02.12.91

Tissue Culture of <u>Hevea</u> <u>brasiliensis</u> (Seedling Shoot Tip Culture)

Ву

Iranganie Damayanthi Goonetileke

B.Sc Chem Sp. (Sri Lanka)

Thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science of the Faculty of Applied Science,
University of Sri Jayewardenepura, Nugegoda, Sri Lanka.

1989

116090

Tissue Culture of <u>Hevea</u> <u>brasiliensis</u>
(Seedling Shoot Tip Culture)

Iranganie Damayanthi Goonetileke

ABSTRACT

Shoot tips of <u>Hevea brasiliensis</u> Muell.-Arg removed from aseptically grown seedlings could be established on liquid or solid media supplemented with or without growth regulators. Four different basal media were tested. The Murashige and Skoog medium (1962), which is a high salt strength medium was found to be the most suitable for axillary shoot proliferation. The other media tested were, the Woody Plant Medium (WPM), the White's medium (1943) which is a low salt strength medium and a modified medium containing MS (1962) salts and vitamins used in Skirvin and Chu (1980) medium. Different cytokinins were tested for shoot proliferation. Benzylaminopurine (BAP), gave better results than zeatin, kinetin or N^6 (Δ^2 - isopentenyl) adenine (2iP). It was found that axillary bud proliferation could be easily induced. The elongation of these buds into shoots however, was a very slow process. The newly formed shoots with 2-4 axillary

buds had to be separated and subcultured. They had to be incubated for a long period for them to get stabilized in the medium. After this period, buds in the separated segments showed rapid elongation into shoots which could then be subcultured again, inducing further proliferation of buds. A multiplication rate of 30 $\frac{+}{2}$ 2.0 shoots per explant was obtained after 165 days of incubation. A concentration of 0.5 mg/l of the Gibberellic acid GA $_3$ had no effect on shoot elongation, whereas at a concentration of 2.0 mg/l dwarf shoots were produced.

Rooting of the proliferated shoots was not attempted. Shoot tips removed from aseptically grown seedlings could be rooted on solid & liquid media. The rooting medium was supplemented with indolebutyric acid (IBA) and activated charcoal (AC) or IBA, BAP and AC.

Table of Contents

List of Photographs						
Abbreviations						
Acknowledgements						
Abstract						
1.	Intro	ntroduction				
	1.1.	Plant	Tissue Culture	3		
	1.2	Embryo	genesis and Organogenesis	4		
		1.2.1.	Axillary Bud Proliferation	4		
	1.3	Advant	ages of <u>In</u> <u>Vitro</u> Micropropa-			
		-gatio	n techniques	6		
	1.4	Select	ing a Suitable Explant for			
		Asepti	c Culture	7		
		1.4.1.	Age and Location	7		
		1.4.2.	Season	8		
		1.4.3.	Size of the Explant	8		
	1.5	Sterilization of Explants		9		
	1.6	Stage I		12		
		1.6.1	Growth Regulators Used in			
			Stage I	14		
		1.6.2	Light	14		
		1.6.3	Temperature	15		
		1.6.4	Humidity	15		

	1.7	Stage II	16
		1.7.1 Growth Regulators Used in Stage II	16
		1.7.2 Orientation	17
		1.7.3 Incubation	18
	1.8	Stage III	18
		1.8.1 Transfer to Soil	20
	1.9	Tissue Culture of <u>Hevea</u>	21
2.	Mater	ials and Methods	26
	2.1	Preparation of Glassware	26
	2.2	Media	26
	2.3	Stock Solutions	29
		2.3.1 Inorganic Salts and Vitamins	29
		2.3.2 Growth Regulators	29
	2.4	Preparation of Media	30
	2.5	Selection of Plant Material	
		for Innoculation	31
	2.6	Aseptic Manipulation	32
	2.7	Incubation	33
	2.8	Surface Disinfection of the Explant	33
	2.9	Selection of the Explant	35
	2.10	Preparation of Explant for Culture	36
	2.11	Assessment of Growth	38

3.	Resu	lts		39	
	3.1	Establ	ishment of Shoot Tips in Culture	39	
	3.2	Orientation			
	3.3	Basal	Media	46	
	3.4	Growth	Regulators	49	
		3.4.1	Effect of Different Combinations		
			of BAP and IBA	49	
		3.4.2	Different Combinations of BAP & IBA	57	
		3.4.3	Effect of Different Combinations		
			of 2iP and IBA	59	
		3.4.4	Effect of Different Combinations		
			of Kinetin and IBA	63	
		3.4.5	Effect of Different Combinations		
			of BAP and NAA	66	
	3.5	Subculture		71	
		3.5.1	Subculture of shoots in Expt 3.3	71	
		3.5.2	Subculture of shoots in Expt 3.4.2	73	
		3.5.3	Proliferation and Elongation of		
			Axillary Buds with Repeated		
			Subculture	76	
		3.5.4	Effect of Gibberellin on Shoot		
			Elongation	81	
4.	Disc	ussion		83	
5.	Refe	rences		101	