DFID Project

R.P.FIII FINAL REPORT

1. Institute Code No.: F11-99/P-CI-F30/0311

2.I.C.A.R. Code No:

3.Name and address of Research Institute/Centre:
Central plantation Crops Research Institute , Kasaragod-671 124,Kerala

4. Title of the project: **Increasing efficiency of embryo culture to promote germplasm collection (DFID Phase-1)**

5. Name and designation of Principal Investigator:
Anitha Karun and V.A. Parthasarathy

6. Name(s) and designation of Associate(s) and establishment(s) on which borne
a. Whole time
b. Part time (indicate proportion of time to be devoted and other area(s)

Anuradha Upadhyay

7. Location of the Research project with complete address:
Crop Improvement Division, Biotechnology Section

8. Date of Start: August 1998

9. Likely date of completion : 2000 March

A) **Objectives: (not more than 150 words)**

1. To compare the efficacy of four embryo culture protocols viz., PCA, Philippines, UPLB, Philippines, IRHO, France and CPCRI India and to study the effect of genotype on *in vitro* embryo culture.

2. To study the effect of growth hormones on embryo maturation and subsequent germination.
   Growth hormones- GA_3 and ABA

3. To study the effect of osmotica on embryo maturation and subsequent germination.
   Osmotica: Mannitol, PEG (Polyethylene Glycol) and proline.

b)**Practical utility** (not more than 100 words)

Collection and exchange of coconut germplasm is difficult and also expensive because of the bulkiness of the seed. Due to short dormancy the seeds germinate during transit and cannot be stored for long term. Standardisation of embryo culture techniques provides an easy and safe alternative for the movement of coconut germplasm (FAO/IPGRI). *In vitro* culture protocol for coconut zygotic embryos have established by various coconut research institutions with different cultivars and their degrees of success has been published. To improve/upgrade these existing protocols for mature (11 months after fertilisation) and immature (9 months after fertilisation) embryos and their high survival rate for large number of cultivars and *International Coconut Embryo Culture and Acclimatisation Workshop* was conducted at PCA, Philippines during 27-31 October 1997.

Technical Programme(indicate briefly plan of procedure , techniques, instruments and special materials, organisms, special environments etc.)

PROTOCOL AND VARIETY INTERACTION

Experimental design – RBD; Treatment details: Protocols (4): PCA, Philippines; UPLB, Philippines; CPCRI, India and IRHO, France. Cultivar (4): MYD, LCT, COD and WCT

No. of replications: 3 No. of embryos /treatment : 20

Total no. of embryos inoculated: 960

EFFECT OF GROWTH HORMONES

treatment details (8 treatments)

GA_3 (4 concentrations1.0 µM, 0.5 µM, 0.1 µM, and 0.05 µM; Age of the embryo (2 types) : 9 month and 11 month oldExperimental design : RBD

No. of replications: 3 No. Of embryos /treatments: 20 Total no. of embryos inoculated : 480

ABA (3 levels) 10 µM, 20 µM and 30 µM) Age of the embryo (2 types) : 9 month and 11 month old. No of embryos/treatment: 20 . No. of replication : 3

Control : 20 (mature) and 20(immature)- Total no. of embryos inoculated : 400

EFFECT OF OSMOTICA ON MATURATION AND GERMINATION OF COCONUT ZYGOTIC EMBRYOS

Treatment details (8 treatments) Mannitol (4 levels) : 0.2 M, 0.3 M. 0.4 M

Age (2 levels) : Mature(11 month old) and Immature (9 months old)

No.of replication : 3 No. of embryos /treatment: : 20 Control: 20 (mature)+20(immature)-40 Total number of embryos inoculated: 400

Treatment details (6 treatments)

Proline (2 levels) : 10 mM, 20 mM, Age (2 levels) : 2 levels (9 month and 11 month) No of replications : 3 No of embryos /treatment20 Control : 30 (mature)+30(immature) Total No of embryos inoculated : 300

Treatment details (8 treatments)

PEG (Poly ethylene Glycol) – 4 levels : 0.5 %,1 %, 2 %, and 3 % with control

Age (2 levels) : 2 levels (9 month and 11 month)No of replication : 3

No. of embryos /treatment : 10 Control : 30 (mature)+30(immature)

Total No of embryos inoculated : 300

Annual Report on the Project: (A summary of results not exceeding 2 pages precisely and concisely stating the fundamental and/or practical significance there)

At CPCRI, India, six experiments were conducted to compare the four protocols and to study the effect of growth regulants and osmotica on embryo germination and culture. Four embryo culture protocols *viz.*, UPLB Philippines, PCA, Philippines, CPCRI, India and IRHO, France were tried with 4 cultivars viz., West Coast Tall (WCT), Laccadive Ordinary (LCT) Chawghat Orange Dwarf (COD) and Malayan Yellow Dwarf (MYD). Interaction between the protocol and time was highly significant. The CPCRI protocol showed slow growth compared to other three protocols, which were on a par. The vitrification was noticed only in liquid medium. Transplant shock was observed more in UPLB, Philippines and IRHO, France. From the latest observations it was found that the maximum plantlets survived in PCA irrespective of the cultivar and were on a par, followed by CPCRI and UPLB protocols. In protocol of IRHO, maximum plantlets were found to be dead both ***in vitro*** and ***ex vitro.*** Other 5 experiments were conducted to study the effect of growth hormones and osmotica on maturation and germination of embryos. GA3, ABA, Mannitol, PEG and Proline were used as treatments. The GA_3 showed no effect between treatment and time for length of shoot. The growth of the shoot depends on the maturity of embryos. However, for the root development there is a significant maturity and GA_3 interaction. It was also observed significant interaction between maturity of embryos and levels of ABA for initial shoot length (60 days) and root length (120 days). The initial observation in mannitol showed all the three levels of mannitol inhibited the germination. With regard to vitrification, the mannitol level * age interaction was significant. PEG and proline did not affect the germination The growth of plants was also found to be influenced by the age of embryos. Significant differences among levels of proline were observed for the shoot length and weight gained For both these characters, there is an increase corresponding to the increase of proline level. The multivariate test showed significant difference among PEG levels as well as between age of embryos. Shoot length is significantly different among the levels of PEG. Significant difference for weight of embryos was noticed between the ages of embryos.

The physiological parameters for acclimatisation like net photosynthetic rates, stomata conductance and transpiration rates were measured at the time of transfer of plants to pots. Net photosynthetic rates were negative in all four cultivars raised in four media. The stomata conductance was less than 0.01 $mol.m^{-2}.s^{-1}$. Transpiration rates significantly varied due to the medium. Transpiration rates and stomatal conductance were higher in seedlings raised on UPLB, Philippines whereas; seedlings raised on PCA Philippines had lower transpiration rates.

13.Progrss of work in relation to the time targeted for completion of work and reasons for non achievement of targets, if any.

Nil

14.**Publication during the period**: (two copies each to be supplied with this proforma)

a) Research papers:

Anitha Karun, Anuradha Upadhyay and Parthasarathy. V.A. 1998

Status of research on coconut embryo culture and acclimatization techniques in India.Coconut embryo culture in vitro cultureProceedings of the first workshop on embryo culture held at Albay research Centre Philippines Oct 27-31 1998. pp.29-36

Anitha Karun,Anuradha Upadhyay, and V.A. Parthasarathy 1998

Report on Coconut embryo culture research in CPCRI *Coconut embryo culture net work Newsletter* **1**(1) :11-12.

Anitha Karun, Anuradha Upadhyay, and V.A. Parthasarathy 1999

Increasing the efficiency of embryo culture to promote germplasm collecting. India.*Coconut embryo culture net work Newsletter* **2**(1) :8-9.

Anitha Karun,Anuradha Upadhyay andParthasarathy. V.A. 1998

Status of research on coconut embryo culture and acclimatization techniques in India*Treeworld* **8**(7):3.

Anitha Karun, Sajini K.K. Upadhyay A. and V.A.Parthasarathy. 2000. Comparison of embryo culture protocols among coconut cultivars. *Journal Of Plantation Crops (communicated)*

Anitha Karun, Sajini K.K. Upadhyay A. and V.A.Parthasarathy. 2000. GA_3 and ABA mediated effect on maturation and germination of coconut zygotic embryos. *Journal of Horticultural Sciences*. (*communicated).*

Anitha Karun, V.A.Parthasarathy and Upadhyay A. 2000. Improvement of *In vitro* techniques for collecting and exchange of coconut (*cocos nucifera* L) germplasm *Coconut Embryo culture Network Newsletter* . 3(1) : 11-12.

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15. Signature of Principal Investigator: Anitha Karun Anuradha Upadhyay

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16. Signature of the Head of Division/Section:

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17. Signature of Director:
