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AGROBACTERIUM MEDIATED HAIRY ROOT INDUCTION AND SOLASODINE PRODUCTION IN *SOLANUM MELONGENA* L. VAR INSANUM (L.) PRAIN.

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ABSTRACT

Investigation was carried out in *Solanum melongena* L. var. *insanum* (L.) Prain. in terms of hairy root induction and solasodine production. Hairy roots were induced from regenerated shoots using *Agrobacterium rhizogenes* ATCC 15834. The hairy roots were characterized by their vigorous growth branched in nature and negative geotropism. The hairy roots are isolated and subjected into mass cultivation. Mass cultivated hairy roots were harvested and dried for solasodine production. The solasodine content of hairy root was higher than that of field grown sample and callus derived samples.

***Keywords*:** *Agrobacterium rhizogenes*, Hairy root, Solasodine

Introduction

Agrobacterium rhizogenes is the largest producer of medicinal herbs and is appropriately called the Medicinal plants constitute a very important bioresource of India because it has one of the richest plant based ethno medical plants and herbal medicines were estimated to be worth US $80 billion a year. Hence, India is known as botanical garden of the world (Ahmedulla and Nayar, 1999) appropriately. International export trade in medicinal plants from India was 3.26 tonns a year. The need for medicinal plants increased due to the revival of interest in herbal medicine (Shawl and Oazi, 2004). Higher plants have continued to play a dominant role in the maintenance of human health since ancient times due to presence of bioactive compunds (Farombi, 2003). Natural products from higher plants used as pharmaceuticals, flavor, food additives and agrochemicals in current and future efforts toward sustainable conservation and rational utilization of biodiversity (Balandrin and Kloucke, 1988). Among the various plants associated with the life of Indians, one such family is *Solanaceae* that is economically very important as several of its species are sources of food, fodder and drugs. *Solanum insanum* (L.) Prain, an Indian member of *Solanaceous* family.

Solasodine, which is a nitrogen analogue of diosgenin and can be similarly converted to starter compound 16-dehydropregnenolone (Sato *et al*., 1951) has been used for the semi synthetic production of pharmaceutical and contraceptive steroids in many parts of the world, especially the former U.S.S.R and Eastern Europe, India and China (Mann, 1978) and it continues to be a potentially important source in many other countries.

Genetic transformation of plants using the causative agent of hairy root disease *A. rhizogenes* in several plants, has emerged as an important substitute to intact plant as well as cultures for the production of secondary metabolites (Giri and Narasu, 2000). Hairy roots have been reported to yield higher amounts of secondary metabolites than cell suspension cultures and in some cases, intact plant roots (Allan *et al*., 2002).

Agrobacterium rhizogens inoculation is arising hairy root system became popular in the last decade as a system to produce secondary metabolites synthesized in plant roots (Toivonen, 1993 and Palazon *et al*., 1997 b). Genetically transformed organ cultures, such as hairy roots and shooty teratomas obtained following transformation with *Agrobacterium* Ri/Ti plasmids are interest for study of secondary metabolite formation or for high production of specific secondary metabolites (Jung and Tepfer, 1987; Saito *et al*., 1992).

High growth rate and ability to synthesize root derived secondary metabolites are the main characteristic of Hairy root cultures. Root cultures need an Exogenous

phytohormone supply normally and grow very slowly, resulting in negligible secondary metabolite or poor synthesis. Conversely, the use of hairy root cultures has modernized the role of plant tissue culture for secondary metabolite synthesis. These hairy roots are distinctive in their genetic and biosynthetic permanence. Their low doubling time, fast growth, ability to synthesize a range of chemical compounds and ease of maintenance offers an additional advantage as a continuous source for production valuable secondary metabolites.

Materials and Methods

Hairy root induction

Plant material

The *in vitro* raised shoots of ***Solanum melongena* L. var *insanum* (L.) Prain.** were used for hairy root induction (Infection with *Agrobacterium rhizogenes* (ATCC 15834)

Bacterial strain

Wild type strain of *Agrobacterium rhizogenes* (ATCC 15834), obtained from Microbial Type Culture Collection, Institute of Microbial Technology Chandigarh, India (MTTC), were used for infecting the plant material. Nutrient broth (NB) and nutrient agar used for growth and maintenance of the bacterial culture. The strain showed better growth response in nutrient broth. Therefore, nutrient broth was used for culturing of *Agrobacterium rhizogenes* ATCC 15834.

Plant tissue culture medium

MS basal medium was used for maintenance and growth of hairy roots in slants.

Induction and establishment of hairy root culture

For hairy root induction, 48-hrs old culture of *Agrobacterium rhizogenes* ATCC 15834 was first centrifuged at 3000 rpm for 5 min and the resultant cell suspension was resuspended in 5 ml of sterile MS medium. This suspension was used for infecting the plant material.

Co - cultivation

The *in vitro* grown shoots were placed on glass petridishes. Bottom of the shoots were purposely -wounded using sterile needle. A 10 µl aliquot of prepared suspension was applied at a base of shoot and placed in to the culture tubes containing the MS basal medium. The infected shoots were subjected to co cultivation for 48 hrs.

Proliferation

After 48 hrs of co-cultivation of shoots and bacterial cells, the shoots were removed and basal portion of shoots were washed thoroughly with sterile MS medium containing 250 mg /l cefotaxime. Washed shoots were blot dried with sterile filter paper and placed on proliferation medium. Cultures were maintained at 25 ± 2^0C with 16/8 hr photoperiod 3000-lux.till inductions of hairy roots. After initiation of the hairy roots the fast growing root tips were sub cultured and maintained on solid MS medium .The hairy root cultures were also maintained in MS liquid medium on a rotary shaker (80 rpm) in complete darkness. Various growth characters of established root clones were observed.

PCR analysis of hairy roots

Isolation of genomic DNA

Genomic DNA was extracted using CTAB method (Doyle and Doyle 1987) from each of the hairy root lines as well as from control (non-transformed roots).

Confirmation of transformation-PCR

PCR primers specific for the amplification of the 780 bp fragments of the *rol* B genes were used. A 50 µl PCR mix contained 200mg of DNA, 10 pmoles primers, 200 µM dNTP mix, 1U of Taq DNA polymerase, 1X PCR buffer and 2 mM $MgCl_2$ .PCR conditions were 94^oC for 5 min and 72^oC for 10 min .The sequences of the primers used in the PCR are as follows.

1. 5' ATGGATCCCAAATTGCTATTCCCCCACGA3' and

2. 5'TTAGGCTTCTTTCATTCGGTTTACTGCAGC3'

Solasodine production

Field grown whole plants of *Solanum melongena* L. var. *insanum* (L.) Prain. were collected. The whole plants were washed with tap water and shade tried. Then the plants were separated into leaf, stem, root, pericarp, and seed. All the separated parts were subjected to shade dry during for 10-15 days. Then the dried parts were ground in to powder and solasodine was estimated using HPLC.

Results and Discussion

Induction of hairy roots and solasodine production

Hairy roots were induced from multiple shoots obtained via micropropagation were infected with *Agrobacterium rhizogenes* ATCC 15834. The multiple shoots were infected with *Agrobacterium rhizogenes* with different time intervals (2, 4, 6, 8, and 10). Before infection with *Agrobacterium rhizogenes* the basal portions of the shoots were pricked thoroughly (0.5 cm) to facilitate the infection. After infection the infected shoots were transferred to MS basal medium for co cultivation. The co cultivation period was 24, 48, and 72 hrs. Among the various infection periods six mts duration was found suitable to induce hairy roots. The infection period beyond six mts leads to over growth to bacteria. The co cultivation period of 48 hrs facilitate hairy root induction without any over growth of bacteria. The antibiotic cefotoxime 250 mg/ L was used to control the over growth of bacteria. The same antibiotic concentration also used for washing of basal portion of the shoots, before transferred to selection media.

The fast, vigorously growing hairy roots were isolated and sub cultured on hormone free MS liquid medium for mass production (Fig. 1 & 2). After 45 days the hairy roots were harvested, dried and used for solasodine estimation by HPLC. The solasodine content in transformed hairy root were recorded as 4.52 mg/g dwt.

PCR Analysis

PCR analysis was carried out to confirm the presence of rol B gene fragment DNA of transformed hairy root. The expected 780 Pb rol B fragment was found in the positive hairy roots. Control roots did not show the fragment (Fig. 3).

In this investigation hairy roots established in *Solanum melongena* L. var. *insanum* (L.) Prain. The extensive hairy roots were induced from *in vitro* grown shoots using

Agrobacterium rhizogenes ATCC 15834. Though a lot of work has been done on induction of transformed hairy roots from various plants of different genera there is no report on induction and establishment of hairy roots in *Solanum melongena* L. var. *insanum* (L.) Prain.

The earlier workers reported that the extensive branching of hairy root was due to the presence of many meristems, which accounted for high growth rate of hairy roots. This particular characteristic was observed to be the most common for members of *Solanaceae* (Flores and Filner, 1985). Similarly Alvarez *et al*.(1994) initiated hairy roots and the produced solasodine in some species of *Solanum*, e.g. *S. eleagnifolium* and *S. mauritianum* (Drewes and Van Staden, 1995).

Like that of our present study, Yu *et al*.(1996) and Kittipongpatana *et al*. (1998) extensively examined the improved growth and solasodine production in hairy root cultures of *S. aviculare*. The hairy roots transformed by *A. rhizogenes* improved higher yield of secondary metabolites compared to those from natural plant material in *Solanum aviculare* This result was similar to those in hairy root cultures of *Solanum melongena* L. var. *insanum* (L.) prain.

Similar observations were made in several *Solanum* sp. and the enhanced solasodine production was reported. Hairy roots in *Solanum aviculare* grew faster and also accumulated up to 4.2 times more solasodine when grown under dark, but not in light conditions (Argolo *et al*., 2000. Mukundan *et al.,* 1998), mentioned that the transformed roots of many plant species have been widely studied for the *in vitro* production of secondary metabolites. Hairy root cultures are promising source for the standardized and continuous production of secondary metabolites over several successive generations without losing genetic or biosynthetic stability. This property can be utilized by genetic engineering to enhance their biosynthetic capacity. It is understandable to note that, in this present research work the transformed roots were confirmed by polymerase chain reactions. Earlier workers like Bonhomme *et al.,* 2000 confirmed the hairy roots *Atropa belladonna* by PCR technique. Godo *et al.,* 1997 confirmed the transgenic plants of *Nierembergia scoparia* (*Solanaceae*) derived from hairy roots by PCR analysis.

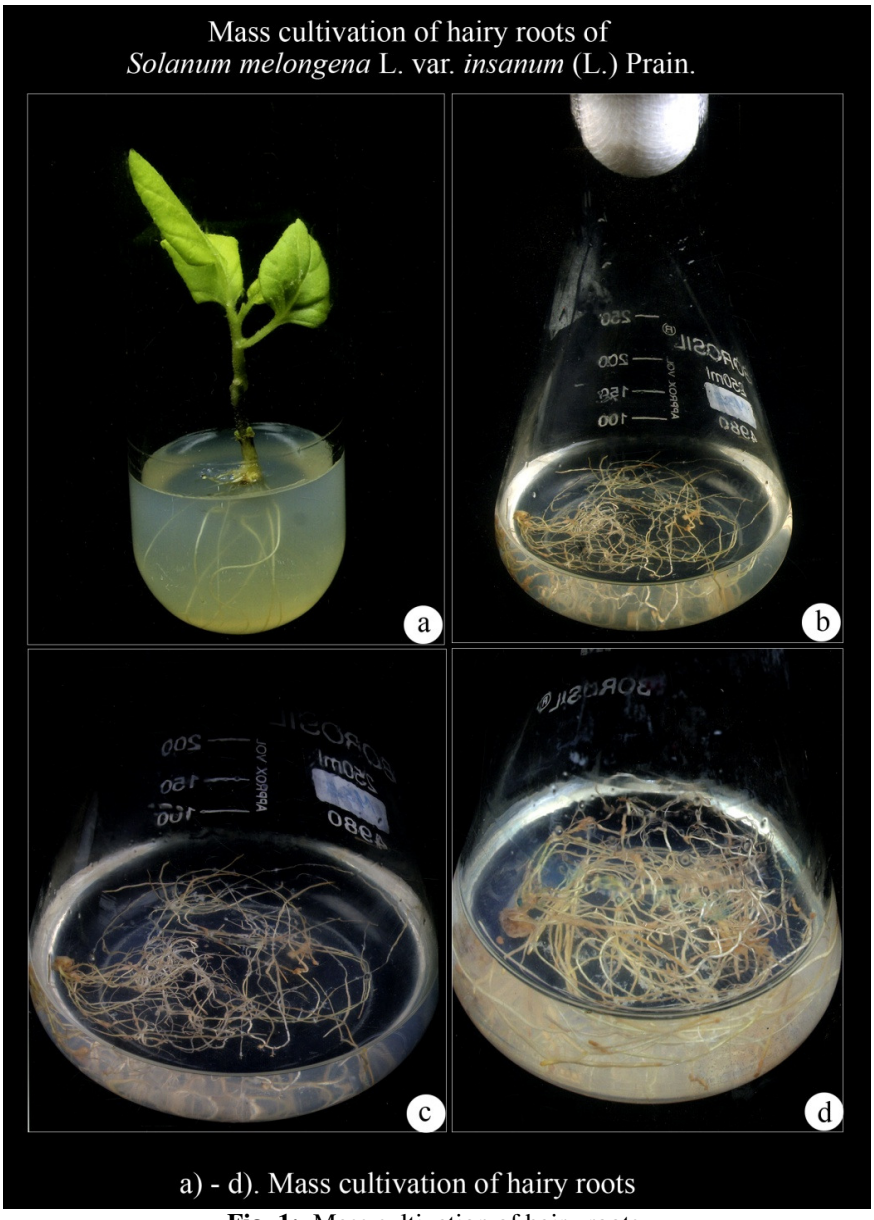

Fig. 1: Mass cultivation of hairy roots.

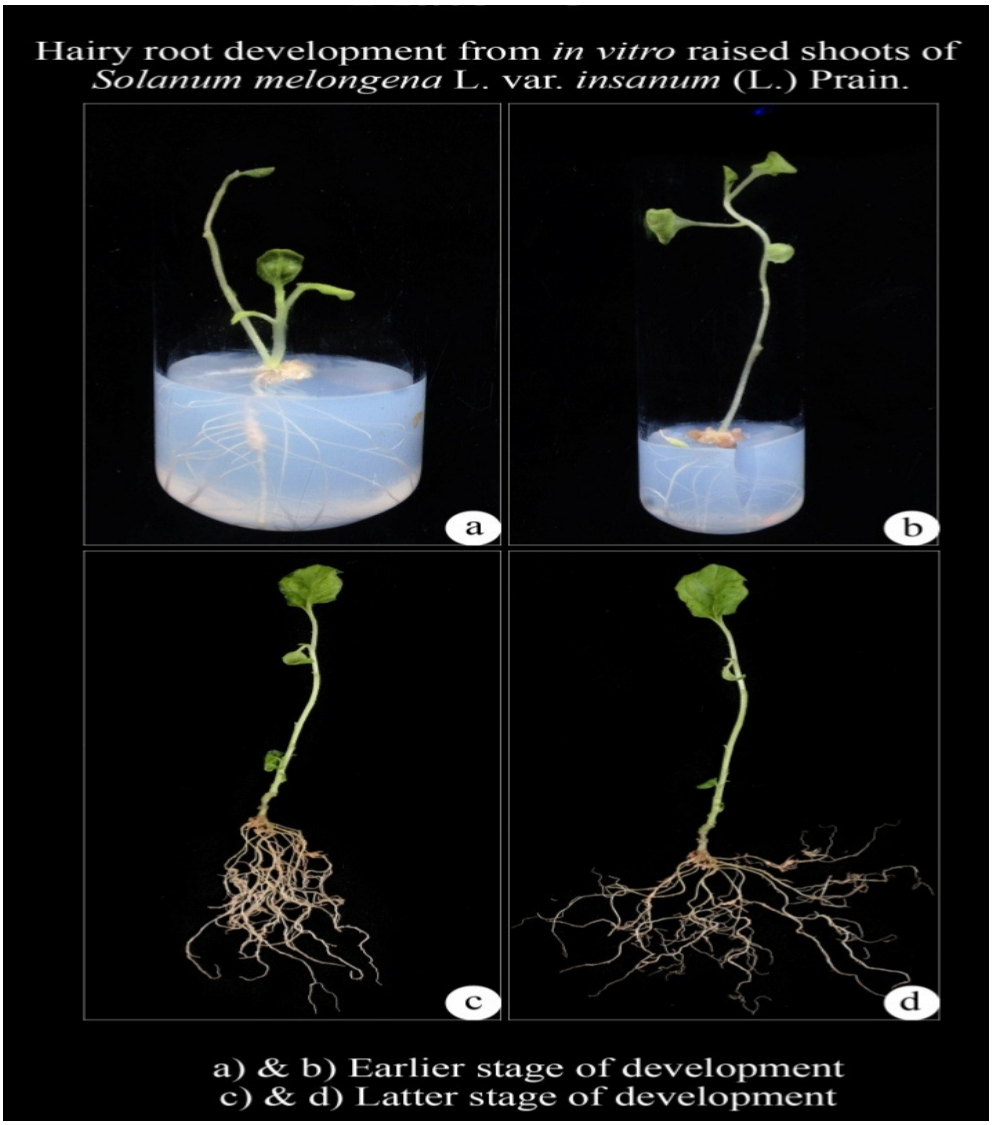

Fig. 1 : Hairy root development from *in vitro* raised shoots.

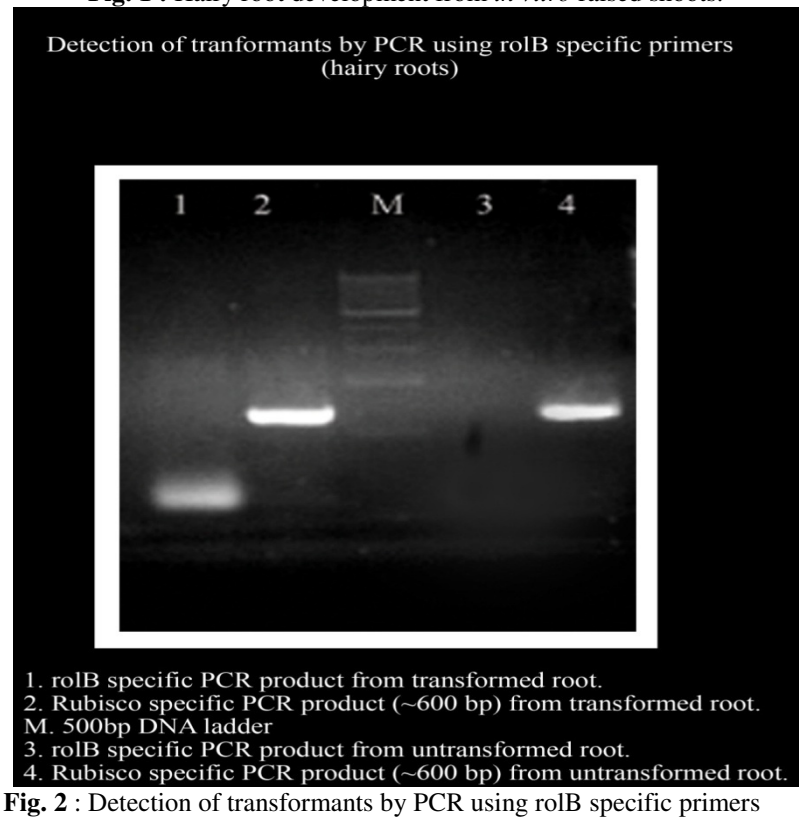

Fig. 2 : Detection of transformants by PCR using rolB specific primers

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