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PROGRESS REPORT NO. 4

RI-PLASMID INDUCED ROOT CULTURE AS A POTENTIAL SUBSTRATE FOR IN-VITRO PROPAGATION OF MYCORRHIZAL FUNGI

4. The Development of the Root VA Mycorrhizal Fungus Culture System

A RESEARCH PROJECT

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Submitted by

Malee suwana-adth
Project Leader

Kasetsart University Research and Development Institute
Kasetsart University
Bangkhen, Bangkok

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Project Profile

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Project Leader	:	Malee Suwana-adth, Ph.D.
Organization	:	Kasetsart University Research and Development Institute Kasetsart University, Bangkok.
Co-investigators	:	Ms. Poonpilai Suwanarit Dr. Wiwut Daengsubha Ms. Yupa Mongkolsook
Project Consultants	:	Professor Dr. Chua Nam Hai, The Rockefeller University Professor Dr. John Monge, University of California (Riverside)
Authorized Officer	:	Associate Professor Dr. Thira Sutabutr Director, Kasetsart University Research and Development Institute
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Introduction

VA mycorrhizal fungi, which form symbiotic association with plants, cannot be propagateds on artificial media but has been shown to infect root cultures of tomato (Miller - Wide man and Watrud, 1984). Infection was, however, constrained by root thickening and lignification as well as by limited formation of lateral roots. Furthermore, the cultivation of roots depended on plant hormones in the culture medium.

On the other hand, Agrobacterium is known as an oncogenic agent and can neoplastically transform cells of many kinds of plants into tumour cells. Agrobacterium - induced tomour can be removed and cultured in vitro where they can continue to grow indefinitely (Hooykaas and Schilperoort, 1986).

It is therefore proposed to use Agrobacterium to induce and accelerate root formation in selected plants and to develop bacteria - free, fast-growing (tumour) root cell lines suitable for mycorrhizal association with useful VA mycorrhizal fungi. The two-member root-mycorrhizal fungus culture system could subsequently be used to propagate, aseptically, VA mycorrhizal fungi inocula.

Material and Method

Bacterial Culture

Agrobacterium tumefaciens ATCC 15955 and Agrobacterium rhizogenes ATCC 15834 were obtained from the American Type Culture Collection, U.S.A. through the Bangkok Microbiological Resource Center for SE Asia at Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. The culture were maintained on a nutrient agar medium under refrigeration. Inoculum was prepared by suspending cells from a 48-hour slant culture in a small amount of distilled water to obtain approximately 10^9 cells per ml.

VA mycorrhizal fungi

Glomus fasciculatus KUMD 8605 was isolated from soils in the legume field and shown to infect mung bean and potato in pot culture (Progress Report No. 3).

Plant materials

Model plants used were carrot and potato. Others tested plants were five different leguminous plants, i.e. soybean, mung bean, peanut, winged bean and sugar pea. All tested plant materials were obtained as seed from local markets. Carrots and potatoes were obtained as fresh materials.

Tomour induction

Fresh carrots and potatoes were peeled and then washed clean and surface-sterilized according to the procedures of Lippincott and Lippincott (1969). They were then aseptically cut into disks 0.5 cm. thick and 1.0 cm. diameter. Legume seeds were similarly treated and allowed to germinate on Murashige and Skoog (1962) medium. Wounds were inflicted on the stem of seedlings. Inoculation was performed by dropping two drop of 10^9 cells suspension to the cut tissue (disc) surface or the cut wound of plant materials maintained on the same medium at 23-25° c under light.

Nutrient media for root cultures and tomour root cultivation.

The growth media for tumour root cultures was according to Linsmaier and Skoog (1965) with the addition of 0.5 percent carbenicillin but without the addition of plant hormones. Tumour roots were removed from plant seedlings and repeatedly transfered to fresh plates every 1-2 weeks to remove the agrobacteria after decontamination, root cultures were maintained on the same medium with no carbenicillin.

Results and Discussion

Pathegenicity of Agrobacterium

Both A. tumefaciens and A. rhizogenes caused tumour growth formation when tested with the model tissue system of potato. A. tumefaciens produced unorganized compact clumbs of tissue growth which were hard and brittle in texture while A. rhizogenes produced white tender lateral roots (Figure 1). A. rhizogenes was chosen for further studies with other test plants.

Susceptibility of test plants to A. rhizogenes infection

Infection experiments with test plants revealed that soybean seedlings showed no response while winged bean seedling showed slight abnormal fluffy cell differentiation at the wound sites. Mung bean, peanut and sugar pea showed positive response with varying degrees of tomour root formation.. Figure 2 shows varying degrees of susceptibility of test plants to A. rhizogenes infection.

Mass Progagation of Ri-induced root cultures

Serial transplantation of Ri-induced roots of carrot, potato and peanut could be successfully made while that of mung bean and sugar pea showed degeration of growth.

Transplanting of tumour roots, after bacterial decontamination by successive cultivation on a carbenicilllin-containing medium, was most successful with only peanut and with the two model systems of potato and carrot. Root tumor cell lines of peanut, potato and carrot could be and are being maintained through periodic transplantation.

Table 1-3 show growth responses of Ri-induced root cultures transplantation of mungbean, peanut and sugar pea respectively. Figure 3. shows series of transplanted Ri-induced root culture of peanut.

VA mycorrhizal infection of A. rhizogenes-induced root cultures

Potato tumour root cultures, with extensive branching and root hair formation were used, initially, to develop the two-member (root-VA mycorrhizal fungus) culture system. It was found that A. rhizogenes induced roots were susceptible, as hosts, to mycorrhizal association with the test fungus, Glomus fasciculatus (Figure 4)

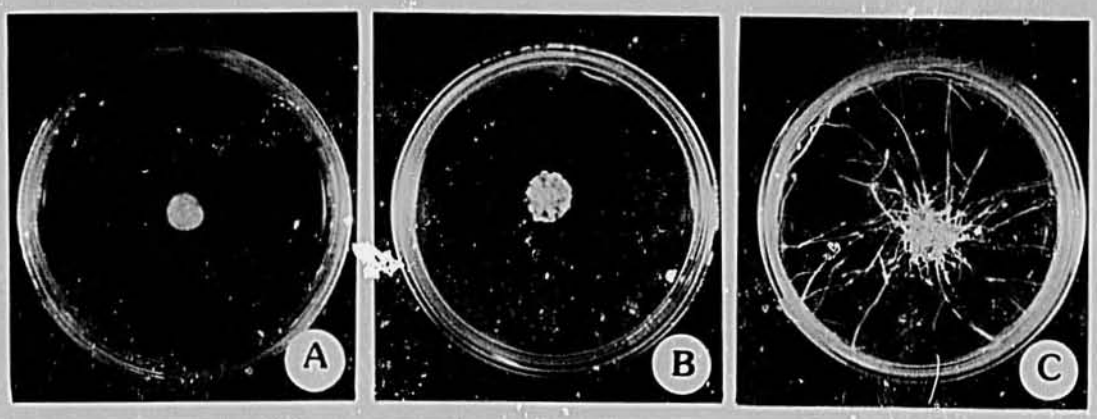

Figure 1. Tumour formation on potato disc
(A) control (B) infected with A. tumefaciens
(C) with A. rhizogenes

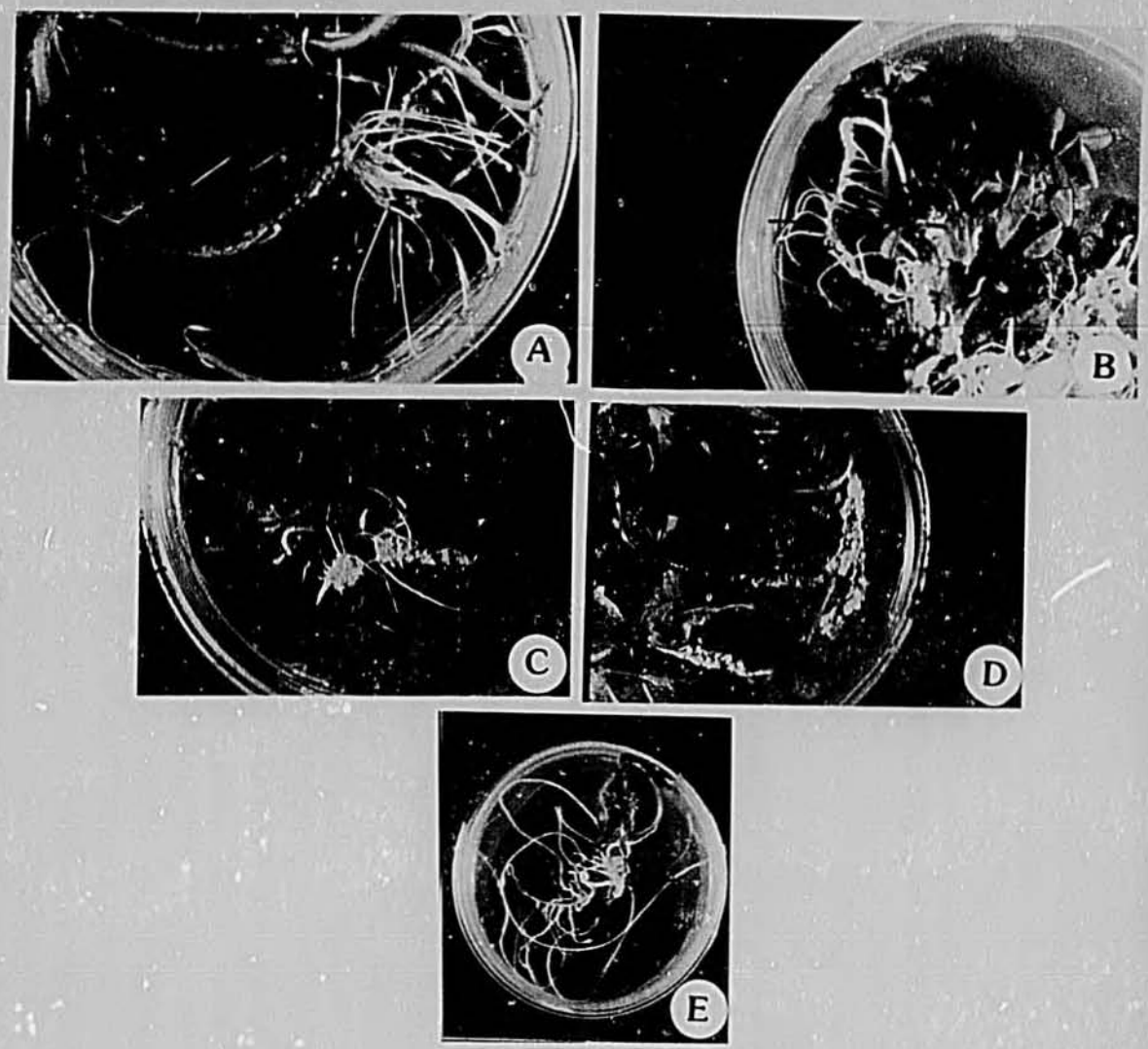

Figure 2. Variation in susceptibility of test plants to *A. rhizogenes* infection.
(A) soybean (B) peanut
(C) Mung bean (D) winged bean
(E) Sugar pea

Figure 3. Series of transplanted Ri-induced root culture of peanut

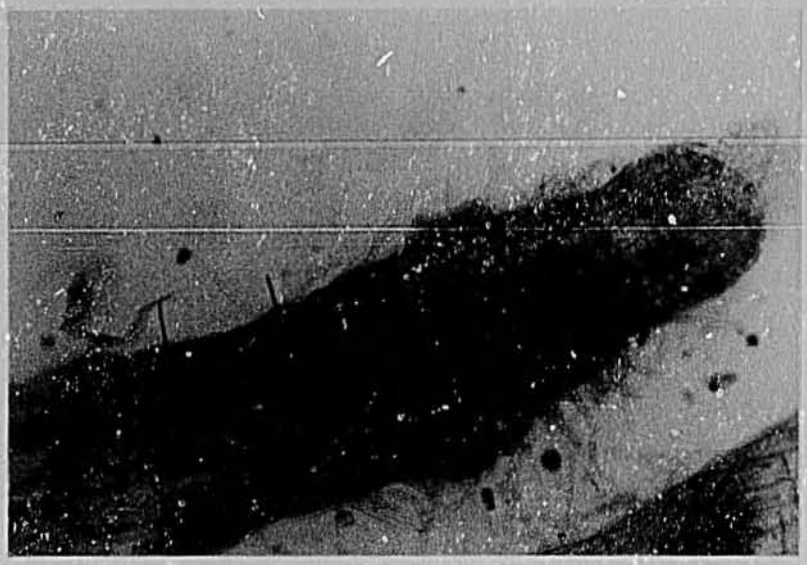

Figure 4. The root-VA mycorrhizal fungus system of potato and *Glomus fasciculatus* showing vesicle formation (arrows)

Table 1. Growth response of Ri-induced root culture of mung bean after serial transplantation.

culture No.	initial weign (gram)	change in wet weight per gram after 30 days of transplantation.
1	0.08	2.88
2	0.17	1.88
3	0.04	1.0
4	0.02	5.5
5	0.10	1.4
		average = 2.53

Table 2. Growth response of Ri-induced root culture of peanut after serial transplantation

Culture no.	Initial weight (gram)	Change in wet weight per gram after 50 days of transplantation
1	0.02	199
2	2.23	28.65
3	0.08	13.50
		average = 80.38

Table 3. Growth response of Ri-induced root culture of sugar pea after serial transplantation.

Culture No.	Initial weight (gram)	Change in wet weight per gram after 50 days of transplantation.
1	0.40	0.28
2	0.42	1.60
3	0.51	0.96
4	0.76	0.05
5	0.45	0.38
		average = 0.65

Conclusions :

1. Bacteria - free hairy-root tumour cell lines of carrot, potato and peanut have been successfully developed and maintained.

2. Root cultures with extensive lateral root formation could be successively transplanted and propagated to obtain effective *in vitro* substrate for cultivation of mycorrhizal fungi based on the two-member culture system of potato tumour root and *Glomus fasciculatus.*

Future Research plan :

1. Studies on the effectiveness of the mycorrhizal root inocula.

2. Induction of sporulation of mycorrhizal fungi

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