Ministry of Agriculture, Livestock and Irrigation
Department of Agriculture
Perennial Crop Division

Callus Induction and development from Mature Anther Culture of *Hevea brasiliensis* Muell Arg.

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Introduction

- Rubber – *Hevea brasiliensis* Muell. Arg.
- Family – Euphorbiaceae
- An economically important perennial tree crop grown in Myanmar and Southeast Asia as the source of natural rubber
- It is of a major economic importance because of its timber and latex that can be collected and is the primary source of natural rubber
- Rubber is one of the main raw materials for industry and has many uses in traffic, industry, national defense etc.

Introduction (cont.)

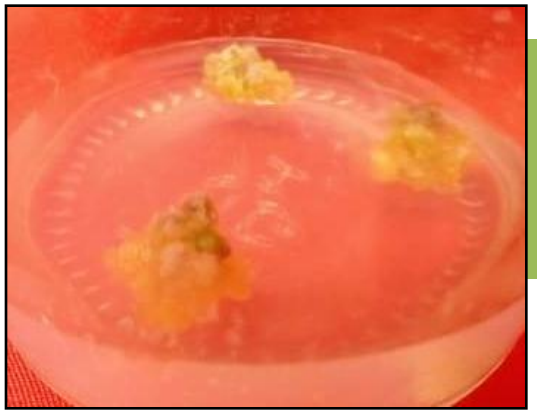
- There are several reports on *Hevea* micro-propagation using different explants, mostly derived from seedlings (Jayashree *et al*., 2000).
- Thereafter, plantlets with shoots and roots were successfully developed by different investigators (Gunatilleke *et al*., 1988; Carron *et al*., 1989; Sompong and Muangkaewngam, 1992; Kyte and Kleyn 1996; Paranjothy and Glandimethi, 1976.
- Tissue culture technique is applied in many plant species including rubber tree

Introduction (cont.)

- Several reports of rubber tree micropropagation using explants raised in different culture media selected according to the objective of the study
- Somatic embryogenesis is one of the powerful tissue culture technique for mass propagation of elite *Hevea* clones
- *Hevea* somatic embryogenesis was firstly developed in China and Malaysia, using the anther as initial mother tissue explants (Venkatachalam, 2006)

Problem Statement

- The conventional method of propagation of rubber may lead to undersirable stock - scion interaction.
- If rubber can be culture by clonal propagation, it will be possible to produce a large number of rubber seedling with desirable traits true to type plant.

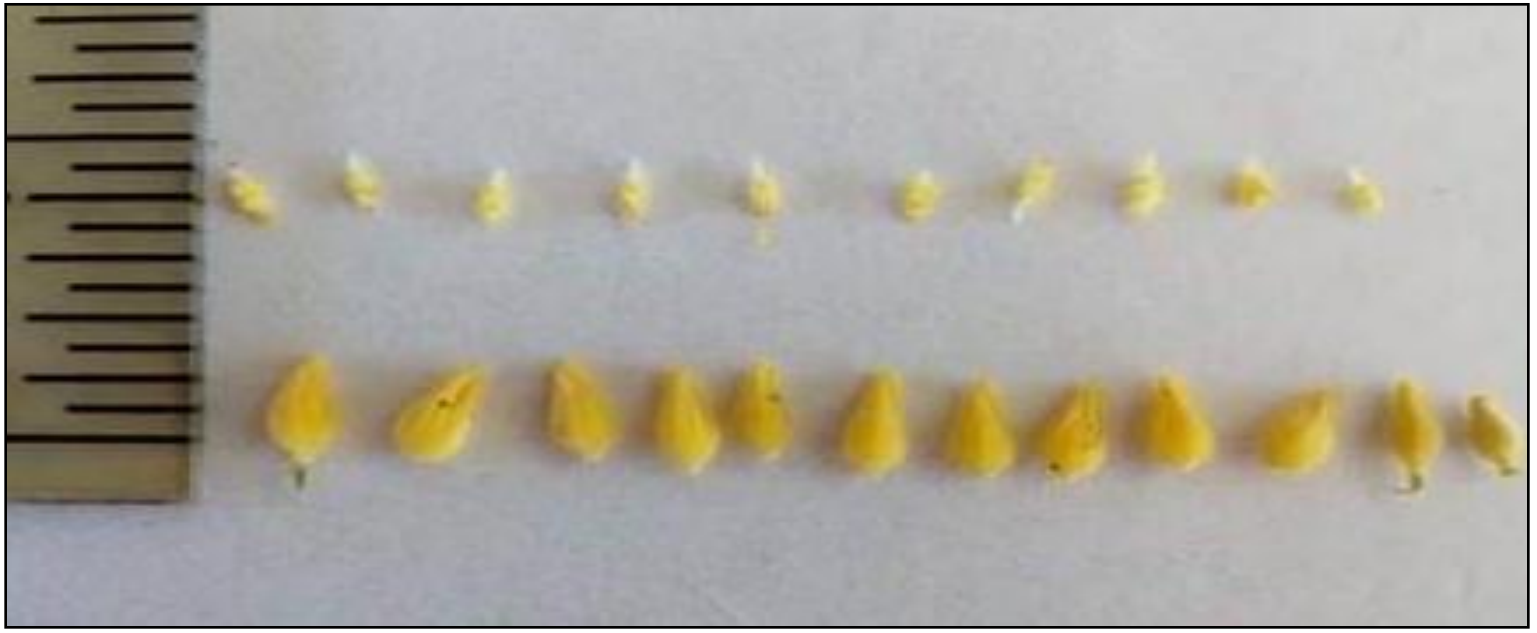


Objectives

- To investigate the induction of callus from mature anther of *Hevea*
- To evaluate the effects of hormone concentrations on callus proliferation and development.

Materials and Methods

- Plant materials
  - Collected from PCRDC, Mawlamyine, Mon state
  - Variety : PB 260, RRIM 717
  - Explant: Mature anther



Materials and Methods (Cont.)

Method of sterilization:

- Flowers bunch were collected from the tree and wash in running tap water for 10 min
- After that wash with detergent powder for 15 min, and sterilized in 0.1% $HgCl_2$ for 3-5 min, and then sterilized in 10 % Clorox for 10 min
- The explants were then thoroughly washed in sterile distilled water for four or five times
- Inoculated onto the media

Materials and Methods (Cont.)

- Media

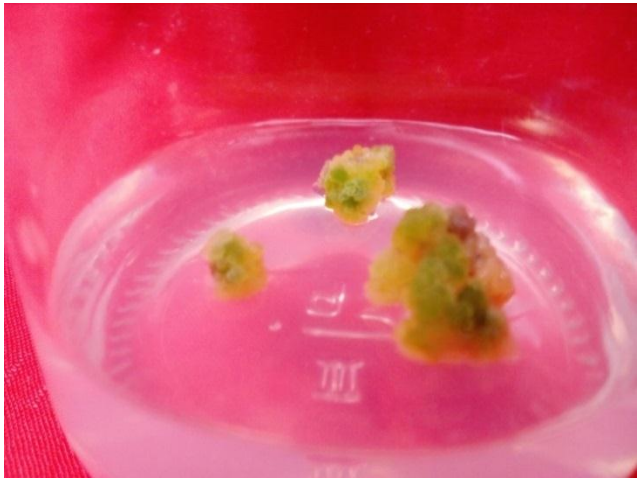
- Basal Media: MS (Murashige and Skoog ,1962)

- Plant growth regulators: 2,4-D, NAA, BA, Kin

- pH – 5.7

- Sucrose – 30 gm/l

- Subculture – 4 week intervals

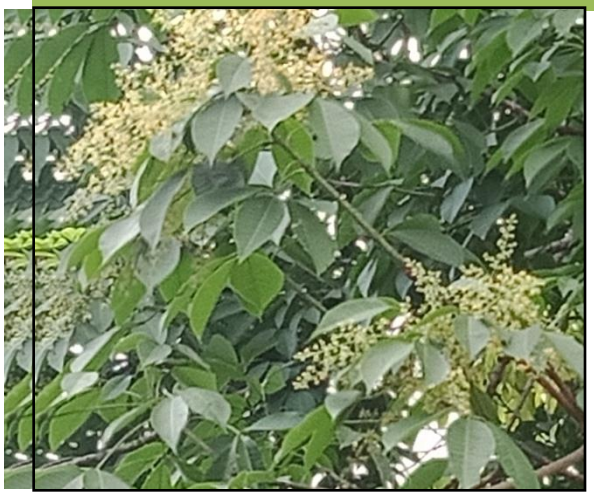


Materials and Methods (Cont.)

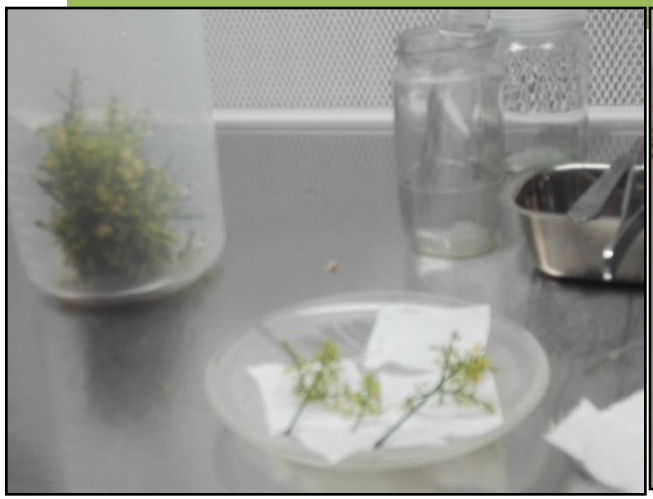
Media combinations

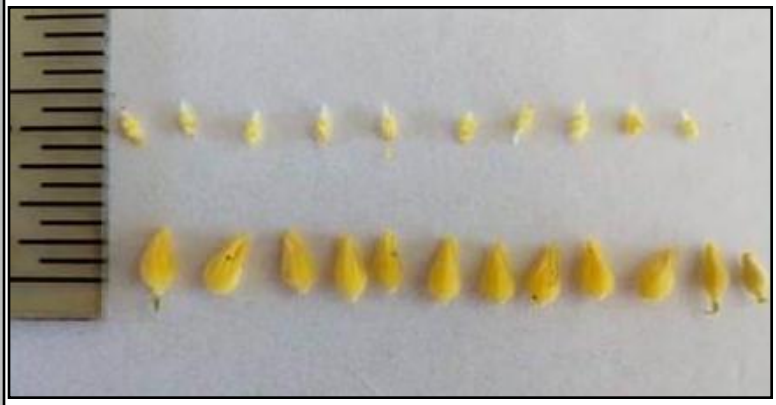
Treat-ment	Medium	Plant Growth Regulators (mg/l)				Sucrose (gm/l)
		2,4-D	NAA	Kin	BA	
A 1	MS	1.0	2.0	2.0	1.0	30
A 2	MS	0.2	0.5	0.2	-	30
A 3	MS	0.3	-	1.0	0.5	30
A 4	MS	-	-	-	-	30

Steps in rubber anther culture



Steps in rubber anther culture





Results

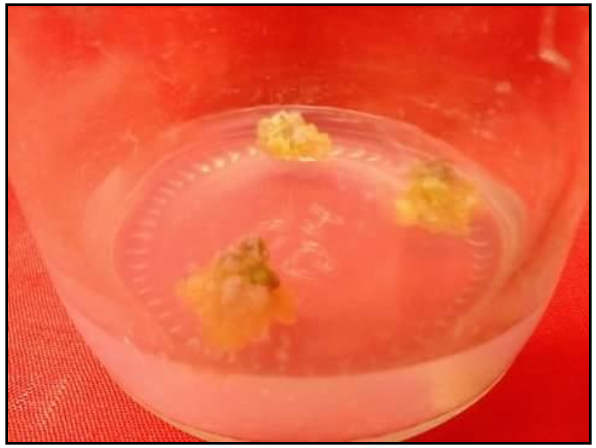
*In vitro r*esponses of cultured mature anther of rubber (*Hevea brasiliensis* Muell Arg.)

Treatment	Callus Induction (%)		Callus Structure		Callus Color	
	PB 260	RRIM 717	PB 260	RRIM 717	PB 260	RRIM 717
A 1	45	50	Compact and hard	Friable and moist	Yellowish Pale yellow	Pale yellow White
A 2	25	33				
A 3	22	27				
A 4	0	0				

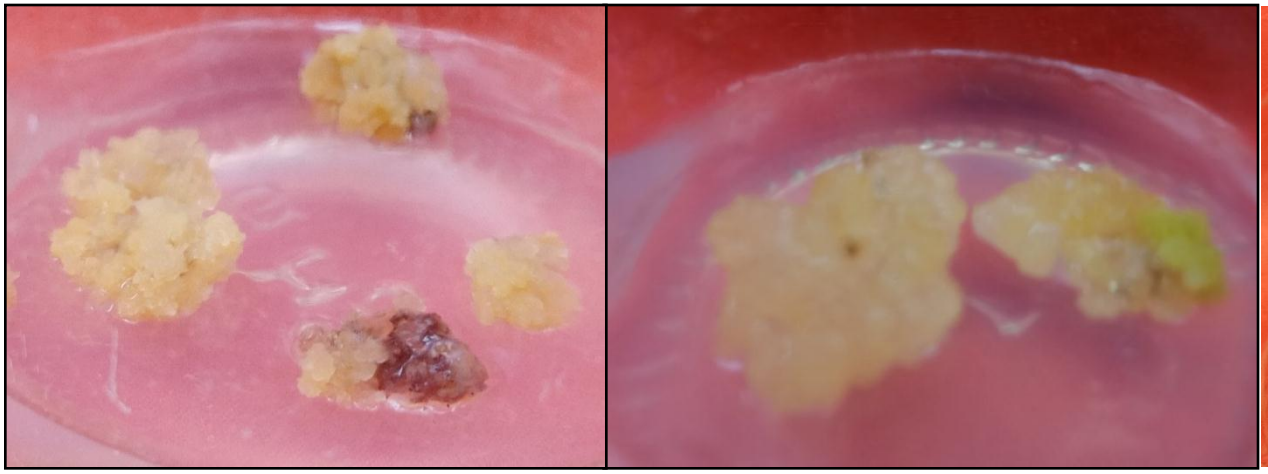
Results (Cont.)

Initial culture

After 2 weeks

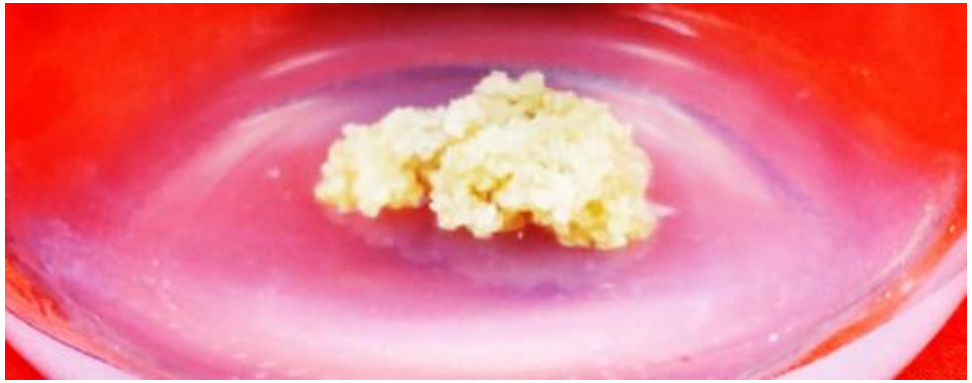


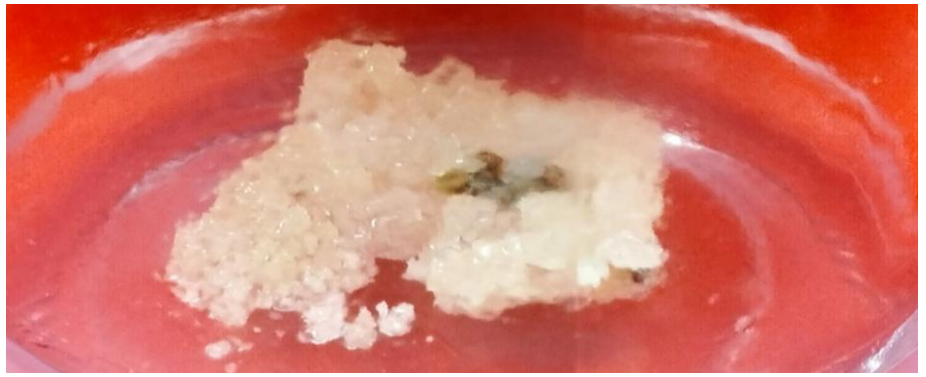
After 1 month



After 3 months

The characteristics of callus formed on mature anther of PB 260 & RRIM 717





Discussion

- In Hevea, 2,4-D or a combination of 2,4-D and NAA were used for callus induction. (Carron and Enjalric 1965; Chen et al 1979, Sushamakumari *et al* 2000)
- On rubber tissue culture, where the application of 2,4-D (1.0 -2mg/l) produce callus. (Rahman *et al*.)
- In the present study, the presence of 1 mg/l 2,4-D in culture medium was found to stimulate callus proliferation.
- Callus amount, texture and color were varied depending on the type and concentration of auxins, especially 2,4-D (Martin 2003)
- In Hevea, a compact callus with highly embrogenic nature was obtained from integumental tissue of clone PB 260 (Montoro *et al*.1993)

Discussion

- In this study, the color of callus was dominted by yellowish, pale yellow, white cream and friable to compact in structure
- Influence of NAA on embryogenesis from mature anther of Hevea was show by Chen *et al*. (1979)
- From this study, embryogenic callus formation varied according to the plant genotype, type of explants, medium composition, plant growth regulator concentration and culture condition

Conclusion and Suggestion

- This paper describes the first report in vitro culture of anther of *Hevea brasiliensis* Muell Arg
- The application of basal medium supplement with 2,4-D 1 mg/l + NAA 2 mg/l + kin 2 mg/l + BA 1 mg/l is the best combination for callus induction and multiplication.
- The proliferated callus was believed to have the opportunity to develop embryogenic properties and regenerate somatic embryos.
- To that end ,the next stage of research would be the optimization of culture medium to induce somatic embryogenesis.

References

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Thank you