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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/4/88

MEMORANDUM

TO: AID/PPC/CDIE/D1, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 5. 2/4
PRH 1

Attachment

PN-ABA-545

5.244

PROGRESS REPORT NO. 1

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

A RESEARCH PROJECT

USAID/PSTC PROGRAM

Grant No. 036 5542-G-00-F077-00

Submitted by

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Project Leader

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Kasetsart University
Bangkhen, Bangkok

Rec'd in Sci JUN 19 1966

Project Profile

Country : Thailand

Grant No. : 936-5542-G-00-5077-00

Program : Program on Science and Technology Cooperation

Project Title : Ri-Plasmid Induced Root Culture as a Potential
Substrate for In-Vitro Propagation of Mycorrhizae
Fungi

Project Leader : Dr. Malee Suwana-adth

Organization : Kasetsart University, Bangkok, Bangkok

Authorized Officer : Dr. Kampo! Adulvit
Director, Kasetsart University Research and
Development Insitutte (KURDI)

Total Project Budget : 2,585,250 bahts

Project Duration : 2 years and 4 months

Reporting Period : August 1, 1985 - January 31, 1986

Budget Allocation for This Period : 1,427,000 bahts

DATE May 1986

BIANNUAL PROJECT PROGRESS REPORT

PROGRAM USAID/PSTCPROJECT Ki-Plasmid Induced Root CulturePERIOD August '85 - January 1986Grant Number 936-5542-G-00-5077-00

Activities Planned for This Period	Activities Accomplished During This Period	Explanation for Variance	Activities planned for Next 6-month Period
<p><u>Administrative</u></p> <ul style="list-style-type: none"> ● Initial Project Preparation ● Recruitment of personnel ● Procurement of equipments <p><u>Technical</u></p> <ul style="list-style-type: none"> ● Selection of suitable strains of mycorrhiza ● Induction of root culture ● Training and consultation 	<ul style="list-style-type: none"> ● Establishing internal project accounting and project management procedures including arrangement for research facilities and services by KURDI ● Two research assistants recruited - one M.S. and one B.S. graduate in microbiology and biology respectively ● Locally made equipments and materials purchased and foreign-made (US.) equipments to be purchased specified for purchasing order ● Additional collection of mycorrhiza strains from various sites and screening of existing collection ● Procurement of strains of Agrobacterium from foreign sources and verification studies ● Establishing procedures for culturing root of selected plants ● Initial consultation with plant molecular biology expert in USA and visiting Japanese cell culture expert 	<ul style="list-style-type: none"> ● KURDI management office handling accounting on behalf of project personnel and providing necessary basic research facilities and services ● purchasing (L/C issuance) order awaited fund-in-hand status. ● unable to find strains from local sources. ● change of institution for training of researcher 	<ul style="list-style-type: none"> ● Completion of acquisition of equipments ● continuation of collection and selection of mycorrhiza strains ● Study of effectiveness of <u>Agrobacterium rhizogenes</u> on various plant roots and initiate larger scale of propagation ● develop method for inoculating mycorrhiza into infected roots ● technical training of Thai researcher at US Universities
<p><u>Problems encountered</u></p> <p><u>Administrative</u> : Lack of access to dividend fund during the initial period caused delay in implementation.</p> <p><u>Technical</u> : ● Lack of local expertise and materials on plant genetic vectors, i.e. Ti and Ri plasmids.</p> <p>● Limited access to limited availability of training facilities in the field</p>		<p><u>Remedies effected</u></p> <p><u>Administrative</u> : Establishment of departmental research fund reserve</p> <p><u>Technical</u> : ● Acquisition from foreign sources</p> <p>● Seeking additional training assistance from Europe</p>	

I. INTRODUCTION

A USAID/PSTC research grant was awarded to Kasetsart University for a research project in the area of agriculture biotechnology. The project has been designed to develop research capability at Kasetsart University as well as to investigate new method for efficient production of mycorrhizal fungi for use to enhance nutrient uptake of plants.

The Project covers a 28-month period with the total budget of 2,585,250 bahts.

II. PROJECT DESCRIPTION

Over the years numerous attempts have been made to culture vesicular-arbuscular mycorrhizal fungi on artificial media but without significant success. They are normally maintained in pot cultures with their host plants. With the recent advancement in biotechnology it is now possible to obtain mass culture of plant cells of increasing varieties.

The Project attempts to use Ri-plasmids from Agrobacterium rhizogenes to induce active growth of plant roots and to study the suitability of root culture as a living substrate for mycorrhizal growth and eventual production of their spores, propagules.

III. SCOPE OF ACTIVITIES DURING THE REPORTING PERIOD

Major activities during the initial Six months of the project were divided into :

- (a) Administrative activities involving mainly Administrative preparations for effective implementation of the project, and
- (b) Technical activities involving technical preparations including, collection and acquisition of necessary scientific and technical materials, preliminary investigations and development of methodologies.

3.1 Administrative Activities

- 3.1.1 Establishment of administrative procedures in relation to project personnel, financial management and acquisition of supplies and materials. The authorized official and project administrative manager is Dr. Kampol Adulvit, Director of KURDI.

Under the arrangement, KURDI, the University's central research laboratory, is responsible for the provision of necessary

basic research facilities and services for the Project as well as for undertaking project accounting.

3.1.2 Recruitment of technical/research assistants for the project as follows:

- Ms. Rapeepan Chewatanaruk M.S. (Microbiology) from September to November 1985 at 3,000 Baht/m. December 1985 - present at 5,000 baht/m.
- Ms. Thanusara Vinothai B.Sc. (Biology) from September 1985 - present at 3,000 baht/m.

3.1.3 Key project personnel working during this reporting period are Dr. Malee Suwana-adth, Ms. Poonpilai Suwanarit, Dr. Wiwat Duengsubha and Ms. Yupa Mongkolsook.

3.2 Technical Activities

3.2.1 Screening for and collection of Mycorrhizal fungi from soils associating with legumes and corns which are Thailand's major cash crops. Soil samples were collected from the north, northeast and eastern regions of the country.

Seven species were isolated from soils from the north, one species was isolated from soils from the east, and nine species were isolated from soils from the Northeast.

Names of these species are listed in Appendix A.

In addition, ten species of pot-cultured mycorrhizal fungi maintained in the fungi laboratory of the KU Department of Microbiology were subcultured and purified for future experimentation.

A total of 27 species of mycorrhizal fungi have been isolated/collected and maintained.

3.2.2 Acquisition of Agrobacterium cultures.

There has been no collection of Agrobacterium culture in Thailand and cultures have been obtained from foreign sources as follow :

- Agrobacterium tumifaciens ATCC 15955 (USA)
- A. rhizogenes ATCC 15834 (USA)
- A. rhizogenes IFO (Japan)
- A. rhizogenes TJ 001 (Japan)

Strains were checked for their effectiveness in inducing rhizoid growth of roots. A. rhizogenes ATCC 15834 and TJ1 were found to be effective.

3.2.3 Establishment of method for active cultivation of plant roots

Two representative types of plants were chosen as follow :

tuber plants - white potato, carrot
non-tuber plants- tomato, and legumes.
(mung bean soybean
and peanut)

In order to lengthen the active growth period of root cultures the strength of culture medium had to be reduced and the half-strength concentration was found to support good and satisfactory growth of plant roots. The appropriate medium was found to be Murashige & Skoog at half strength with 3 per cent sucrose.

IV. CONCLUSIONS

1. Necessary preparations have been made for efficient implementation of the project.
2. Technical materials such as strains of mycorrhizal fungi and Ri-plasmid containing agrobacterium have been acquired and tested for their effectiveness
3. Methodologies have been established for actively growing plant root cultures and infecting them with Ri-plasmids from Agrobacterium rhizogenes.

V. Plan for the Next Period

Major activities for the 2nd six-month period from February - July 1986 are as follows :

- 5.1 Completion of procurement of equipments from abroad (USA)
- 5.2 Develop procedure for large-scale cultivation of selected plant roots
- 5.3 Determine appropriate conditions for plant root culture and mycorrhizal fungi association.
- 5.4 Training of project's researcher, Ms. Yupa Mongkolsook at the U.S. Institutions (Colorado State University) on tissue culture techniques.
- 5.5 Preparation of at least one technical paper for publication.

Appendix A
List of Mycorrhizal Fungi Isolated from Soil
Samples from Different Regions in Thailand

North

1. Gigaspora Gigantea
2. G. margarita
3. Gigaspora sp.
4. Acaulospora sp.
5. Sclerocystis sp.
6. Unidentified sp. N1
7. Unidentified sp. N2

East

8. Gigaspora gigantea

Northeast

9. Gigaspora gigantea
10. Gigaspora sp. No.1
11. Gigaspora sp. No.2
12. Gigaspora sp. No.3
13. Gigaspora sp. No.4
14. Acaulospora sp.
15. Glomus sp.
16. Glomus monosporus
17. Unidentified species NE1

KU Department of Microbiology Collection

1. Acaulospora spinosa
2. A. scrobiculata
3. Gigaspora gigantea
4. G. margarita
5. G. heterogama
6. Gigaspora sp.
7. Glomus monosporus
8. Entrophospora sp. No.1
9. Entrophospora sp. No.2
10. Entrophospora sp. No.3