"IN VITRO" POLLEN GERMINATION OF HEVEA CAMARGOANA[1]

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ABSTRACT - A study of pollen viability was carried out aiming to obtain the best medium concentration for pollen germination. Different concentrations of sucrose, glucose, galactose, lactose and mannitol in distilled water with and without boric acid were tested as media for germination of pollen of *Hevea camargoana* in the laboratory. Twenty percent sucrose with 0.01% boric acid at 25 to 30°C gave the best results. Tube lengths of 74.78 μm were obtained. Pollen tubes emerged within 30 minutes and completed full growth within six hours. Adding 0.01% boric acid increased the percentage germination markedly in all media except in mannitol.

Index terms: rubber tree, *Hevea brasiliensis*, pollen viability, sugar medium concentration, pollen length.

GERMINAÇÃO DE PÓLEN DE HEVEA CAMARGOANA "IN VITRO"

RESUMO - Foi conduzido um experimento em laboratório visando obter o melhor meio para determinar a viabilidade de pólen de *Hevea camargoana*. Foram utilizadas diferentes concentrações de sacarose, glicose, galactose, lactose e manitol diluídos em água destilada, com e sem ácido bórico, como ambientes para germinação do pólen. Foi observado que sacarose a 20% com 0,01% de ácido bórico na amplitude de 25 a 30°C de temperatura apresentou o melhor resultado. Foram obtidos tubos polínicos de 74,78 μm. Os tubos polínicos emergiram dentro de 30 minutos, e após seis horas já se encontravam completamente desenvolvidos. A adição de 0,01% de ácido bórico contribuiu marcadamente para o aumento da germinação, em todos os meios, exceto em manitol.

Termos para indexação: seringueira, *Hevea brasiliensis*, tubo polínico.

INTRODUCTION

Information on pollen germination of *H. amargoana*[4] is nonexistent and there appears to be ittle information in the literature on pollen ;ermination of *Hevea* under artificial medium, ;enerally. However, pollen of *H. brasiliensis* Muell. \rg. has been sucessfully germinated *in vitro* Heusser 1919, Dijkman 1938, Majunder 1964).

According to Heslop-Harrison (1971), germination of pollen *in vitro* is important from several points of /iew such as the determination of pollen fertility, /iability, physiological development, incompatbility, allergy reactions etc.

The components of the artificial media for *in vitro* ›ollen germination largely depend on the species in ıuestion. Ramaer (1932) reported no germination in ın aqueous solution of sucrose or glucose in *H. brasiliensis*. Hrabetová (1964) evaluated the effects of different sugars in the media on the growth of pollen from 49 plant species. He reported that pollen tubes of 41 species had most growth on a sucrose medium, while pollen tubes of seven species grew best on a glucose medium, and pollen tubes of *Salix carea* L. grew only on a fructose medium. Similar investigations by Hrabetová & Tupi (1963) on the effects of 20 different sugars on the growth of apple pollen tubes reported that the longest tubes were obtained in solutions of raffinose, followed, in decreasing order of effectiveness, by sucrose, lactose, melibiose, maltose and cellobiose. Other studies by Cook & Walden (1965) showed that sucrose and raffinose produced the highest germination of maize pollen, and Majunder (1964) stated that *Hevea brasiliensis* pollen could germinate in 15% sucrose solution of distilled water plus 0.01% boric acid at a temperature of 25°C and recommended this for routine tests of *H. brasiliensis* pollen germination.

This experiment was initiated to develop a suitable

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[4] *Hevea camargona* used to be known as *H. marajoensis* (Pires, J. Murça, 1980, personal communication).

artificial medium for *H. camargoana* pollen germination *in vitro* in order to facilitate further studies on pollen storage, viability, incompatibility, and other possible pollen physiological problems of this species.

MATERIAL AND METHODS

In this study, only mature but still enclosed pistillate male flowers were used. They were cut at 8-9 a.m. between 2-3 hours before anthesis. The cut flowers were placed in a Petri dish on moistened cotton.

The staminate flowers possess ten anthers, sessile and arranged in two regular whorls of five anthers on a central column. This column was taken out at the base of the male flower with tweezers. Such columns were ready for anthesis but had not dehisced. In the laboratory, pollen was removed from the anthers with a brush and dusted on to the various media plated on microscope silides wihich were placed to allow the drops to hang. The silides were not covered with a cover slip, to allow free access of air, which is necessary for germination. The pollen was incubated in laboratory, where the temperature ranged from 25 to 30°C, this being the best range for *H. brasiliensis* (Majunder 1964).

Germination percentage was based on observation of 150 to 300 pollen and of at least two replicates.

In the main set of tests described here, the artificial media used were sucrose, galactose, glucose and lactose at concentrations of 10, 15, 20 and 25% and mannitol in distilled water and with and without 0.01% boric acid. Each medium contained only one sugar. All solutions of sugars were made up by weight in distilled water.

Scoring was carried out on the percentage of germinated, nongerminated and bursted pollen, five hours after sowing, and pollen tube length was measured six hours after sowing had stopped (Fig. 1).

Pollen was removed at intervals and a germination study was carried out. A pollen tube which had attained a length of at least half the diameter of the pollen grain was considered to have germinated. For each period, pollen grains were counted and the percentage germination determined. The tube length was measured in all different medium concentrations. Five samples were taken from each slide and an average was calculated.

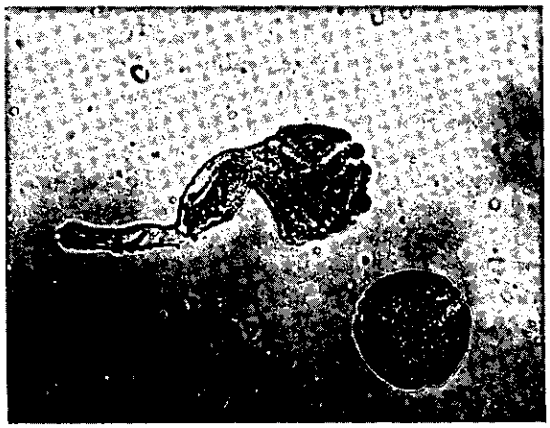

FIG. 1. Germinating pollen of *Hevea camargoana* six hours after sowing when germination pollen tube growth had stopped.

RESULTS AND DISCUSSION

Responses of pollen germination and tube length to five sugar media over a range of medium concentrations from 10% to 25% are presented in Table 1. All five sugars tested supported germination of *H. camargoana* pollen.

Except for mannitol, the results indicated that boric acid was essential for the pollen germination in all of the media used. It was very beneficial when the compound was added to the medium at 0.01% level. The high percentage of pollen grain germination and the rapid growth of tubes showed that the nutrient solution was indeed the key to the successful culture *in vitro* of *H. camargoana* pollen. According to Linskens (1964), boron is involved in the translocation of carbohydrates as well as pectin synthesis in germinating pollen (Stanley & Loewus 1964) and boric acid consistently enhanced germination of pollen.

On sowing the pollen on to the solution, activity commenced immediately. Protoplasm streaming was visible within ten minutes and pollen tube emerged within 30 minutes. For sucrose, after three hours the tubes were very well defined.

Although, in the best cases (sucrose 20%), up to 23% of the sown pollen grains germinated, variation in germination was found even when the duplicated slides were made from the same inflorescence. One possible cause of variation probably is the time of the day when pollen is sown. Usually, germination was higher in the morning than in the afternoon. This rhythm coincided with the time of the pollen shedding in *Hevea* sp. (Dijkman 1938).

The bursting of pollen grains soon after sowing reduced germination. Except for sucrose (25%) and lactose (10%) without boric acid, in all other media the proportion of pollen bursting was less than 25% in all medium concentration. The pollen grains swelled and burst, sometimes sending out protoplasmic extrusion through all pores. In the presence of boric acid, bursting occurred in 25% to 55% of all grains. At the absence of boric acid, bursting was reduced to approximately 10% to 20% in all medium concentration. Obviously, the relative osmotic

TABLE 1. Different medium effects on the *in vitro* germination of *Hevea camargoana* pollen grains - Manaus, AM, 1980.

Characteristic	Media	Concentration percent	First slides				Second slides				Tube length (μm)
			Number grains	Percent			Number Grains	Percent			
				G.N.G.	G.G.	B.G.		G.N.G.	G.G.	B.G	
Medium without boric acid	Sucrose	10	184	53.80	18.47	27.21	273	54.94	10.25	34.79	36.41
		15	195	60.00	18.46	21.53	278	61.15	14.38	24.46	38.38
		20	257	45.14	23.73	31.12	299	46.82	23.74	29.43	55.10
		25	180	41.11	16.66	42.22	258	36.04	14.34	49.61	49.20
	Galactose	10	395	75.94	10.12	13.92	312	79.16	9.61	11.21	40.34
		15	347	76.36	8.35	15.37	292	79.10	7.53	13.35	29.52
		20	390	78.20	6.41	15.38	383	79.63	6.00	14.36	26.57
		25	350	82.00	4.00	14.00	308	83.44	4.87	11.68	22.50
	Glucose	10	152	75.65	18.42	5.92	298	62.41	18.79	18.79	20.66
		15	194	65.97	23.71	10.30	219	61.18	21.46	17.35	41.33
		20	198	69.69	15.65	14.64	273	64.83	13.91	21.24	31.49
		25	223	70.40	12.55	17.04	222	63.96	12.61	23.42	24.60
	Lactose	10	348	43.67	5.17	51.14	225	45.77	4.44	47.77	14.76
		15	314	58.91	15.60	25.47	278	55.39	12.23	32.37	25.58
		20	331	67.97	10.57	21.46	292	58.90	8.21	32.87	23.61
		25	303	72.27	7.26	20.46	293	63.82	6.48	29.69	20.66
	Mannitol	10	183	64.43	10.21	25.35	139	62.89	12.66	24.43	15.74
		15	197	67.69	8.59	23.71	133	65.19	11.27	23.52	13.77
		20	183	68.79	6.76	24.43	164	70.68	7.32	21.98	11.80
		25	226	82.18	2.18	15.63	196	79.03	2.01	18.95	11.80
Medium with boric acid	Sucrose	10	369	49.05	14.63	36.31	279	49.10	14.69	36.20	47.23
		15	355	45.07	19.71	35.21	294	45.23	19.04	35.71	48.22
		20	334	36.82	28.14	35.03	314	39.17	30.57	30.25	74.78
		25	331	31.41	27.79	40.78	332	35.24	27.10	37.65	69.86
	Galactose	10	226	28.31	25.66	46.01	294	28.91	24.83	46.25	62.98
		15	291	31.27	24.05	44.67	262	32.06	23.66	44.27	53.14
		20	273	37.36	22.34	40.29	254	38.12	21.65	40.15	42.31
		25	280	46.79	20.00	33.21	245	44.08	19.59	36.32	37.42
	Glucose	10	312	20.51	26.28	53.20	323	24.76	24.45	50.77	32.47
		15	271	35.06	24.72	40.22	295	36.27	21.35	42.37	47.23
		20	244	49.18	18.44	32.37	292	47.26	18.49	34.24	37.39
		25	241	62.65	12.03	25.31	260	55.00	11.92	33.07	35.42
	Lactose	10	254	49.21	7.08	43.70	272	50.36	6.25	43.38	19.68
		15	271	5.39	15.12	32.47	243	51.85	16.04	32.09	29.52
		20	218	58.25	11.00	30.73	236	60.59	11.44	27.96	24.60
		25	265	61.13	6.79	32.07	219	64.38	8.67	26.94	18.69
	Mannitol	10	261	31.03	10.72	58.23	527	34.63	11.28	54.08	23.61
		15	217	36.86	8.29	54.83	235	38.29	10.21	51.48	21.64
		20	248	41.53	7.25	51.20	229	40.61	9.17	50.21	19.68
		25	272	46.32	6.25	47.42	246	46.34	6.09	47.52	15.01

G.N.G. = Pollen grains not germinated
G.G. = Pollen grains germinated
B.G. = Bursted pollen grains

pressure of the solution and that of the pollen affected the extent of bursting.

Best germination was obtained in 20% sucrose with boric acid, followed by 10% galactose + boric acid. The addition of boric acid increased 12 to 15% the amount of germination in 20% sucrose. Low percentage of germination was obtained in all galactose concentrations. However the increased germination ranged from 4% to 10%, to 20%, to 26% when boric acid was used. For other sugar concentrations, the increase in germintion seems not to be significant.

The effect of the different media at various concentrations on pollen tube length is shown in Fig. 2. The pollen tubes on 20% sucrose were longer than in the other media with the same concentration or with sucrose at a higher concentration of 25%. Good tube length, however, was obtained for 10% galactose with boric acid and this was better than for 10% sucrose with boric acid. Tube length decreased with increasing galactose concentration. In lactose and galactose media, the pollen grains germinated but the resultant pollen tubes were sometimes

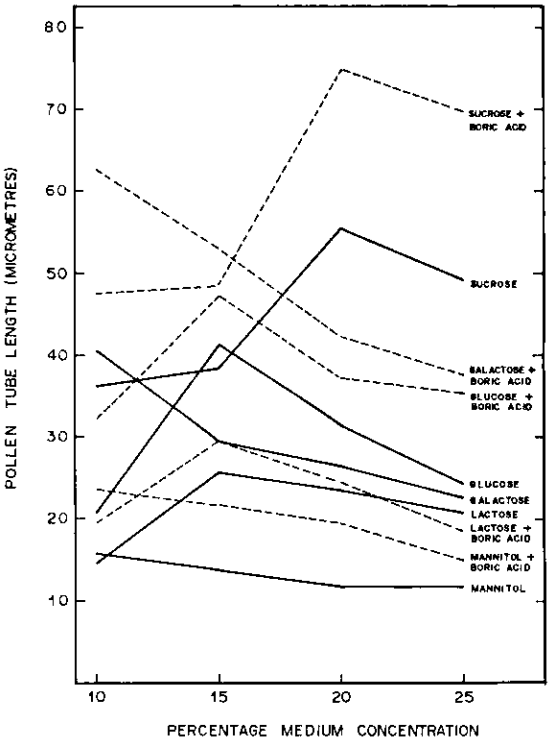

FIG. 2. The effect of various concentrations of different media on pollen tube length at six hours after sowing.

FIG. 3. Abnormal pollen tube development in liquid medium containing 15% galactose.

abnormally developed (Fig. 3). The reason for this usual behavior is unknown since similar technique was used in all media. The addition of 0.01% boric acid increased the tube length to about 50% in both media.

CONCLUSIONS

1. Germination in aqueous solution of sucrose, galactose, glucose, lactose and mannitol have found to be successful with *H. camargoana* pollen.

2. Twenty percent sucrose in distilled water at a temperature range of 25 to 30°C is suggested as the medium for routine test of pollen germination of this species.

3. *In vitro* germination of *H. camargoana* pollen, however, might not reflect the elongation of the pollen tube *in vitro* as pointed out in *H. brasiliensis* (Majumber 1964). The longest tube length observed in the present study was considerable shorter than for the other species.

Boric acid showed an effect of increasing the percentage of grain germination and rapid growth of pollen tubes. For these media, boric acid was indeed the key to successful *in vitro* culture of the studied species.

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