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Full Length Research Paper

Hairy root induction from hypocotyl segments of groundnut (*Arachis hypogaea* L.)

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Hairy roots were induced from hypocotyl explants excised from seven day old aseptically grown seedlings of groundnut using *Agrobacterium rhizogenes* 15834. The percentage of hairy root induction and number of hairy roots per ex-plant varied with infection period. The suitable co-cultivation period was 48 h. The hairy roots were fast growing, thin, slender and sometimes having branches which varied in their morphological nature. The cefotaxime concentration of 250 mgL^{-1} was found to be most suitable for hairy root induction in groundnut.

Key words: *Arachis hypogaea*; *Agrobacterium rhizogenes*, hairy root induction.

INTRODUCTION

Highly productive and stable hairy root culture has been obtained by the genetic transformation of plant tissue by the pathogenic soil bacterium *Agrobacterium rhizogenes*. The infection of dicotyledonous plants by *A. rhizogenes* caused roots to proliferate rapidly at the infection site. This phenotypic change results from the insertion into the plant genome of t-DNA carried on the bacterial Ri-plasmid coding for auxin synthesis and other rhizogenic functions. *A. rhizogenes* is well known to induce hairy roots when it infects plant tissues (Mugnier, 1988) and used as a vectors of foreign DNA for a wide range of dicotyledonous plants (Simpton et al., 1986; Morgan et al., 1987; Rech et al., 1989; Visser et al., 1989). Plant tissue transformed by wild-type strains of *A. tumefaciens* or *A. rhizogenes* can be readily distinguished by their oncogenic phenotype – tumours or hairy roots respectively (Spano et al., 1982). The ongogenic strains of *A. rhizogenes* can be conveniently used to transform a range of plant species; since they induced hairy roots which can be regenerated into whole fertile plants (Sevon and Marja, 1995; Puddephat et al., 2001; Christey and Sinclair, 1992; Christey et al., 1997; Hatamoto et al., 1990). In legumes proliferous root growth and abundant lateral branching of hairy roots are considered to be useful for improving nitrogen fixation. Peanut or groundnut (*Arachis hypogaea* L.) is a popular and important food legume known for its high protein and oil content (Cheng et al., 1992). It has a high energy value (Cobb and Johnson, 1973) and suitable for wide variety of agroecological conditions (Norden et al., 1982). Tissue culture studies in groundnut have been well documented including some recent studies (Palanivel and Jeyabalan, 2000, 2002; Palanivel et al., 2001; Palanivel et al., 2002). But in terms of hairy root induction only very few reports are available. So, the present study was aimed to induce hairy roots from isolated hypocotyl segments of groundnut.

MATERIALS AND METHODS

Plant material

The commonly cultivated groundnut cultivar VRI-2 that was used as an experimental material obtained from Anna Agricultural Farm, Kudumiyanmalai, Pudukkottai district, Tamil Nadu, India.

Bacterial strain

A. rhizogenes ATTC 15834 was obtained from Microbial Type Culture Collection Centre (MTCC), Chandigarh, India.

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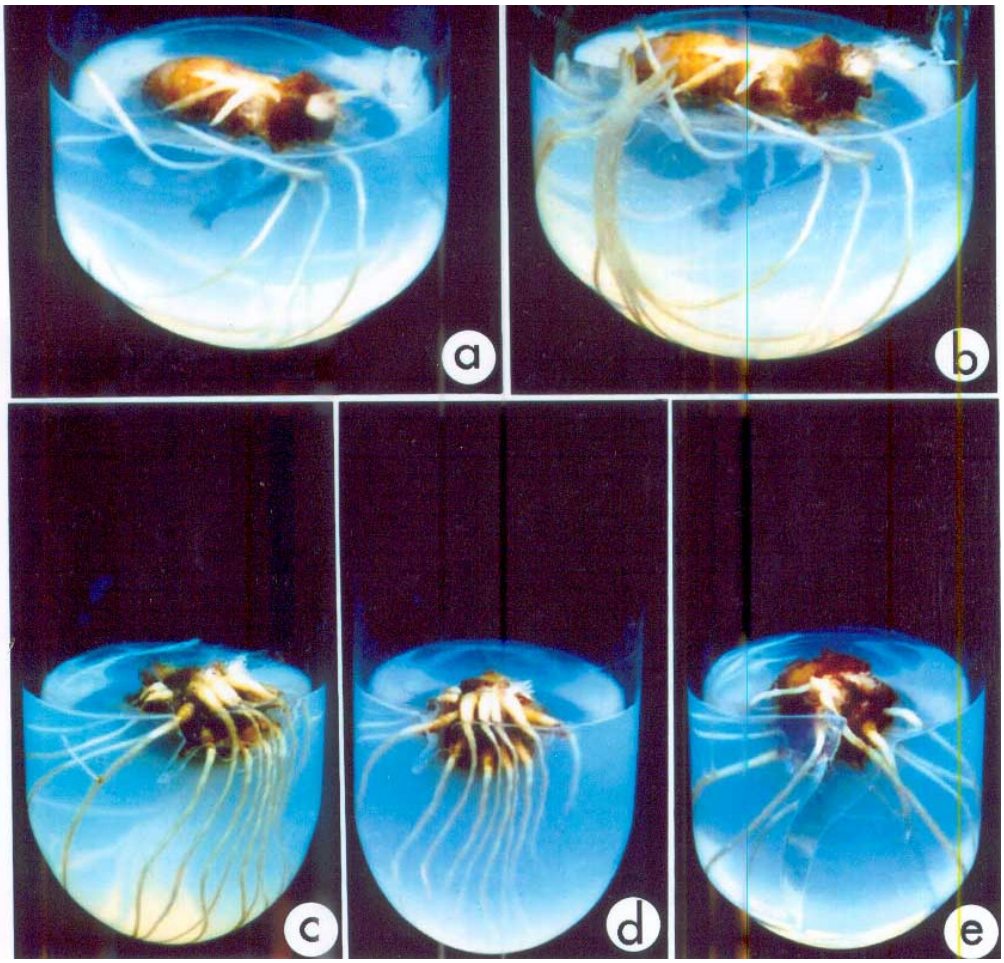

Figure 1. The frequency of root formation and number of roots per explant was reduced with increasing infection period, a - e.

Surface sterilization

Seeds of groundnut cultivar VRI-2 were washed with tap water for 10 - 15 min followed by immersion in liquid detergent solution for 5min. After washing with distilled water, the seeds were again washed with 70% alcohol for few seconds and rinsed three times with distilled water. Then the seeds were brought to the inoculation chamber and surface sterilized with 0.1% $HgCl_2$ for 8 to 10 min and again rinsed with sterile distilled water for 5 to 7 times.

***In vitro* germination**

The surface sterilized seeds were aseptically transferred to pre-sterilized culture tubes containing moistened cot-ton/ MS basal medium. Then the culture tubes containing seeds were maintained in culture room for germination.

Preparation of bacterial strain for infection

The bacterial culture was prepared by culturing a loopful of bacteria in 25 ml of nutrient broth medium for 48 h at 160 revs/min at 28ºC in the dark. The bacterial pellets were collected by centrifugation at 10,000 rpm for 15 min and again re-suspended with liquid MS medium at a density of $X10^8$ cells/ml and used for infection.

Collection of ex-plant and infection with *A. rhizogenes*

From 7-day-old aseptically germinated seedlings, the hypocotyl segments were excised and cut into small pieces (1 cm^2). The hypocotyl segments were soaked in the bacterial suspension for different time intervals (5 to 25 min) for infection. The hypocotyl segments were injur-ed all over the surface to facilitate the infection process before treatment with *A. rhizogenes.*

Co-cultivation and hairy root induction

After infection, the hypocotyl segments were placed on sterilized whatman filter paper in petridish to remove ex-cess of moisture present on the surface of the explants and inoculated on MS basal medium for co-cultivation. The duration was 24, 48 and 72 h. After co-cultivation period was over, the hypocotyl segments were washed with MS basal medium with cefotaxime (250 mgL^{-1}) to remove over growth of *A. rhizogenes* on the surface of the ex-plant. After washing, the hypocotyl segments were transferred to culture tubes containing MS basal medium with cefotaxime (250 mgL^{-1}) for hairy not induction.

Collection of data

After 25 - 30 days of inoculation, the number of responsive explants

Table 1. Effect of *Agrobacterium rhizogenes* on hairy root induction from hypocotyl segments of groundnut cv VRI-2.

Infection period (min)	Co-cultivation period (h)	Percentage of response	Number of roots per ex-plant
5	48	73.5 ± 1.07	8
10	48	68.3 ± 1.90	10
15	48	60.0 ± 2.16	20
20	48	54.6 ± 1.77	13
25	48	48.2 ± 1.65	12

and number of hairy roots/explants were recorded and the data were calculated.

RESULTS AND DISCUSSION

Hairy roots were induced from hypocotyl explants of groundnut when infected at different time intervals with *A. rhizogenes*. The percentage of responsive explants and numbers of hairy root per explants was varied in terms of infection period (Table 1). The hairy roots from hypocotyl segments were thin with faster growth rate and they also have branches unlike normal roots. These findings have been also reported in groundnut by other workers. The epicotyl explants derived from mature embryo axis of groundnut were infected with wild type strain of *A. rhizogenes* MAFF-02-20266 produces hairy roots (Akasaka et al., 1998). The frequency of root formation and number of roots per explants was reduced with increasing infection period (Figures 1). The above fact was also found to be true with the earlier reports. A delay in root induction was observed when the hypocotyls segments were infected with *A. rhizogenes*. The similar trend was already reported in *Ericax darleyensis* (Viemont and Lambert, 1994). The delayed root formation may be due to substances released by the bacterium. The hairy root was different in their morphology when compared to control. This was also reported in several plant species by several workers (Handa, 1994; Tepfer, 1983; Godo et al., 1997; Daimon and Mii, 1995; Kumar et al., 1991; Gautheret, 1985; Ooms et al., 1986; Jaziri et al., 1994; Mazur and Falco, 1989). The infected hypocotyl segments, from young seedlings produced hairy roots from the infection site. Like that of present research work, hairy roots were induced from *Hyoscyamus muticus* and some tropical pasture legumes (Kamble and Eapen, 2005). Hairy roots were also induced from several leguminous seedlings with different *A. rhizogenes* strains like LBA 9402 and 2659 by wounding stem or hypocotyl portions. The hairy roots induced from hypocotyl segments of groundnut may be used for obtaining transgenic groundnut plants.

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