

PL - ABB-096

AGENCY FOR INTERNATIONAL DEVELOPMENT  
WASHINGTON, D.C. 20523

DATE: 8/25/88

MEMORANDUM

TO: AID/PFC/CDIE/DT, 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. 399  
Half Yearly Report - First Half 1988

Attachment

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USAID/NARESA RESEARCH GRANTS-RG/AID/7 2/1

HALF YEARLY REPORT-FIRST HALF, 1988

1. Grantee (1) : Y.M.H.B. Yapabandara

Address : Minor Export Crops Research Station  
Matale

Grantee (2) : M.D. Dassanayake

Address : Department of Botany, Faculty of Science  
University of Peradeniya,  
Peradeniya

2. Grant No : RG/AID/7

3. Date of Award : 10<sup>th</sup> August, 1985

4. Title of Project : In vitro Propagation of Clove and  
Nutmeg

5. Brief statement of the methodology that was used

Recd in US

AUG 25 1988

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N U T M E G

- (A) Experiments with in vitro-multiplied shoots, originating from 2-3 year old plants (juvenile)

The experiment with shoot tips from immature plants of nutmeg were very successful. An acceptable protocol was made during the project period. According to the theoretical calculations a 1 cm piece of nutmeg shoot we can produce about 3000 plant per year. Investigators are already making arrangements to publish these findings in the Journal of Plant Tissue, Cell and Organ Culture.

- (B) Shoot tip culture experiments with plants grafted on scions from known mocher trees, maintained in the greenhouse.

Experiment 1

Objective : To find out suitable hormones and concentrations for culture establishment and proliferation.

Materials and Methods : Newly sprouted shoots were collected from the grafted nutmeg plants grown under greenhouse conditions. All the leaves and stems were discarded leaving about 4 cm shoot tip. After sterilization with 0.1% HgCl<sub>2</sub> for 10 minutes shoot tips were thoroughly washed in sterilized distilled water three times. Three sizes of the shoot tip explants were made (0.5, 1.0 and 1.5 cm) and introduced to the following hormonal combinations as treatments. Basal

medium was Anderson's with 1.5 mg/litre of BA.

1. Factorial combination of NAA (0, 0.5 and 1.0 mg/l) with GA<sub>3</sub> (0, 0.5 and 1.0 mg/l).
2. Factorial combination of IBA (0, 0.5 and 1.0 mg/l) with GA<sub>3</sub> (0, 0.5 and 1.0 mg/l).
3. Factorial combination of kN (0, 0.5 and 1.0 mg/l) with GA<sub>3</sub> (0, 0.5 and 1.0 mg/l).

Results : The smaller shoot tips (0.5 cm long) died after about 4 weeks. Larger explants survived for about 12 weeks. The hormonal combinations of NAA and GA<sub>3</sub>, and kN and GA<sub>3</sub> failed to induced axillary buds. However the explants in IBA and GA<sub>3</sub> treatments survived longer and produced some axillary buds. But these axillary buds did not grow further. This experiment was repeated two times and the same results observed.

#### Experiment 11

Objective : Comparison of liquid and solid medium on culture establishment.

Materials and Methods : The same procedure was followed as Expt. 1 and explants were introduced to Anderson's liquid medium (1.5 mg/l BA) with following hormonal combinations:

Factorial combinations of IBA (0, 0.5 and 1.0 mg/l) with GA<sub>3</sub> (0, 0.5 and 1.0 mg/l).

Results : No satisfactory results were obtained.

#### Experiment 111

Objective : Manipulation of various media on culture establish-

ment

Materials and Methods : Explants were prepared as in Expt. 1 and introduced to the following media: (Hormonal combinations were common for all media, IBA and GA<sub>3</sub> 0, 0.5 and 1.0 mg/l).

1. B5 medium
2. MS medium
3. Anderson's
- MS ( $\frac{1}{2}$  macro salts)

#### Experiment 1v

Objective : To find out the effect of various transferring periods on culture establishment.

Materials and Methods : Explants were prepared same as Expt. 1 and introduced to a common medium (Anderson's with 1.5 mg/l BA). Transferring explants to fresh medium as follows:

1. Every week
2. Every two weeks
3. Every three weeks
4. Every four weeks
5. Every five weeks

Results : The cultures were transferred at one or two week intervals died within three weeks. other cultures were still alive and the experiment was continuing.

(C) Experiments with materials directly from the mother trees (about 20-40 year old trees).

#### Experiment 1.

Culturing axillary buds induced at the mother tree.

Materials and methods : Newly emerged shoots were selected and pretreated by spraying a solution of fungicide. Then shoot tips of the branches were trimmed in order to induce axillary buds at the remaining portion. These portions with initiated axillary buds were collected after 3-4 weeks and used as explants.

The basal medium was Anderson's with a range of BA

concentrations (0, 1, 2, 4, 6, 8, 10 and 12 mg/l).

Results : Although the explants survived for a long time (6-8 weeks) none of the treatments was able to induce axillary buds.

Experiment 11 : Culturing of shoot tips from the mother trees.

After the major rainy season in December, newly emerged pretreated shoots (about 6") were collected from the mother trees around the Kandy area. All the leaves were discarded and shoot tips were dissected to about 4 cm long. These shoot tips were washed in running water and then sterilized with 0.1%  $HgCl_2$  for 10 minutes. After three rinses with sterilized distilled water shoot tips were dissected to about 1.5-2.0 cm long. These explants were introduced to Anderson's medium with following hormonal combinations: (BA at the concentration of 1.5 mg/l was added to all of the treatment except: IV and V).

1. Factorial combinations of NAA (0, 0.5 and 1.0 mg/l) with  $GA_3$  (0, 0.5 and 1.0 mg/l)
2. Factorial combinations of IBA (0, 0.5 and 1.0 mg/l) with  $GA_3$  (0, 0.5 and 1.0 mg/l)
3. Factorial combinations of kN (0, 0.5 and 1.0 mg/l) with  $GA_3$  (0, 0.5 and 1.0 mg/l)
4. BA (0, 2, 4, 6, 8, 10, 12, 15 and 20 mg/l)
5. kN (0, 2, 4, 6, 8, 10, 12, 15 and 20 mg/l)

Results : the cultures in experiment 1, 2 and 5 did not survive beyond four weeks. The cultures in other treatments survived a longer period (6-12 weeks). But axillary bud development was not observed.

C L O V E

(A) Experiment with two months old greenhouse-grown seedlings.

Successful results were obtained through the culture of above materials. Appropriate media requirements were found for establishment, multiplication and rooting of clove. Already arrangements are made to publish these findings in the Journal of Plant Tissue, Cell and Organ Culture.

(B) Experiments with materials from selected mother trees (20-25 year old).

Experiment 1. Nodal culture experiments

Actively growing shoot tips were taken from the selected mother trees and brought to the laboratory. Stems were prepared for sterilization, discarding all the leaves. Sterilized explants were washed three times in sterilized distilled water and second or third nodal sections were separated as explants.

Medium was prepared according to the results from the earlier experiment as Anderson's basal medium with 8.0 mg/l BA. Nodal explants were introduced to this medium in each month (December, January, February, March, April and May).

Results : The nodal explants collected in the month of December were observed to show the best establishment. However, very few cultures were established.

## Experiment 11

Three different concentrations of basal medium (Anderson's) at 100%, 50% and 25% were prepared with equal amounts of BA (1.5 mg/l). Shoot tips from the grafted plants were introduced to this medium with 10 replicates. The explants lived a few weeks but no regeneration was achieved.

### (6) Conclusions drawn from the work

Nutmeg : Entire procedure was developed in order to regenerate plantlets from shoot tips of juvenile nutmeg plants. But regeneration of material from the selected clonal nutmeg trees were still a failure. Regeneratio of nutmeg explants from very lod trees (that bore fruits for many years) could not be achieved through media manipulations.

Clove : A procedure is available for regeneration of clove plantlets with seedling origin. Very few cultures could be established from mature selected trees. Further, very slow rate of growth and multiplication was observed.

### (7) Is the work on schedule? Yes

### (8) Plan of work for the next half year.

Various phases of the explants of both species, ie; orthotropic shoots, induced shoots, shoots from the regrafted plants and newly emerged axillary buds will be cultured in various media.

### (9) Any other comments :

This project will be over on 10<sup>th</sup> of August, 1988.

A six months of extention of the project will be needed to complete some of the on-going experiments and to tryout a few new ideas.

Signature of grantee/s :

1. Y.M.H.B. Yapabandara. *M.H.B.*.....

2. M.D. Dassanayake. *M.D.*.....

Comments (if any) of Head of Department/Section

*M.D.*.....  
Signature of Head of Department