNA detection, electrodes and electrical coatings.

Microbes used in the production of hairy roots :
Agrobacterium rhizogenes strains and selected plant source used for generation of hairy roots and production of respective secondary metabolites are listed in table :

Strains of microorganism	References	Plant Explants	Secondary metabolite produced
1.*Agrobacterium rhizogenes* strain MTCC532	Surender Khatodia1 and Kakoli Biswas2* (2014)	*Aegle marmelos, Boerhavia diffusa, Datura innoxia* and *Solanum xanthocarpum*	Comparative study of all medicinal plants
2.LBA 9402, LBA 9360 and A4	A.Giri et.al(1997)	*Acinitum heterophyllum*	Aconites, heteratisine, Atisine, Hetidine
3.ATCC 15834	Shrutika Dhakulkar(2005)	*Gmelina arborea* Roxb.	
4.RI000	Kyung - Hwan Han(1997)	Popler (woody plant)	Hairy Root Production
5.LBA 9402.	M. Petrova et al.(2013)	*Arnica montana L.*	Hairy Root Production
6.EHA105	Zhanyuan Zhang et.al(1999)	*Glycine max*	Hairy Root Production
7.*Agrobacterium rhizogenes*	Maria Petrova(2013)	*Arnica montana L.*	
8.*Agrobacterium rhizogenes*	Xiaoning Ni(2011)	*Camptotheca acuminata*	Camptothecin production
9.NCIM-5140		*Justicia adhatoda*	
10.ATCC 15834.	Gauri Abhyankar(2013)	*Phyllanthus amarus*	Genomic and metabolic fingerprinting
11.1855	Celine Dechaux, A MICHELE BOITEL-CONTI(2005)	*Datura innoxia*	Scopolamine
12.strain6 R1601	H. N. Murthy et al	*Withania sominifera*	Withanolide A
13.MTCC 2364 and MTCC 532 strain	Pawar and Maheshwari(2003)	*Withania sominifera and Solanum surrattense*	Withanoloid and solasodine
14.strain A4	Mohammad Hossein Mirjalili (2009)	*Withania somnifera*	nicotine, *Molecules* Hyoscyamine and scopolamine
15.ATCC 15834, R1000 and K599	Ananthapadmanaban Saravanakumar et al(2012)	*Withania somnifera*	Withanin A
16.A4, MTCC 532, TR105 and LBA 5402	C. G. Sudha et al(2012)	*Decalepis arayalpathra*	2-hydroxy-4-methoxy benzaldehyde
17.A4, ATCC 15834 and MTCC 2364	Smini Varghese et al(2014)	*Withania somnifera*	Withaferin A
18.A13	A.Ohara et al(1999)	*Crotalaria juncea L.*	Hairy root production
19.MTCC532	Surender Khatodia et al(2013)	*Solanum xanthocarpum*	Hairy root production
20.A4 and LBA	T. M. Martins et al(2003)	*Vitis vinifera*	Hairy root production
21.15834	Valerie Bonhomme et al(2000)	*Atropa belladonna*	Tropane alkaloid
22.MAFF 03-01724	M.Sauerwein et al(1994)	*Hyoscyamus albus*	Alkaloid production
23.*Agrobacterium rhizogene*	Ricardo Luís Mayer Weber et al(2011)	*Glycine max*	Hairy root production
24.MTCC 532	K.M.Mariya John(2009)	*Camellia sinensis L.* (Tea plant)	Hairy root production
25.strain R1000	Samir Kumar Gunjan et al(2013)	*Solidago nemoralis*	Hairy root production
26.NCPP 1855	Annalisa Giovannini et al(1996)	*Datura arborea and Datura sanguinea*	Hairy root production
27.LBA 9402	Smita Ray et al(2014)	*Rauvolfia serpentina*	Hairy root production
28.strain 8196	K.H.Beach	Forage legume	Hairy root production and characterization
29.*Agrobacterium rhizogenes* (ATCC 15834, A4 and LBA 9402)	Gangopadhyay M	*Plumbago indica*	Root biomass and plumbagin production
30.*Agrobacterium rhizogenes* (ATCC 15834, A4, WC, WR)	R. Pratap Chandran and V.P.Potty	*Ipomoea batatas,Solenostemon rotundifolius, Vigna vexillata, Pachyrrhizus erosus, Canavalia spp.*	Hairy root production

CONCLUSION

Hairy root is unique application of plants for higher production of valuable products. The above reports by various authors proved that laboratory production of hairy roots are cost effective, high yield in less time than conventional way. By using genetic engineering fabrication hairy root produced pharmaceutically important products, improved quality of proteins, vitamins, used in biofortification, bioaccumulation of heavy metals, phytoremediation and production of artificial seeds.

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