

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/27/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, 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. 5.389
Just copy - 1987

Attachment

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USAID/NARESA RESEARCH GRANTS

5.399

HALF YEARLY REPORT - FIRST HALF, 1987

1. Grantee I. : Y.M.H.B. Yapabandara
Address : Minor Export Crops Research Station, Matale.
II Prof. M.D. Dassanayake
Department of Botany
Faculty of Science
University of Peradeniya
Peradeniya.
2. Grant No. RG/ADD/7
3. Date of award : 10th August 1985
4. Title of Project : In vitro propagation of clove and nutmeg
5. Brief statement of the methodology that was used:



N U T M E G

A) Experiments with in vitro multiplied shoots, originating from two year old plants.

Experiment I : Evaluation of rate of proliferation of in vitro grown shoots at four different cytokinine levels.

Objective : To find out the most suitable cytokinine and its concentration for optimum rate of proliferation.

Method and material : About 4 cm long in vitro grown nutmeg shoots were collected and about 2 cm long shoot pieces were taken leaving about 5 cm from the apex. All leaves were discarded and stems were used as explants.

Rec'd in DCI AUG 27 1987

Medium:- Anderson's medium was used as basal with the following cytokinins and its concentrations

| Cytokinin | | Concentrations mg/l | | | |
|----------------|---|---------------------|-----|-----|-----|
| | | 0 | 0.5 | 1.0 | 1.5 |
| BA | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
| Zip | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
| Z | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
| K _n | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
| PBA | 0 | 0.5 | 1.0 | 1.5 | 2.0 |

Results:- The trial is continuing. But at present it seems that the rate of proliferation is very much higher in benzyladenine than in the other cytokinins.

Experiment No. 2

Objective :- In vitro grown plantlet establishment in soil.

Method and:- Rooted in vitro grown shoots in Anderson's medium materials with 2% activated charcoal and NAA or 4BA were taken from test tubes and thoroughly washed free of excess agar and dipped in 0.1% benlate solution for 30 seconds. These plantlets were transferred to pots containing sterilized mixture of 1:1:1 top soil sand and well decomposed cowdung. These plants were kept in the growth room for 2-3 weeks and transferred to the green house.

Results :- About 80% of plantlets were successfully established in soil mixture.

Experiment No. 3:

Objectives : To compare physical status of the medium on rate of proliferation (solid/filter paper bridge)

Method and material : About 1 cm long shoot pieces were used after dissecting out the leaves. These explants were kept in an erect position on solid medium whereas for liquid medium filter paper bridges were used for holding explants.

Results: Observed a number of axillary buds which were greater and in better health on solid medium.

- B) Experiments with shoot tips from Matale Research station, about seven year old plants, sex still not determined.

These trials were not continued because more emphasis was given to selected mother plants from the field.

- C) Experiments with grafted plants, scion from known mother tree.

Experiment No. 1

To find out basic hormonal requirements for establishment, proliferation and rooting of nutmeg shoot tips.

Method and : Lateral shoot tips obtained from grafted nutmeg plants

Material were introduced to medium containing factorial combination of BA (0, 0.5, 1.0, 2.0, and 5.0 mg/l) with NAA (0, 0.2, 0.5 mg/l) replicated five times.

Results : Very low rate of fungal infection (about 5%) was observed. But about 25% - 40% bacterial contamination showed 10 - 17 days after cultivating. Best establishment with a very slow growth was found in medium containing 2 mg/l BA with 0.1 NAA.

Experiment No. II

Objective : Somatic embryogenesis or organo-gencsis from leaf discs.

Method and : leathery type of leaves (third leaf) were collected

Material from green house grown plants and 1 cm^2 pieces were introduced to WPM medium containing combination of 2, 4-D (0, 0.25, 0.50, 1.0, 2.0, 5.0 mg/l) with kn (0, 1.0, 2.0, 5.0, 10.0 mg/l) kept in continuous dark.

Results : Only friable callus was found around cut edges in following treatment -
2, 4-D 0.5, 1.0, 2.0, and 5.0 mg/l without or with 0.1 mg/l kn.

Experiment No. III

Objective : Somatic embryogenesis or organogenesis from leaf callus.

Method and : Medium B5 - 2 medium

Material Glutamine 0.4 gms

Sucrose 50 gms.

Hormones: All possible combinations of 2, 4 -D (0, 0.5, 1.0, 2.0, 3.0 ng/l) and kn (0, 1.0, 2.0, 3.0 mg/l).

Material : Nearly emerged, very small, whole leaf.

All the cultures were kept in the dark.

Results : Trial 1. continuing.

Experiment No. IV

Objective : Use of antibiotics for eradication of bacteria infecting explants.

Method and : The following mixture of antibiotics were used for study -

| | |
|--------------|-------|
| Cefelotaxmin | 25 mg |
| Tetracycline | 25 mg |
| Rifampicin | 6 mg |
| Polymixine B | 6 mg |

Four concentrations of the above mixture (0, 1x, 2x, 3x) used as liquid basal medium . Infected nodal segments were introduced to liquid medium and kept at continuous agitation for 3 days and introduced to fresh solid medium.

Results : Observed no infection when the mixture of antibiotics was incorporated at any concentrations (1, 2x, 3x).

D) Materials directly from mother trees (about 20-40 years old)

Experiment No. 1

This was difficult to establish in medium , main reason being the large amount of contaminants surviving even after sterilization. Increasing the concentration and time resulted in death of the shoot tips.

However, axillary buds are more resistant to sterility. Therefore used many steps for sterilization as follows:-

- i. Wash one hour in running water
- ii. Brush axillaries with liquid soap
- iii. 1 minute in 70% Ethanol
- iv. 1 minute in ethanol
- v. 10 minutes in 01% $HgCl_2$
- vi. Kept overnight and re-sterilized with 01% $HgCl_2$ for 10 minutes.

This procedure proved to be a little better. About 60% of the nodal explants treated appeared to be free of infection.

Experiment II

Experiment same as b. III. But incorporated benlate into the medium after the selection of correct concentration. 100 % contamination observed . Most of the cultures were infected with fungi.

Experiment III

Embryo/Endosperm culture.

Objective : To find out suitable medium for somatic embryogenesis.

Methods and : Used B5-2 medium with/ascorbic acid 0.1 gms, glutamin material 0.4 gm and 30 gm of sucrose with four concentrations of 2, 4-0 (0, 0.5, 1.0, 2.0 mg/l).

1 - 2 months old small fruitlets were collected and sterilized in 10% clorox solution for 10 minutes . fruitlets were split into halves and placed on medium with the cut side downwards.

Results : The larger explants (< 0-5 cm) were dead after 4 - 6 weeks, smaller explants were still alive. The trial is continuing.

C L O V E

A) Experiments with 1 - 2 month old seedlings.

Experiment No. 1

Objective : To find out the hormonal requirement to achieve optimum proliferation rate.

Method and: In vitro multiplied (in 2 mg/l BA), 0.5 cm long
Material shoots were used for this study. Prepared medium contained the range of concentration of Benzyladenine as 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mg/l.

Results : Higher concentration of BA seems to result in better growth. Trial is continuing.

Experiment No. 2

Objective : To find out a suitable medium for root initiation and elongation.

Method and: In vitro multiplied about 1.5 cm long shoots were
Material placed in medium containing various concentrations of IBA or NAA with or without 2% activated charcoal (0, 0.2, 0.5, 1.0, 2.0 mg/l).

Results : Trial is continuing.

B) Experiments with Epicotyl/Hypocotyl/Cotyledons of clove.

Method and: Epicotyl and hypocotyl segments were introduced to
Material medium containing all possible combination of 2, 4-D (0, 0.25, 0.50, 1.0, 2.0, 5.0 mg/l) with kn (0, 1.0, 2.0, 5.0, 10.0 mg/l). All treatments were kept in the dark.

Results : Large callus was produced in medium with 2,4 -D 0.5, 1.0, 2.0, and 5.0 without kn. These callus turned brown after about 8 weeks and died after 4 weeks time.

C) Experiments with material from mother trees (15 - 25 years)

Experiment No. 1

Objective : Embryogenesis/organogenesis through ovule culture/embryo culture.

Method and: Medium B5-2 medium supplemented with ascorbic acid
Material

0.1 gms, glutamine 0.4 gms and sucrose 30 gms.

Four concentrations of 2,4 - D were used (0, 0.5, 1.0, 2.0 mg/l).

Ovules were taken from clove flowers in three stages I Fully matured unopened, II One week after opening, III 3 - 4 weeks after opening.

Immature embryos were extracted from small fruits 4 weeks after pollination.

Results : Trial is continuing.

Experiment No. 2

Objective : Shoot tip culture/nodal cultures

Very difficult to sterilize materials, mother plants cannot be maintained in the green house because grafting/budding methods are impossible. Even very complex procedures were used for sterilization but very high % of contamination observed.

6. Conclusion drawn from the work.

Nutmeg - Materials from Juvenile plants.

- I. In multiplication phase solid medium is better than filter paper bridge. Fig. I
- II. In vitro rooted nutmeg shoots can be easily established in soil. Fig 2.
- III Cytokinine, other than BA may not be suitable for multiplication of nutmeg cultures.

Nutmeg - Material from grafted plants

- I. In leaf culture experiment only callus was found in some combinations of 2,4 - D with km and without km.
- II. Better establishment of shoot tip/nodal explants were found in Anerson's medium with 2.0 mg/l BA and 0.1 mg/l BAA. Fig 3
- III. A mixture of antibiotics is capable of eradicating bacterial infection of cultures.

Nutmeg - Materials directly from mother trees

- I. A complex procedure has to be followed to obtain sterile explants.

Clove - juvenile

- I. Axillary bud could be established and multiplied in Anderson's medium with 2 mg/l BA . Fig. 4

Clove - Adult trees

Shoot tip/nodal explants directly from the field showed very high incidence of contamination.

Is the work on schedule? Yes.

7. Plan of work for the next half year.

Nutmeg

- 1. More experiments will be done with mature/grafted material (shoot tip cultures/nodal cultures/leaf cultures/Embryo cultures)

Clove

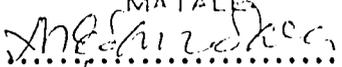
- 1. Continuation of trials with juvenile material upto field planting.
- 2. Try to regenerate plants using explants other than shoot tips/nodal (ovule etc.)

8. Any other comments:

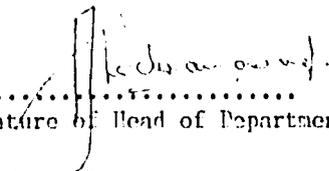
- 1. The consultant, Prof. P.E. Litz who visited our project has given us valuable suggestions regarding our current problems.
- 2. We are experiencing long delays in the supply of chemicals and equipment from foreign sources, and this is affecting our programme adversely.

Y. M. H. B. YAPABANDARA
 Plant Tissue Culture Laboratory
 Minor Export Crops Research Station
 MATALE

Signature of grantee/s

- 1. Y.M.H.B. Yapabandara : 
- 2. Prof. H.D. Dassanayake : 

Comments (if any) of Head of Department/Section



 Signature of Head of Department.

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Fig. 1 - Multiplication of ...
per bridges and ...

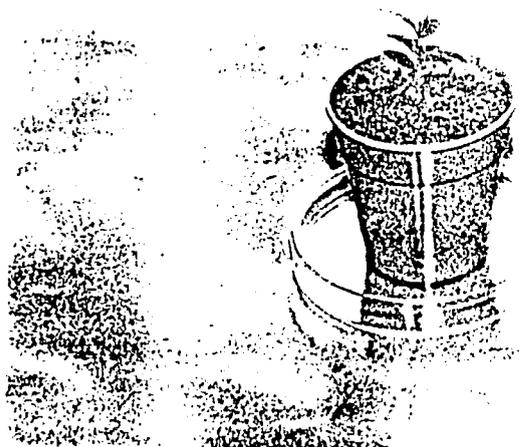


Fig. 2 - ...meg plantlet

Fig. 1 - Multiplication of nutmeg in filter
paper bridges and solid medium

Fig. 2 - Nutmeg plantlet established in soil

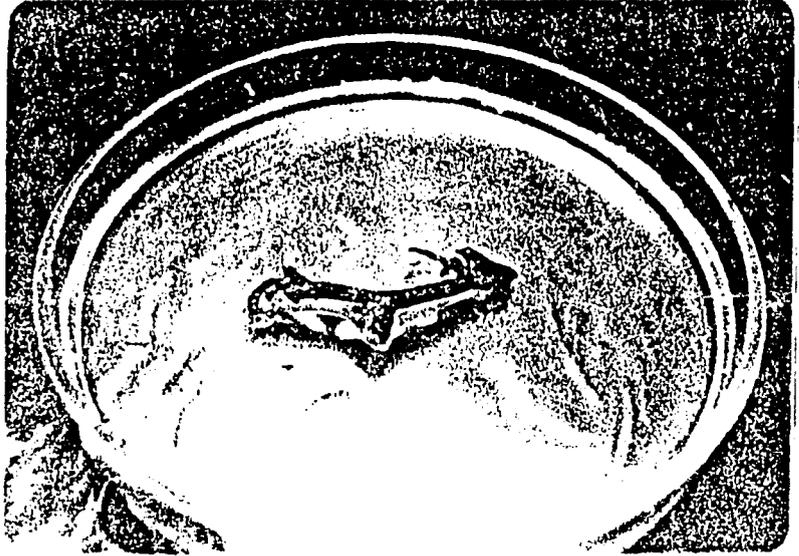


Fig. 3 - Establishment of growth of axillary bud from grafted nutmeg plants in Anderson's medium with 2.0 mg/l BA and 0.1 mg/l BAA

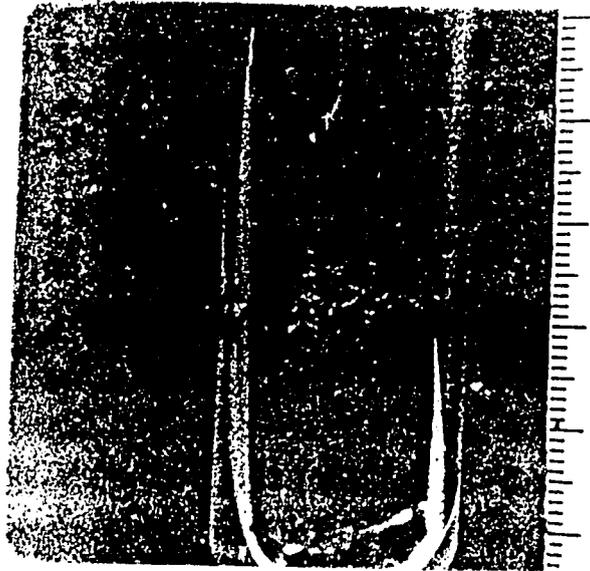


Fig. 4 - Multiplication of clove in Anderson's medium with 2 mg/l BA (juvenile material)