စိုက်ပျိုးရေး၊ မွေးမြူရေးနှင့် ဆည်မြောင်းဝန်ကြီးဌာန

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In vitro callus induction and somatic embryogenesis from mature zygotic embryo of oil palm (*Elaeis guineensis* Jacq.)

ဆီအုန်း သန္ဓေလောင်းအရင့်မှ ကျိုးပေါင်းဆဲလ်ဖြစ်ပေါ်ခြင်းနှင့် သန္ဓေလောင်းအဆင့်ဆင့်ကြီးထွားမှု ကိုလေ့လာခြင်း

ဒေါ်တင်တင်ဝင်း

ဒု-ဦးစီးမှူး

၂၀၂၂ ခုနှစ် ဖေဖော်ဝါရီလ (၉) ရက်

Introduction

- Oil Palm (*Elaeis guineensis* Jacq.)

➢ one of the most oil bearing crops in the world

➢ conventional breeding of oil palm often takes about more than 10 years per generation, and is extremely slow and costly (Jayanthi *et al*., 2015)

➢ brought about interest in vegetative propagation of oil palm *via* tissue culture

Introduction (cont.)

- Much research has been carried out from different explants culture in oil palm---Mature zygotic embryos (Teixeira *et al*., 1993, 1994), Immature zygotic embryos (Te-chato & Sanputawong 2008), Seedlings (Anitha & Sajinii, 1996), Young leaves (Mondjeli Constantin & *et al*., 2015) and inflorescences (Elis Kartika, *et al*., 2019)
- The establishment of plant regeneration in oil palm by somatic embryogenesis is satisfactory
- Embryo explants are more convenient because fruits are readily available, have a high degree of physiological uniformity, and can be shipped to a long distances (Te-chato *et al*., 2011)

Introduction (cont.)

- Since the introduction of oil palm tissue culture in the 1970's, clonal propagation has proven to be useful, not only in producing uniform planting materials, but also in the development of the genetic engineering programme
- As a monocotyledonous species with a single growing apex, the plant cannot be multiply vegetatively
- For the extension of oil palm cultivation, a large amount of selected planting materials will be required.
- To solve this problem, micropropagation is one of the most important ways. So, efforts are being made to advance oil palm tissue culture in PCRDC, Mawlamyine.

Process of oil palm tissue culture in PCRDC

- ortet selection in the field
- sampling of explant from ortet
- callus initiation and multiplication
- embryogenesis and multiplication of polyembryoids
- shoot regeneration and development
- rooted plantlets
- hardening and establishment in the nursery
- field planting and evaluation

Problem Statement

- Oil palm seed production is very restricted and time taken to obtain the require amount of hybrid seeds
- If oil palm clonal propagation has become a commercial output of clonal plantlets, it can constitute the require amount of the annual oil palm planting material in Thanintharyi Region.

Objectives

- To investigate the induction of callus from mature zygotic embryo
- To evaluate the effects of hormone concentrations on callus proliferation ,embryoid production and plant regeneration

Materials and Methods

Plant materials

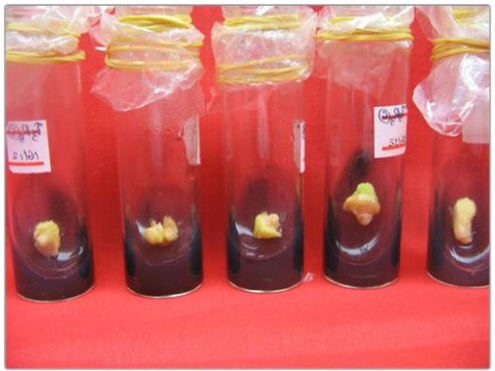
- Collected from the Baeyan Research farm, Mudon, Mon state,
- Tenera hybrid plants (Dura x Pisifera)
- Mature zygotic embryos




Materials and methods (cont.)

Sterilization technique

- Embryos were extracted from the seeds and sterilized in 10 % Clorox for 20 min.
- The embryos were then thoroughly washed in sterile water for four or five times
- Inoculated onto the media



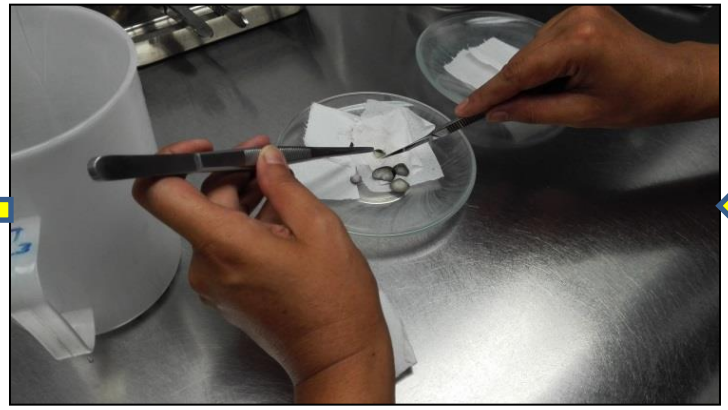
Mature zygotic embryo culture

Fruit bunch

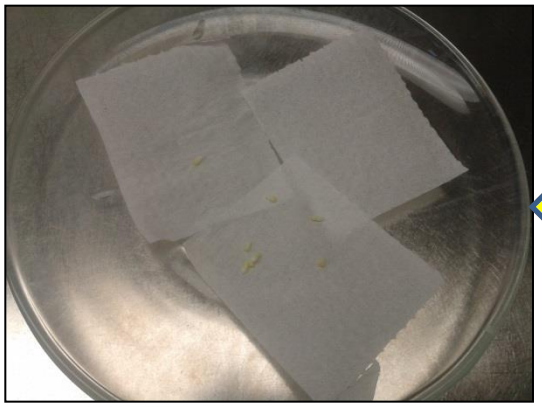
Hybrid seeds

Isolated seeds

Isolated seeds



Extracting the embryo

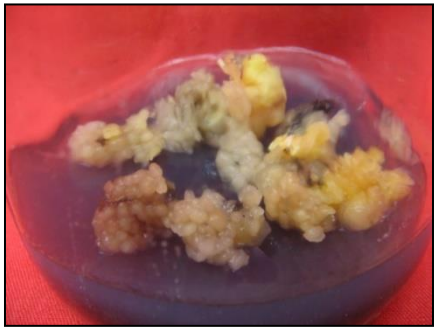


Embryo

Materials and methods (cont.)

Culture media

- Teixeira *et al*., (T) (1995) (Modified MS) – callus initiation
- Murashige and Skoog (MS) media (1962) – callus multiplication and shoot initiation
- Woody plant medium (WPM) – rooting



Callus induction and maintenance

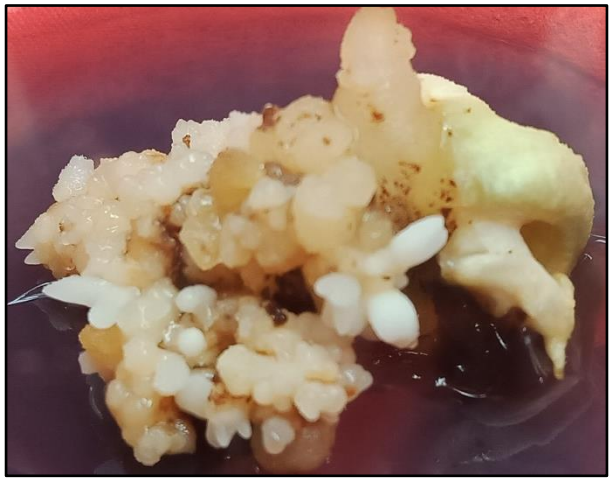
- Growth regulators
  - 2,4-D - 0, 50, 70 and 90 mg/l (T_0,T_1, T_2,T_3,)
- Incubated under light at 27 ± 2°C until sufficient callus was obtained
- sub-cultured – monthly intervals

Materials and methods (cont.)

Induction of embryoids and plantlet regeneration

For callus multiplication and embryoid production

- ½ MS macro-nutrients
- Full strength MS micro-nutrients
- Biotin – 0.02 mg/l
- D-Ca panthothenate - 0.2 mg/l
- Glycine – 2.0 mg/l
- PVP - 40 – 0.5%
- Activated charcoal – 0.3 %
- BAP – 2.0 mg/l
- NAA – 1.0 mg/l



Results

*In vitro r*esponses of cultured mature zygotic embryos of oil palm (*Elaeis guineensis* Jacq.)

Teixeira *etal*., media+ 2,4-D (mg/l)	Callus induction (%)	Compact embryogenic tissue (%)	Single shoot formation
0	0	0	45
50	25	5	15
70	45	21	0
90	40	10	0

- Callus multiplication, embryoid production and shoot initiation were observed on MS basal medium supplemented with BAP – 2.0 mg/l , NAA – 1.0 mg/l and Activated charcoal – 0.1% (B1)

Time-frame for mature embryo culture

Stage of culture	Time taken for each stage	Cumulative time
Callus induction	3 months	3 months
Embryoid induction	9 months	12 months
Shoot initiation	4 months	16 months
Plantlet formation	2 months	18 months
Rooting	2 months	20 months
Hardening	4 months	24 months

Discussion

- In the previous studies, embryogenic calluses in oil palm were induced and maintained in a medium containing growth regulators, especially auxins and cytokinin (Rajesh *et al*., 2003)
- The induction of somatic embryogenesis from immature embryos showed a pattern of development district from all other reports on oil palm (Teixeira *et al*., 1993)
- Most of the reported embryo induction media used for oil palm was MS based
- However, in the present study, Teixeira *et al*., 1995 medium was found to be the best based on earlier experience

Discussion (cont.)

- Jones (1974) suggested that embryogenesis in oil palm cultures is a function of explant source rather than of culture medium and culture conditions

- From this study, callusing rate per plant varied according to the genotype of the palm, the level on the embryo and, very significantly, with the phytohormone concentration.

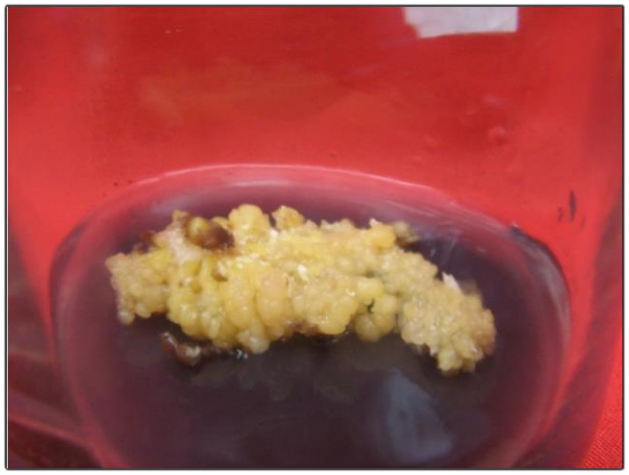
- Growth regulator concentrations and culture conditions appear to be important for the formation of somatic embryoids.

Mature zygotic embryo culture

1. Initial culture

2. After 2 weeks

3. After 5 weeks

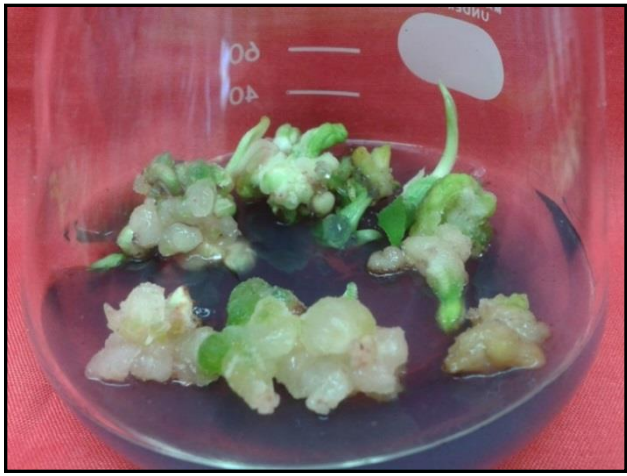


4. After 3 months

Mature zygotic embryo culture

Callus proliferation after (5) months

Somatic embryoids after (9) months



Multiple shoot developing from somatic embryos after(4) months

Rooted plantlets after 2 months

Hardened plantlets(4) months

Oil palm plantlets planted in pre-nursery and main nursery

Oil palm clonal plants planted in the field

Conclusion

- The first report of the use of mature zygotic embryos for induction of callus, somatic embryogenesis and subsequent plant regeneration in oil palm
- The highest percentage of callus induction was obtained on Teixeira *et al*., basal medium supplemented with 2,4-D - 70 mg/l
- MS medium supplemented with BAP – 2.0 mg/l , NAA – 1.0 mg/l and Activated charcoal – 0.1% (B1) gave the best response on callus multiplication, embryoid production and shoot initiation
- For a commercially viable clonal propagation unit, technical know-how and efficient management are essential. It is reliable methods for inducing embryogenesis, together with genetic uniformity and stability of the cultures are of paramount importance.

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THANK YOU