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7.086

"CLONING OF ELITE CASHEW TREES BY GENERATING JUVENILITY
THROUGH MICROGRAFTS USING TISSUE CULTURE TECHNOLOGY" (7.086)

GRANT NO: 493-5542-G-00-9102-00

FINAL REPORT
(PROGRESS REPORT NO. 4)
(FOR THE PERIOD APRIL - SEPTEMBER 1991)

SUBMITTED BY
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1

SCIENTIFIC SUMMARY

NOTE: Minimal experiments were conducted after March 1991 when PLANTEK was notified by USAID that the Cashew project is suspended due to the military coup in Thailand.

The following experiments were carried out:

- (a) Protocol development for the sterilisation of plant materials from the field;
- (b) In vivo grafting of cashew; and
- (c) Micropropagation of mature cashew.

We have been moderately successful in the sterilisation of field plant materials. The success rate range from 30 to 70% depending on the raining season.

The sterilised mature explants were used for in vitro micrografting. There was no success in using this technique for grafting because the grafted explants (a small piece of shoot tip circa 4 mm²) dried up in culture. The sterilisation protocol which was found to be most effective for the sterilising mature plant materials from the field is as follows:-

- (1) wash explants in running water plus a few drops of Tween 20;
- (2) half an hour in 500ml sterile water + 5 drops Tween 20 + 5ml 30%(v/v) Hydrogen peroxide;
- (3) sterilise in 10% (v/v) Cetricide for 15 min;
- (4) rinse once in sterile water;
- (5) wash explants in 96% alcohol for 30 sec then trim the cut ends of the explant;
- (6) sterilise explant in 10% (v/v) Clorox for 1 min;
- (7) Rinse explant in sterile water 3 times;
- (8) dip explant in 96% alcohol for 20 sec; and
- (9) rinse explant in sterile water 3 times before inoculating the explant in a suitable medium.

In vivo grafting was conducted. The percentage of successful grafts range from 2 to 20%. The method which we found to be most suitable was to dip the scions in 10% (w/v) Benlate (Benomyl) for 15 secs and then shake off any excess fungicide before grafting. The 2nd grafting after the first graft was attempted but all were infected by fungus.

We have attempted both in vitro and in vivo graftings. Will low level of success, we decided to attempt micropropagation of mature plant materials. We compared two major method of propagation.

- (1) M & S medium (full & half concentrations) + 3% sucrose + 0.5% activated charcoal + 0.01mg/l NAA + BAP; and
- (2) K(h) medium (Cheng, 1975) (full & half concentration) + 3% sucrose + 0.5% activated charcoal + 0.01mg/l NAA + BAP.

The BAP concentration used was 0 - 15 mg/l.

We found that using K(h) medium gave the best result while M&S medium cannot maintain viable cultures. K(h) medium can maintain viable initiates as well as causing the dormant buds to break within 2 weeks in culture for all concentrations of K(h) and BAP level range of 2 to 6 mg/l. A comparison of the M&S media and K(h) medium is given in Appendix 1.

Due to the lack of time available in the project we did not proceed to induce multiplication of the sprouted buds.

2. SCIENTIFIC ISSUES

At Plantek our research had been impeded by the lack of elite plant materials for experiments. We had made headway in the use of a medium that could maintain viable mature explants and bud sprout. This method is highly significant for future research into the micropropagation of cashew. With this in mind we have written to USAID, Washington in four occasions since August 20th, 1991 requesting for an extension as well as revision to the research project. Our proposal request for the use of micropropagation method instead of micrografting method for the propagation of mature cashew trees. Unfortunately, PLANTEK has yet to receive a reply to our proposal.



PHOTO 1 Axillary bud enhancement of seedling shoot tips in M&S medium (Full strength) + 3% sucrose + 0.5% activated charcoal + 0.01mg/l NAA + 8 mg/l BAP. The buds are very compact and does not elongate.



PHOTO 2 Sprouting of mature explant in K(h) medium (Full strength) + 3% sucrose + 0.5% activated charcoal + 0.01mg/l NAA + 0 mg/l BAP.



PHOTO 3 Sprouting of mature explant in K(h) medium (Full strength) + 3% sucrose + 0.5% activated charcoal + 0.01mg/l NAA + 2 mg/l BAP

3. MANAGERIAL ISSUES

In March 1991, PLANTEK received a telephone call from the Principal Investigator of the project (Prof Pranom Prutpongse) informing us that due to the military coup in Thailand, the US government had suspended financial aid to Thailand. We were officially informed by USAID, Thailand on March 18, 1991 through telephone conversation with Dr Jaroon Kumnuanta and a letter from Mr Neil C Edlin on April 5, 1991. PLANTEK had taken immediate action by minimising all research activities related to the cashew project.

4. SPECIAL CONCERNS

No special concern had arise.

5. COLLABORATION, TRAVEL, TRAINING AND PUBLICATIONS

No activities.

6. REQUEST FOR AID OR BOSTID ACTIONS

PLANTEK would appreciate an extension as well as a revision to the project objectives. We have sent our proposal for the revision and extension of research plan to the following address:

The Director
Science Program
Office of the Science Advisor
Room 302 SA 18
Agency for International Development
2201 C Street, N W
Washington D C, USA

Fax No. 703-875-4394

Thus far, we have yet to receive a reply to the proposal.

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APPENDIX 1MEDIUM COMPOSITION FOR USE IN CASHEW TISSUE CULTURE

<u>CHEMICALS</u>	<u>M&S</u>	<u>K(h)</u>
NH ₄ NO ₃	1650	825
KNO ₃	1900	950
CaCl ₂ .2H ₂ O	440	220
MgSO ₄ .7H ₂ O	370	185
KH ₂ PO ₄	170	85
NaEDTA	37.23	7.2
FeSO ₄ .7H ₂ O	27.95	6.0
KI	0.83	0.8
H ₃ BO ₃	6.2	6.2
MnSO ₄ .4H ₂ O	22.3	1.4
NaMoO ₄ .2H ₂ O	0.25	0.4
Zinc sulphate hepta hydrate	8.6	10.6
CuSO ₄ .5H ₂ O	0.025	0.02
CoCl ₂ .6H ₂ O	0.025	0.02
Inositol	100	250
Nicotinic acid	0.5	--
Pyridoxine-HCl	0.5	--
Thiamine-HCl	0.1	2.5
Glycine	2.0	--
Sucrose	3%	3%
Activated Charcoal	0.5%	0.5%
Agar	0.7%	0.7%