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AGENCY FOR INTERNATIONAL DEVELOPMENT  
WASHINGTON, D.C. 20523

DATE: 4/15/88

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. 399

half yearly - 2d half, 1987

Attachment

*1 copy*

USAID / NARESIA RESEARCH GRANTSHALF YEARLY REPORT - SECOND HALF , 1987

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1. Grantee I : Y. H. S. Yapabandara  
Address : Minor Export Crops Research Station, Matile  
II : Prof. M. D. Dassanayake  
: 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.

**Best Available Document**

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NUTMEG

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 cytokinines, each at five levels (0,0.5,1.0,1.5 and 2.0 mg/l).

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

Materials & Method : About 4 cm long in vitro grown nutmeg shoots were collected and about 1 cm long shoot pieces were taken leaving about 1.5 cm from the apex. All leaves were discarded and stems were used as explants. These explants were introduced to five concentrations (0,0.5,1.0,1.5,2.0 mg/l) of four different cytokinines (BA, 2ip, 2, Kn).

Results

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Table -I Average number of axillary buds produced from  
2 cm long shoot pieces of nutmeg after 5 weeks.

Concentrations mg/l Cytokinene	0	0.5	1.0	1.5	2.0
BA	1.2	3.2	4.75	4.75	5.2
Zip	1.2	1	0.8	1	1.2
Z	1.2	0 (callus)	1.6 (C & R)	1 (C & R)	1.2
Kn	1.2	1.8	0.8	1.2	1.2

C = Callus

R = Roots

The rate of proliferation is very much higher in benzyladenine than in the other cytokinins. Lower concentrations of Zetin (0.5, 1.0 and 1.5 mg/l) produced callus and subsequently roots.

Experiment No. 2.

Objectives- To compare physical status of the medium on rate of proliferation (solid/liquid agitated)

Materials & Method : About 1 cm long shoot pieces (1.5-2.5 cm below the apex) were used after dissecting out the leaves. These explants were introduced to stationary solid medium and liquid medium in orbital shaker (50 rpm).

Results : Explants in liquid medium produced greater number of axillary buds (6-10) than the cultures on solid medium. Also shoots were thicker and well grown in liquid medium.

Experiment No. 3. The effect of light intensity on proliferation.

Objective : To find out the suitable light intensity for optimum multiplication.

Materials & Method : 1 cm shoot pieces were introduced to shoot multiplication medium (1.5 mg/l BA) and kept under three different light intensities (20,40,60 foot candles) and the number of axillary buds under each light intensity was counted.

Results : No treatment effect on axillary bud proliferation.

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B) Nodal culture experiments with grafted plants, scion from known mother trees, maintained in the green house.

Experiment I

Objective: To find out optimum hormonal requirement and dark/light period for establishment and induction of axillary buds from the internodal section (nodal culture).

Materials & : Newly emerged branches were collected from the green-

house all the leaves were discarded and the stems were sterilized in 0.1%  $HgCl_2$  solution for 10 min. After 3 rinses with sterilized distilled water 2<sup>nd</sup> or 3<sup>rd</sup> internodes were cultured in Anderson's medium with ten different concentrations of benzyl adenine or Kinetin (0, 1, 2, 4, 6, 8, 10, 12, 15, 20 mg/l). Prepared ten cultures for each treatment and five of them were kept in total dark for first two weeks and then at 16 hr light. Other five cultures were kept in 16 hr light from the beginning.

Results : None of the treatments could induce the growth of the axillary buds. But cultures were alive for about 12 weeks.

Experiment II The effect of temperature on induction of axillary buds from nodal cultures.

Material & : Sterilized nodal sections were used as in expt. I.

Method These explants were introduced to ten different concentration of BA(0, 1, 2, 4, 6, 8, 10, 12, 15, 20 mg/l)

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Prepared ten cultures for each treatment and five of  
of them were kept at 32°C whereas other five at 28°C.

Results : All of the cultures at the temperature 32°C died within  
in 2 weeks. Cultures at 28°C not produced any axillary  
ies but were alive for about 12 weeks.

Expt. III Effect of auxin (NAA) for axillary bud induction in  
nodal cultures.

Materials & : Nodal explants were introduced to the medium contained  
five different concentration of NAA (0, 1, 2, 4, 6 mg/l).  
Basal medium was that Anderson with 1.5 mg/l BA.

Results : Some callus formation was observed at the higher con-  
centration of NAA. Axillary bud induction were not  
observed in the treatments.

C) Shoot tip/meristem culture Experiments with grafted plants, scion  
from known mother trees, maintained in the green house.

Exp. I:-

Objective : To find out suitable hormones and their concentration  
for establishment and proliferation.

Materials & : Newly sprouted shoots were collected from the green house.

Method: All the leaves and stems were discarded leaving about 4 cm  
shoot tip.

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After sterilization followed by three rinses with sterilized distilled water three sizes of the explants were obtained. These explants were introduced to following media with 10 replicates, Basal medium was Anderson's medium with 1.5 mg/l BA.

- i. Factorial combination of NAA (0,0.5,1.0 mg/l) with  $GA_3$  (0,0.5,1.0 mg/l)
- ii. Factorial combination of IAA (0,0.5,1.0 mg/l) with  $GA_3$  (0,0.5,1.0 mg/l).
- iii. Factorial combination of Kn (0,0.5,1.0 mg/l) with NAA (0,0.5,1.0 mg/l).

Results : Small shoot tips, size about 0.5 cm, died after about 4 weeks. NAA  $GA_3$  combination trial didn't give any survival. Other two trials (IAA &  $GA_3$ , Kn &  $GA_3$ ) were continuing.

Expt. II : Callus/somatic embryos/ plant regeneration from leaf culture.

Material & Method : Newly emerged, small, whole leaves were cultured in B5-2 medium supplemented with glutamine 0.4 gms. sucrose 60 gms. All possible combinations of 2,4-D(0,0.5,1.0,2.0,3.0 mg/l) and Kn (0,1.0,2.0,3.0 mg/l) used as treatments. Cultures were kept in the dark.

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Results : No callus was found any of the treatments. At higher concentration of 2,4-D leaves became crinkled.

with final report.

D) Experiments with the materials directly from the mother tree (about 20-40 years old trees ).

Experiment I :- Node culture

Materials & Method : About 6" long shoots were collected from the mother trees and all the leaves were discarded and adopted following procedure for sterilization.

- i. Wash one hour in running water.
- ii. Brush axillaries with liquid soap.
- iii. 10 minutes in 0.1%  $HgCl_2$
- iv. Washed with sterilized distilled water, covered with aluminium foil and kept overnight in the cabinet.
- v. Resterilized with 0.1%  $HgCl_2$  & thoroughly washed with sterilized water.

Then internodal sections were obtained after dissecting out unnecessary portions. Prepared Anderson's medium with a range of concentration of BA or Kn (0, 1, 2, 4, 6, 8, 10, 12, 15, 20 mg/l). Prepared twenty cultures for each treatment and few of them kept in total dark for first two weeks and then at 16 hr light. Other five cultures were kept in 16 hr light.

Results : Much less contamination was observed with the sterilization procedure. Cultures were healthy and alive for about 6-8 weeks but no axillary bud break was observed.

Experiment II Shoot tip/meristem culture.

Materials : After the major rainy season, newly emerged pretreated shoots (about 6") were collected from mother trees at Kandy area. All the leaves were discarded and dissected shoot tips about 4 cm long. These shoot tips were washed with running water then sterilized with 0.1%  $HgCl_2$  for 10 minutes. After three rinses with sterilized distilled water one or two leaves which covered the meristem portion were dissected out and prepared 1.5-2 cm long explants. These explants were introduced to the Anderson's medium with following hormonal combinations. BA at the concentration of 1.5 mg/l was added to all of the treatments except IV and V.

- i. Factorial combination of NAA (0, 0.5, 1.0, mg/l) with  $GA_3$  (0, 0.5, 1.0 mg/l)

α'

- ii. Factorial combination of IBA (0, 0.5, 1.0 mg/l) with  
GA<sub>3</sub> (0, 0.5, 1.0 mg/l)
- iii. Factorial combination of Kn (0, 0.5, 1.0 mg/l) with  
NAA (0, 0.5, 1.0 mg/l)
- iv. BA (0, 1, 2, 4, 6, 8, 10, 12, 15 and 20 mg/l)
- v. Kn (0, 1, 2, 4, 6, 8, 10, 12, 15, and 20 mg/l)

Results : Culture contamination was very low (10%). The experiment is continuing.

Experiment III Stimulation of cuttings branches.

Material & Method : About 12" long branches were collected from mother tree and leaves were discarded except few (about 2) at the terminal. Five of them kept in 250 ml conical flask containing 50 ml of liquid Anderson's medium. These shoots were weekly sprayed with 100 mg/l solution of BA and kept in greenhouse.

Results : All of the shoots died within 14 days.

Experiment IV Embryo/Endosperm culture

Material & Method : About 1 month old fruitlets were collected then sterilized and split into two halves. Immature endosperm was dissected out and introduced to following medium with four concentrations of 2,4-D (0, 0.5, 1.0, 2.0 mg/l).

B5-2 B sal medium	Glutamin 0.4 gms
Ascorbic acid 0.1 gms	Sucrose 30 gms.

Results : Smaller ( 0.5 cm) explants survived for a longer time & produced small callus at the cut edges.

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Experiment V Hard pruning of grafted plants to induce new shoots.

Material & : Grafted plants maintained in pots were decapitated at  
Method 6" above the union and paint was applied at the cut end  
and maintained in the green house with regular watering.

Results ; All of the plants died due to this treatment.

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GLOVE

A) Experiments with 1-2 month old Seedlings.

Experiment No. 1

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

Method : From in vitro multiplied (in 2 mg/l BA) shoots

Material : 0.5 cm long lateral shoot explants were used for this study. Prepared medium containing the range of concentration of Benzyladenine 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mg/l.

Results : The cultures grown in the medium 2 mg/l BA produced maximum number of axillary buds.

Experiment No.2

Objective : To find out the suitable medium for shoot elongation.

Material : In vitro multiplied (2 mg/l BA) about 0.5 cm long

Method : lateral shoot clusters (about 3 shoots/cluster) were used as explants.

Media : I. Anderson's + BA 2 mg/l with GA<sub>3</sub> 0/0.2/  
0.5/1.0 mg/l

II. Anderson's medium BA<sub>2</sub> mg/l and increased nitrogen (NO<sub>3</sub><sup>-</sup> & NH<sub>4</sub><sup>+</sup>)

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III. MS medium + 2 mg/l  $3^A$

IV. 35-2 medium + 2 mg/l  $3^A$ .

Results : Introduction of  $3^A$  to the culture medium didn't show elongation. Other trials were continuing.

Experiment III To find out the suitable medium for root initiation and elongation.

Material & Method : In vitro - multiplied about 1.5 cm long shoots were taken and introduced to following media.

- I. Anderson's + IBA or NAA with or without at 2% activated charcoal (0, 0.2, 0.5, 1.0, 2.0 mg/l)
- II. Anderson's + 20% mg/l Fluroglucinol + IBA or NAA with or without at 2% activated charcoal (0, 0.2, 0.5, 1.0, 2.0 mg/l)
- III. 1/3 Anderson's + IBA or NAA with or without 2% activated charcoal (0, 0.2, 0.5, 1.0, 2.0 mg/l)

Results : Full strength of Anderson's medium with any of the concentration of IBA or NAA with or without charcoal failed to produce roots. At lower concentration of Anderson's medium (1/3 strength) was produced an excellent root system with IBA or NAA at the concentration of 1-2 mg/l. The experiment with Fluroglucinol is continuing.

B) Experiments with materials from other trees (15-25 years).

## Experiment No. 1

- Objective :** All of the attempts were a failure on shoot tips either actively growing or dormant due to high sensitivity to sterilant. Lower concentration or less dipping time of the sterilant (0.1%  $HgCl_2$ ) resulted in higher number of fungal contamination. Therefore more emphasis was given to nodal sections which were highly resistant to sterilant.
- Material :** About 6" long shoot tips were collected from the mother trees and all the leaves and shoot portions were discarded to obtain 2<sup>nd</sup> and 3<sup>rd</sup> internodal sections. These internodal sections were sterilized with 0.1%  $HgCl_2$  for 10 minutes. After 3 rinses with sterilized distilled water nodal sections were dissected out and introduced to Anderson's medium with following hormones.
- I. BA (0, 1, 2, 4, 6, 8, 10, 12, 15, 20 mg/l)
  - II. Kn (0, 1, 2, 4, 6, 8, 10, 12, 15 & 20 mg/l)
- Results :** BA at the concentrations 6-10 mg/l showed better establishment and two axillary buds sprouted after 8-10 weeks. All of the treatments with Kn failed to produce any axillary buds.

Experiment No. 2

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

Method & Materials : Medium:- B5-2 medium supplemented with ascorbic acid  
0.1 gms, glutamine 0.4 gms and sucrose 30 mgs. Four  
concentrations of 2,4-D were used (0,0.5,1.0,2.0  $\mu\text{g}/\text{l}$ ).

Ovules were taken from clove flowers in three different  
stages I. Fully matured unopened,

II. One week after pollination,

III. 3-4 weeks after pollination. Immature  
embryos were extracted from small fruits  
(4 weeks after pollination).

Results : Ovules from fruitlets (3-4 weeks after pollination)  
survived for about 6 months, whereas other stages of  
ovules survived more than 4 months.  
However none of the ovules produced any callus.  
Immature embryos (4 weeks after pollination) produced  
large, white callus after 8 weeks at the concentration  
of 2,4-D 1.0 and 2.0  $\mu\text{g}/\text{l}$ .

6. Conclusion drawn from the work.

Nutmeg - materials from juvenile plants

- I. In multiplication phase liquid agitation is better than others.
- II. In vitro produced plants were well grown in the pots.

Nutmeg - materials from grafted plants

- I. Newly emerged shoots after terminal pruning provide clean and healthy materials for future experiments.

Nutmeg - materials directly from mother trees

- I. Nodal cultures failed to produce axillary buds

Clove - juvenile

- I. In vitro multiplied shoots can be rooted in lower concentration of salts.

Clove - Adult trees

- I. Nodal explants can be established in medium containing 6-8 mg/l of BA.

Is the work on schedule? Yes.

7. Plan of work for the next half year.

More experiments will be done with the material from the mother trees of clove/nutmeg and nutmeg grafted plants.

8. Any other comments:

1. Even after 2½ years of commencement of project major equipment did not received.

Signature of Grantee/s :

1. Y.M.H.B. Yapabandara

  
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2. Prof. M.D. Dassanayake

  
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Comments (if any ) of Head of Department/Section:

  
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Signature of Head of Department