

Observations on Pollination, Fecundity/Siring Ability and Seed Germination in *Hevea brasiliensis*

T.R. CHANDRASEKHAR^{*#}, J. ALICE^{*}, T. GIREESH^{*},
G. PRABHAKARA RAO^{*} AND C.K. SARASWATHYAMMA^{*}

The major objective of this paper is to discuss the far higher hand-pollination success noted in some of the crosses of the hand pollination programme of the Rubber Research Institute of India conducted during 2002. As part of the ongoing activity, selected wild accessions (A) of Hevea brasiliensis were crossed with cultivated clones (W) for widening the genetic base of the breeding population. Hand pollinations were carried out following the conventional method for eight days between 9.30 a.m. and 1.30 p.m. on each day. Pollination success rate in W × A crosses ranged from nil to 14.2% while in W × W crosses it was 0.8% to 19%. Among the female parents, clone PB 330 gave an average success rate of 16.7% with more than 50% success in many of the groups of pollinations. Wild accession MT 2226 produced the best pollination success as male parent (12.1%). Between first fruit count and their maturity nearly 100% fruit retention was observed indicating negligible fruit loss in later stages of fruit development. Seed germination percentage in WA crosses ranged from 37.5% to 83.3% with an average of 65% while in the WW crosses it was 50% to 100% with a mean of 82.6%. Seeds sired by MT 2226 germinated within a few days of putting them for germination appeared to possess an inherent capacity for forcing early germination of seeds. The pollination results indicated that cultivated clone PB 330 as having high fecundity and germplasm accession MT 2226 as high siring ability parents.

Key words: *Hevea brasiliensis*; Wickham clones; pollination; fecundity; siring ability; wild germplasm; seed germination

Ever since the introduction of *Hevea brasiliensis* into the South East Asia in 1876 by Sir Henry Wickham¹, different breeding and selection methods have resulted in the development of many improved clones. Like in other crops, production of *Hevea* hybrids of desired genetic constitution through breeding involves

controlled artificial cross-pollination (hand pollination), which is labour intensive. Generally, success rate from hand pollination is poor ranging from 2% to 5%. In the literature, a good amount of information is available on these aspects²⁻¹⁴. In contrast, information on germination of hand pollinated hybrid seeds is

^{*}Rubber Research Institute of India, Kottayam – 686 009, Kerala State, India

[#]Corresponding author: (e-mail: chandratr@rubberboard.org.in)

very little in spite of the availability of vast literature on various aspects of the crop^{2,15-20}. However, good information on germination of assorted seeds is available²¹⁻²⁴. As far as Indian experience is concerned even though many hand pollination programmes have been implemented, published information from such programmes is very meagre. The objectives of this paper are to discuss the far higher hand pollination successes noted in some the crosses along with the general observations noted on pollination, fecundity/siring ability and seed germination from the hand pollination programme of the Rubber Research Institute of India conducted during 2002.

MATERIALS AND METHODS

Study Site and Plant Material

This study was conducted at the Rubber Research Institute of India's Central Experiment Station at Chethackal (9° 22' N, 76° 50' E, 100 m asl). As part of the ongoing activity of involving wild (Amazonian origin) germplasm in *Hevea* breeding for widening its genetic base, seven wild accessions were crossed with four clones of Wickham origin (cultivated clones). Crosses were attempted between Wickham and Wild accessions (WA crosses) and among Wickham clones (WW crosses). Reciprocal crosses of the wild accessions with Wickham clones were not attempted as it was not part of the objective of the programme but was done in WW crosses. Details of the Wickham clones and the wild accessions used in the pollination programme are given in *Table 1*.

Pollinators and Pollination

Six workers — two experienced (P1 and P2) and four first timers (P3 to P6) — were drafted

for hand pollination work. The first timers were trained by dummy pollination work on detached flower branches and after satisfying their competence, they were engaged for the regular work. Hand pollinations (HP) were done following the conventional method^{8,30} on well-grown trees. The trees selected were from an ongoing clone evaluation trial where the plot size is 16 trees planted in square planting. Four healthy trees each of clone RRII 414, RRII 105 and PB 330 and a single tree of clone RRII 429 were used for carrying out pollination. For efficient organisation of pollinations and management of disease protection measures, selection of trees was restricted to a single plot while the plots had good spatial separation (because of the RBD nature of the trial). Time of pollination on each day was from 9.30 a.m. to 1.30 p.m. The controlled pollinations were done over a period of eight days from 13th to 21st February 2002.

Count of Fruit Set, Bagging and Mature Fruit Harvesting

In *Hevea*, fruits take 22 to 24 weeks to attain maturity. Extensive withering of pollinated flowers and loss of developing fruits occur during the first 10 to 12 weeks^{4,8}. Therefore, first count of fruit set was enumerated after 11 weeks of pollination. Number of successes for each label and for each pollinator was noted separately. Labelwise enumeration, based on the pollination record, was carried out. Each developing HP fruit was bagged using 5 mm mesh nylon fish net bag. Seed development would be almost complete when fruits turn yellowish. First harvesting of the fruits was done in the second week of July and subsequent harvests were made every week till its completion by the second week of August. Apart from collecting hand-pollinated seeds, half-sibs (HS = open pollinated seeds) were also collected from all the female parents.

TABLE 1. WICKHAM CLONES AND WILD ACCESSIONS INVOLVED IN THE HAND POLLINATION PROGRAMME

Plant material	Identity ^a	Parentage	Origin	Characteristics
Wickham	RRII 105	Tjir 1 × Gl 1	Secondary clone of Indian origin. Resultant of 1954 HP programme	Mean yield is 1500 to 2000 kg/ha and potential is 2500 to 3000 kg/ha. Most popular clone in India ^{26,27}
	RRII 414	RRII 105 × RRIC 100	Secondary clone of Indian origin. Resultant of 1982 HP programme	Pipe line clone, tree mean yield is 40 % higher than RRII 105 in small-scale trials ²⁸
	RRII 429	RRII 105 × RRIC 100	Secondary clone of Indian origin. Resultant of 1982 HP programme	Pipe line clone, tree mean yield is 30% higher than RRII 105 in small scale trials ²⁸
	PB 330	PB 5/51 × PB 32/36	Secondary clone of Malaysian origin	High yielding, high vigour and wind tolerant clone in Malaysia ²⁹
Wild	AC 2004 (AC/S/10-37/336)	Wild collections from the centre of origin (primary centre of diversity) in the Amazonian forests of Brazil ²⁵	Acre province	^b Profuse branching, very close canopy, small leaves
	AC 2584 (AC/B/19-56/239)			^b Good test tap yield, high girth and bark thickness
	MT 1021 (MT/C/1-18/91)		Matto grosso province	^b Good test tap yield and girth
	MT 1057 (MT/C/10-17/24)			^b Good test tap yield, mature yield comparable to RRII 105
	MT 2226 (MT/C/2-10/151)			^b Moderately tolerant to <i>Phytophthora</i> , and average girth
	RO 1739 (RO/J/6-32/42)	Rondonia province	Rondonia province	^b Good test tap yield
	RO 2629 (RO/CM/10-44/24)			^b Good test tap yield and girth, moderately tolerant to <i>Phytophthora</i>

^aInformation given inside the parentheses for the wild germplasm is their international accession numbers.^bUnpublished data; character classification is based on juvenile evaluation at 4 years of age.

Germination of Seeds

Seeds were germinated in sieved river sand taken in aluminium trays. The trays were kept watered once in two days. After allowing sufficient time for germination, seeds in each tray were examined for any late germination. Wherever initiation of radicle was found, the seeds were maintained for few more days and the seeds that produced plumule were only counted as successful germinations.

Disease Management

The climatic conditions existing in the study location are highly conducive to the development of two serious diseases viz., powdery mildew (*Oidium heveae*) and the abnormal leaf fall (*Phytophthora*) that affect pollination and fruit set, especially in the traditional rubber growing zone in India³¹. The flowers are seriously affected by *Oidium* while the fruits by the *Phytophthora*. *Oidium* was kept under check by weekly dusting of sulphur powder from the ground using a dust blower on all the selected trees. Sulphur dusting was done right from the initiation of flowering till the disease subsided by the maturation of leaves. *Phytophthora* control measures were initiated a week earlier to the onset of pre-monsoon showers (usually by April) by spraying Bordeaux mixture every 10 days till the fruit harvest. Bordeaux mixture spraying was done climbing on the tree and each HP fruit was identified and sprayed, while HS fruits were not given much attention, other than random spraying on completion of spraying on HP fruits.

Statistical Analysis and Weather Data

Pollination and seed germination data were first subjected to chi-square analysis to test

whether the results were homogeneous within and between WA and WW group of crosses and WA, WW and HS group of seeds. In many cases, the expected values were less than five. Therefore, Fisher's exact test was performed to identify significantly different crosses. The cross-combinations where no seed could be obtained were excluded from the analysis. The analyses were performed using R-package³². Percent standard error was worked out for each cross combination success rate as $\{\sqrt{[p(1-p)/n]}\}100$ where, p = success divided by total number³³. Weather data were collected from the meteorological observatory maintained in the station where the pollination work was carried out. Daily pollination successes were correlated with the corresponding, five lag and succeeding days weather parameters to understand their influence on the success rate.

RESULTS

Pollination successes obtained at first count ranged from 0.8% to 19% (Table 2). In WA crosses, accession MT 2226 gave the highest average success rate of 12.1%, while cross combinations RR II 429 $\times$ AC 2004 and RR II 414 $\times$ MT 1057 did not give any success at all. On one of the pollination days, in the crosses of MT 2226 with RR II 429 and RR II 414, success was noted in all the groups of pollinations (178 pollinations in 13 labels) carried out by two pollinators with a few groups of pollinations recording 20% to 33% success. In WW crosses success rate ranged from nil to 19% with PB 330 giving the best results. Clone PB 330 recorded success in all 10 groups of pollinations (90 pollinations) and in a label of eight pollinations success rate was as high as 62.5%. On the last day of pollination, when PB 330 was crossed with RR II 414, success was observed in most groups of the pollinations done by four pollinators (268 pollinations in

TABLE 2. HAND POLLINATION SUCCESSES IN WA AND WW GROUP OF CROSSES

Cross combination	No. of pollinations attempted	Fruit set at first count (No.)	Fruits harvested at maturity (No.)	Fruit set based on pollinations attempted (%) ^{\$}	Fruit retention from first count to maturity (%)
Wickham × Wild (WA group)					
RRII 105 × RO 2629	78	0a	0	0.0 (0.0)	—
RRII 414 × MT 1057	27	0a	0	0.0 (0.0)	—
RRII 429 × AC 2004	89	0a	0	0.0 (0.0)	—
RRII 414 × AC 2004	162	5a	3**	1.9 (1.4)	60
RRII 105 × AC 2004	106	1a	1	0.9 (0.9)	100
RRII 105 × AC 2584	56	1a	1	1.8 (1.8)	100
RRII 105 × RO 1739	112	1a	1	0.9 (0.9)	100
RRII 429 × RO 1739	50	1a	1	2.0 (2.0)	100
RRII 429 × MT 1021	67	3a	3	4.5 (2.5)	100
RRII 105 × MT 1057	43	2a	2	4.7 (3.2)	100
RRII 429 × MT 2226	90	10b	9@	10.0 (3.3)	90
RRII 414 × MT 2226	127	18b	18	14.2 (3.1)	100
Sub-total	1007	42	39	3.9 (0.6)	93
Wickham × Wickham (WW group)					
RRII 105 × IRCA 130	77	2a	2	2.6 (1.8)	100
RRII 105 × PB 330	129	4a	4	3.1 (1.5)	100
RRII 429 × PB 330	123	3a	2@	1.6 (1.4)	67
RRII 414 × RRIM 600	132	4a	1 [#]	0.8 (1.5)	25
RRII 414 × PB 330	197	12b	11@	5.6 (1.7)	92
PB 330 × RRII 429	186	27c	27	14.5 (2.6)	100
PB 330 × RRII 414	290	56c	55&	19.0 (2.3)	98
Sub-total	1134	108	102	9.0 (0.9)	94
Pooled	2141	150	141	6.6 (0.6)	94

*In each group, pollinations successes followed by similar letters are not significantly different from each other according to Fisher's exact test and hence form a homogeneous group.

**One label was found missing while in another label the fruit was found detached from the branch when it was nearing maturity.

^{\$}Figures in parentheses are standard errors in %.

@One label each was found missing

&One fruit withered away due to *Phytophthora* attack

[#]Three labels were found missing.

23 labels) and in a few labels success rate was more than 50%. Four labels (40 pollinations) at initial count and six labels (successes) at harvesting time were found missing. On April 9, 2002 there was a gale resulting in a branch snap in one of the PB 330 pollinated tree producing a loss of seven successes out of 43 pollinations. Fruit loss between first count and harvest time was negligible giving nearly 100% success.

Fisher's exact test resulted in classification of WA crosses into two and WW crosses into three sub-groups based on pollination successes (*Table 2*). The first group included ten crosses, all of which had very low success rates (1.7%). The pooled values for the remaining two crosses gave an average success rate of 12.1%. Likewise, three sub-groups were identified in the WW group of crosses. Cross combination RRII 105 $\times$ IRCA 130, RRII 105 $\times$ PB 330, RRII 429 $\times$ PB 330 and RRII 414 $\times$ RRIM 600 formed the low success rate sub-group (2.0%), PB 330 $\times$ RRII 429 and PB 330 $\times$ RRII 414, the high success rate sub-group (16.7%) and RRII 414 $\times$ PB 330 was intermediate with a success rate of 5.6%. The two highly successful crosses in the WA group were almost as successful as the two most successful crosses in the WW group. Similarly, the lowest success rates in the WW group were not much different from the least successful crosses in the WA group.

In the success rates obtained for different pollinators, in one cross of WA group and in two crosses of WW group, success rate was more than 16% with a maximum of 26% (*Table 4*). In the pollinations where good success was observed, two pollinators did not produce much success. On one of the days, success was noticed in every group of pollinations done by the pollinators P1, P2 and P3. Success rate based on first count was about 95%. Success rates noted for different

pollination days varied from 1.7% to 18.7% (*Table 5*). On day three, success was recorded in all the seven pollination labels (102 pollinations) of P1 and six labels out of seven of P2 (78 pollinations). On the same day out of eight labels of P6 (37 pollinations) only one success was noted. On day seven, success was recorded in all 10 pollination labels of P2 (90 pollinations) and in a label of eight pollinations success rate was as high as 62.5%. Similar success was also noted for P3 who was a first timer. On the last day (day eight), success was observed in all seven labels of P1 (88 pollinations) and all six labels of P4 (53 pollinations). The same day, in a few labels success rate was more than 50%. Most of the successes survived until fruit harvest.

The study location did not receive any rain during the pollination period (*Table 5*). All the weather variables, except minimum temperature, were practically uniform on all the days of pollination. Minimum temperature varied from 18.6°C to 21.7°C. January and February are normally dry months. The hot season from March to May is followed by the Southwest Monsoon from June to September. The Northeast Monsoon occurs during October and November. The annual rainfall ranges from 2000 mm to 4500 mm. June and July are the wettest months. The mean minimum and maximum temperatures are 21°C and 36°C, respectively. Correlations of daily pollination successes with lagged as well as succeeding days weather variables indicated that the influence of maximum temperature was positive while that of minimum temperature and relative humidity was negative (*Table 6*). Most of the other coefficients were of lower magnitude.

Germination percentage in seeds of WA crosses ranged from 37.5% to 83% (*Table 7*) with an average of 66% while in WW crosses it ranged from 50% to 95% with a mean of

TABLE 3. POLLINATOR-WISE POLLINATION SUCCESSES IN HIGHER PERCENTAGE OBSERVATIONS

Pollinator	RRII 414 × MT 2226			RRII 429 × MT 2226			PB 330 × RRII 429			PB 330 × RRII 414		
	No. done	Success		No. done	Success		No. done	Success		No. done	Success	
		No.	Percent		No.	Percent		No.	Percent		No.	Percent
P1	102	18	17.6	—	—	—	—	—	—	88	14	16.0
P2	—	—	—	78	9	11.5	90	19	21.1	85	18	21.2
P3	—	—	—	—	—	—	47	8	17.0	42	11	26.2
P4	—	—	—	—	—	—	—	—	—	53	12	22.6
P5	—	—	—	—	—	—	49	0	0.0	22	1	4.5
P6	25	0	0.0	12	1	8.3	—	—	—	—	—	—

P1 and P2 : Experienced pollinators

P3 to P6 : First timers

— : Pollination not attempted.

TABLE 4. POLLINATION SUCCESSES IN DIFFERENT DAYS

Pollination day number	No. of pollinations attempted	Success	
		Number	Percent
1	69	2	2.9
2	218	6	2.8
3	295	28	9.5
4	355	6	1.7
5	309	10	3.2
6	277	14	5.1
7	318	29	9.1
8	300	56	18.7
Total	2141	151	7.1

TABLE 5. WEATHER CHARACTERISTICS FROM WINTERING TO FRUIT HARVEST OF THE LOCATION WHERE POLLINATION WAS CARRIED OUT

Months	Rain fall (mm)	SSD (Hours)	Evaporation (mm)	Air temperature (°C)		Relative humidity (%)		Wind speed (km/h)
				Min.	Max.	7.20 a.m.	2.20 p.m.	
Nov '01	291	5.6	2.4	21.8	32.4	94	65	0.8
Dec '01	12	7.8	3.2	20.4	33.8	89	52	1.4
Jan '02	29	7.4	4.2	20.9	35.1	85	42	1.9
13 Feb '02	0	9.6	4.2	21.6	34.9	80	40	1.6
14 Feb '02	0	9.6	5.0	21.7	35.0	79	38	2.5
15 Feb '02	0	9.6	4.9	20.0	36.5	96	38	2.2
16 Feb '02	0	9.8	5.8	18.6	37.2	93	39	1.3
17 Feb '02	0	9.5	4.8	21.1	35.9	84	34	1.6
18 Feb '02	0	9.6	4.8	19.6	36.8	91	30	1.6
19 Feb '02	0	9.0	4.6	19.7	36.7	93	38	1.2
20 Feb '02	0	9.5	4.3	20.5	36.5	93	42	1.0
21 Feb '02	0	9.5	4.5	19.3	36.1	94	36	1.0
Feb '02	55	9.0	4.4	20.3	35.3	91	42	1.5
Mar '02	200	9.0	4.7	22.1	36.3	96	44	1.1
Apr '02	354	8.0	3.9	23.1	34.4	95	58	1.0
May '02	300	5.2	2.9	23.7	32.3	96	68	0.8
Jun '02	366	4.0	2.7	23.1	30.4	96	73	1.2
Jul '02	415	3.0	2.7	22.8	27.6	99	80	1.2
Aug '02	400	4.5	2.5	21.3	29.4	96	77	1.6

SSD = Sun shine duration; Min = Minimum temperature; Max = Maximum temperature
 13th to 21st February : Pollination period, except on 17th which was a holiday (Sunday).

82.6%. In seed germination also, success rate was better in WW crosses than in WA crosses. In the HS group, lowest germination percentage (68%) was observed in RRII 414 while maximum (83%) was observed in clone PB 330. Mean percentage germination in the HS was 76%. In some crosses bursting of seed coat was noted due to excess growth of the endosperm. This was more prominent in the

seeds of clone RRII 429. Apart from these, underdeveloped seeds and seeds that appeared to be fully developed but with little or no endosperm was also observed. All the seeds resultant of combination where MT 2226 was the male parent germinated early (within a week of sowing) while the seeds from PB 330 female parent germinated late and took more than two weeks. Seeds from other combina-

TABLE 6. CORRELATIONS BETWEEN DAILY POLLINATION SUCCESS AND THE RESPECTIVE WEATHER PARAMETERS OF FIVE PRECEDING AND FIVE SUCCEEDING DAYS

Weather variable	5	4	3	2	1	Pollination day	1	2	3	4	5
Rainfall (mm)	-0.378	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sunshine (hours)	0.563	-0.006	0.140	0.256	0.207	-0.150	0.387	0.390	0.249	-0.021	0.357
Evaporation (mm)	0.639	0.552	-0.038	-0.152	0.134	-0.308	0.034	-0.040	0.292	0.766*	0.557
Temperature (°C)											
Minimum	-0.672	-0.039	0.197	-0.129	-0.193	-0.340	-0.636	-0.556	-0.709*	-0.731*	-0.215
Maximum	0.850**	0.494	0.277	0.340	0.355	0.177	0.525	0.212	0.026	0.711*	0.565
Relative humidity (%)											
7.20 a.m.	0.226	-0.290	0.039	0.176	0.130	0.581	0.436	-0.778*	-0.803*	-0.104	0.185
2.40 a.m.	-0.522	-0.446	-0.442	-0.306	0.172	-0.021	-0.748*	-0.607	-0.768*	-0.133	0.348
Wind speed (km/h)	0.152	0.023	-0.237	-0.137	-0.142	-0.412	-0.645	-0.077	0.612	0.279	-0.385

NA : Not available as the coefficients could not be worked out because of zeros in the matching rain fall values

*P (< 0.05) = 0.707

** P(< 0.01) = 0.834

TABLE 7. GERMINATION SUCCESS IN SEEDS OF WA AND WW GROUP OF CROSSES AND HALF-SIBS (HS GROUP)

Cross combination	Final success (Fruits harvested)	Number of seeds put for germination	Number of seeds germinated*	Percentage germination ($\pm$ s.e., %)
Wickham $\times$ Wild (WA group)				
RRII 105 $\times$ RO 2629	0	0	0	0.0
RRII 414 $\times$ MT 1057	0	0	0	0.0
RRII 429 $\times$ AC 2004	0	0	0	0.0
RRII 414 $\times$ AC 2004	3	8**	3a	37.5 (17.1)
RRII 105 $\times$ AC 2004	1	3	2a	66.7 (27.2)
RRII 105 $\times$ AC 2584	1	3	2a	66.7 (27.2)
RRII 105 $\times$ MT 1057	2	6	5a	83.3 (15.2)
RRII 105 $\times$ RO 1739	1	3	2a	66.7 (27.2)
RRII 429 $\times$ RO 1739	1	3	2a	66.7 (27.2)
RRII 429 $\times$ MT 1021	3	9	5a	55.6 (16.6)
RRII 414 $\times$ MT 2226	18	54	35a	64.8 (6.5)
RRII 429 $\times$ MT 2226	9	26 ^s	20b	76.9 (8.3)
Sub-total	39	115	76	66.1 (4.4)
Wickham $\times$ Wickham (WW group)				
RRII 105 $\times$ IRCA 130	2	6	3a	50.0 (20.4)
RRII 105 $\times$ PB 330	4	12	10a	83.3 (10.8)
RRII 429 $\times$ PB 330	2	6	6a	100.0 (0.0)
RRII 414 $\times$ RRIM 600	1	3	2a	66.7 (27.2)
RRII 414 $\times$ PB 330	11	36 [#]	21a	58.3 (8.2)
PB 330 $\times$ RRII 414	55	160 [@]	132a	82.5 (3.0)
PB 330 $\times$ RRII 429	27	81	77b	95.1 (2.4)
Sub-total	102	304	251	82.6 (2.2)
Half sibs (open pollinated) ^{&} (HS group)				
RRII 414 $\times$?	72	211	143a	67.8 (3.2)
RRII 429 $\times$?	60	172	129a	75.0 (3.3)
RRII 105 $\times$?	115	335	261b	77.9 (2.3)
PB 330 $\times$?	61	179	148b	82.7 (2.8)
Sub-total	308	897	681	75.9 (1.4)
Pooled	449	1316	1008	76.6 (1.2)

*In each group, number of seeds germinated followed by similar letters are not significantly different from each other according to Fisher's exact test and hence form a homogeneous group. **One seed had suppressed growth. ^sOne seed appeared normal but with shrunken endosperm and embryo. [#]Three fruits were four seeded ones. [@]Five seeds were not well developed. [&]Discrepancies in the number of seeds compared to fruits harvested was due to unviable, not fully developed and aborted seeds.

tions took one to two weeks to complete their germination. In many of the crosses a few precocious germination of seeds was observed. In a fruit of the cross between RRII 105 $\times$ MT 1057, due to delay in harvesting all the three seeds had fully germinated and the seedlings survived until planting in the seedling nursery. In the germination trays, few seeds produced only the roots and no plumule emerged even after allowing them to grow for more than a week and in a few cases the other way round was also noticed. In some seeds the plumule got jammed in between two cotyledon stalks and failed in further development. Some seeds failed to germinate even though they appeared to be healthy on examination by breaking them open. In this study, measurement on radicle, tap root and number of feeder roots was not measured as the seeds were hybrids and needed to be established in the observation nurseries without much delay. However, wide variability in these characters was observed.

For germination successes, Fisher's exact test produced two sub-groups in each of the WA, WW and HS group seeds (Table 7). The first sub-group in WA group included nine crosses with a mean germination of 63% while a cross RRII 429 $\times$ MT 2226 formed a single member group with 77% germination. Similarly, seven crosses of WW group formed a sub-group with a mean germination of 78% and the cross PB 330 $\times$ RRII 429 formed the single member second sub-group with a germination success of 95%. In the HS group, RRII 414 and RRII 429 formed the first sub-group with a mean of 71% germination success while the second sub-group included RRII 105 and PB 330 with a mean of 79.6% germination success. Both in WW and HS groups, germination success in seeds of female parent PB 330, came out as significantly different.

DISCUSSION

There was wide variability in success rates among different cross combinations. In general success rate was better in WW crosses than in WA crosses. Even though overall pollination successes observed were similar to those reported already^{3,4,6-14,34}, in some of the crosses the magnitude of success was far greater. This was especially so in the crosses with PB 330 $\times$ RRII 414, PB 330 $\times$ RRII 429, RRII 414 $\times$ MT 2226 and RRII 414 $\times$ MT 2226. During the study period, weather conditions were typical of the location. The study site has a tropical humid climate, with an aggressive summer and plentiful rainfall³⁵. There were no any extraordinary weather events other than the gale storm on 9 April 2002, which did not produce any major loss in HP trees other than a branch snap in one of the pollinated trees. The real loss due to the gale could not be ascertained as the first census of the pollination success was taken on 9 May, a month after its occurrence.

Among the male parents, wild accession MT 2226 gave the best results. This accession was crossed with only two of the four pistil parents used in this study and unfortunately it was not crossed with the clone PB 330 that gave the best results as female parent. It is reported that wide genetic crosses between cultivated varieties and new germplasm from diverse provenances had not given higher rates of fruiting success and the cause of successful fruit set was mainly due to the fecundity ability of the female flower rather than the pollen parents siring ability³⁴. This notion appears to be flawed because in the present study siring ability of the wild accession MT 2226 was as good as the fecundity ability of the female parent PB 330. Differences in siring success among the wild genotypes could be due to corresponding differences in pollen

performance (germination and growth rate), donor-by-recipient interaction and the frequency of abortion of seeds sired by those donors. Although this was not specifically examined in this study, such differences are common in many angiosperms³⁶⁻³⁹.

Clone PB 330 as pistil parent produced the best results. In many of the groups of pollinations done on PB 330, clusters of fruits were observed. These successes were identified by their fruit bunches. This type of bunching was not observed in other female parents used in this study. Similar results have also been reported⁴⁰. Fruit clusters noticed in PB 330 suggest no maternal resource constraint for the developing fruits. It should be noted here that the fruits of this clone were comparatively smaller than in the other clones. Though this clone, as seed parent gave a higher success in pollination, fruit-set and seed germination, but it was not so when used as a pollen parent. Similar results for clone PB 5/51 and a maximum of 13% success as female parent in some combinations only with substantially lower success in reciprocal crosses has been reported³⁴. In this study the results obtained for PB 330 as pistil parent were twice that of PB 5/51 and in a few cases it was more than 50%. In fact this clone is a progeny of PB 5/51 and its inherent capacity for high fecundity appears to have been inherited from both of its parents. Similar results were also noticed for PB 5/51 in the earlier pollination programme (unpublished, 1993). In general, the fecundity ability of the female parents can be ranked thus: PB 330 > RR II 429 > RR II 414 > RR II 105.

In all the published reports, including this study, massive pollination failures were the order. The causes could be fecundity and siring ability variations, pre and post-zygotic mechanisms, genetic load and maternal resource constraints and other intrinsic factors.

No thorough investigation has taken place on the causes for such failures. The existence of 'receptive' and 'non-receptive' flowers has been proposed for the failures³⁴. The proposition is difficult to accept because in this study apart from recording higher average fruit set than the earlier reports and even higher success rate in some cases. Further, the female flower parts that guide the pollen tube up to the embryo sac is the genetically identical diploid tissue in every flower given a female parent. In this study even though the effect of the time of pollination on success rate was not studied, examination of the pollination log book point that stigma was receptive for a limited time in the afternoon hours as the successes noted appear to be skewed towards the end of the pollination period. This observation along with the significant correlations observed for the 4th day lag maximum temperature and evaporation indicated that receptivity of the stigma appeared to be dependent on maximum temperature.

In seed germination percentages, better results than in the literature were noted. An average germination of 52.5% with a range of 29% to 70% in a study on OP seeds of 20 clones has been reported²³. But this study recorded a better pooled average germination in WW, WA and half-sibs seeds which stood at 76.6%. Like in hand pollination successes, seeds of WW crosses showed the highest average germination that included the highest germination in a particular cross. The male parent MT 2226, appeared to force all its seeds to germinate within a few days indicating to carry an inherent capacity for the early germination of seeds. In the literature, available information on germination, emerging tap root and feeder root characteristics of the germinating seed is very sparse. These aspects need to be studied both for clonal variations and as well as the effect of maternal and paternal parents.

It is reported that in *Hevea* fruit development, embryo was the last to develop and was visible only after fourteen weeks from pollination⁴¹. Nearly 100% success observed between initial count and final harvest induces us to assume that failures due to post-zygotic problems could be very minimal even though initial census on pollination success was done at 11 weeks after pollination. Similar conclusions have been drawn based on pollen tube penetrations into the ovary of the hand-pollinated flowers³⁴. However, post-zygotic effects also cannot be ruled out at least in the initial stages of the development of the fruit after fertilisation, because in this study census of fruit abscission from the pollination day to the initial count was not done. Substantial fruit loss between initial count and harvest time due to premature fruit drop ranging from 10% to 97% have been reported^{3,4,7-9,12-14}. Heavy fruit fall during the first half of the period between pollination and maturity has been reported by Ross⁴. This study recorded nearly 100 percent success between first count and harvesting of fruits, indicating that in general fruit drop in the later stages of fruit development will be almost nil if the fruits escape *Phytophthora* related problems. Few failures recorded in this study could be either due to attack of *Oidium/Phytophthora* diseases. It was difficult to attribute the high success rate obtained in some crosses, either to the weather conditions or to the skill of the pollinators. The weather was typical of the location and four pollinators did the job for the first time in their life. High success rate could be mainly due to high fecundity of the female parent and the positive interaction of the cross combinations. Substantial fruit losses noted by the published reports could have been mainly due to the diseases, but was difficult to confirm because the publications have not reported the disease management practices adopted.

The main focus of this paper was to discuss the far higher hand-pollination success noted in some of the hand-cross pollinations hitherto unreported. The results indicated that cultivated clone PB 330 as having high fecundity and germplasm accession MT 2226 as high siring ability parents. This conclusion should be treated as indicative only because MT 2226 was crossed with only two of the four pistil parents used in this study and unfortunately it was not crossed with the clone PB 330 that gave the best results as female parent. Likewise PB 330 could not be involved in as many crosses as possible. However, the results seem to indicate a possible significant benefit in both the cases. More research is needed for further confirmation.

ACKNOWLEDGEMENTS

We thank Dr. N. M. Mathew, Director, Rubber Research Institute of India, for the encouragement to this study. We are especially grateful to Prof. R. J. Baker, Professor Emeritus, University of Saskatchewan in Saskatoon, Canada, for his expert help/guidance in the analysis/interpretation of the results of chi-square/Fisher's exact tests. Co-operation extended to this work by Jacob Pothan, Deputy Director, O. V. Mathew, Assistant Estate Superintendent, and E. P. Sajikumar, Field Assistant of the RRII's Central Experiment Station, at Ranny, is thankfully acknowledged. Great appreciation is due to C. C. Joseph, Assistant Farm Superintendent for his supervisory efforts during the pollination programme. Thanks to Joy P. Edakkara, Field Assistant, for his general help during the initial stages of this programme.

Date of receipt: July 2003
Date of acceptance: March 2004

REFERENCES

1. BAULKWILL, W. J. (1989) The History of Natural Rubber Production, *Rubber* (Webster, C.C. and Baulkwill, W.J. eds.), pp. 1–56. England: Longman Scientific and Technical.
2. WEBSTER, C.C. AND BAULKWILL, W.J. (1989) *Rubber*. Essex, England: Longman Scientific and Technical.
3. MORRIS, L.E. (1929) Field Observations and Experiments on the Pollination of *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya*, **1**, 41–49.
4. ROSS, J. M. (1960) Observations on the 1959 Hand Pollination Programme at the Rubber Research Institute of Malaya. *Proc. Nat. Rubb. Res. Conf., Kuala Lumpur*, 392–408.
5. RAO, B.S. (1961) Pollination of *Hevea* in Malaya. *J. Rubb. Res. Inst. Malaya*, **17**(1), 14–18.
6. ATTANAYAKE, D.P.S.T.G. AND DHAR-MARATNE, S.C. (1984) Preliminary Observations on Flowering, Pollen Germination and Fruit Set in *Hevea* species. *J. Rubb. Res. Inst. Sri Lanka*, **62**, 41–46.
7. LECONTE, A., NOUY, B. AND NICOLAS, D. (1984) Rate Successes of Hand Pollination and Effects of Some Growth Regulators on Fruit Set in *Hevea*. *Compte-Rendu du Colloque Exploitation-Physiologie et Ame'lioration de l'Hevea, Montpellier, France*, 519–525.
8. YEANG, H.Y. AND GANDHIMATHI, H. (1984) Factors Influencing Fruit Set in *Hevea* Following Hand Pollination. *Compte-Rendu du Colloque Exploitation-Physiologie et Ame'lioration de l'Hevea, Montpellier, France*, 401–418.
9. GANDIMATHI, H. AND YEANG, H.Y. (1984) The Low Fruity Set that Followes Conventional Hand Pollination in *Hevea brasiliensis*: Insufficient Pollen as a Cause. *J. Rubb. Res. Inst. Malaya*, **32**(1), 20–29.
10. SEDGELY, M. AND ATTANAYAKE, D.P.S.T.G. (1986) Floral Biology of Rubber and Recommendations for Increases in Seed Yield for Plant Breeding. *J. Rubb. Res. Inst. Sri Lanka*, **65**, 1–13.
11. SEDGELY, M. AND ATTANAYAKE, D.P.S.T.G. (1988) The Breeding System of Rubber (*Hevea brasiliensis*): An Evaluation of Controlled Hand Pollination Methods. *Euphytica*, **39**, 83–91.
12. OLAPADE, E.O. AND OMKHAFFE, K.O. (1990) Hand Pollinations of *Hevea brasiliensis* in Nigeria (1984–1990): Comparison with Open Pollinated Field Date. *J. Rubb. Res. Inst. Sri Lanka*, **70**, 27–34.
13. MYDIN, K.K., NAZEER, M.A., LICY, J., ANNAMMA, Y. AND PANIKKER, A.O.N. (1989) Studies on Improving Fruit Set Following Hand Pollination in *Hevea brasiliensis* (Willd. Ex. Adr. De. Juss.) Muell. Arg. *Indian J. nat. Rubb. Res.*, **2**, 61–67.
14. MYDIN, K.K., ANNAMMA, Y., NAZEER, M.A., PREMAKUMARI, D., SARAS-WTHYAMMA, C.K. AND PANIKKER, A.O.N. (1990) Controlled Pollination of *Hevea* — Problems and Perspectives. *IRRDB Breeding Symposium 1990, Kunming, China*.
15. WRIGHT, H. (1906). *Hevea brasiliensis* or *Para Rubber*. Colombo, Ceylon: A. M. and J. Ferguson. *Para Rubber: Its Botany, Cultivation, Chemistry and Diseases*. First Indian Edition (1998) New Delhi, India: Biotech Books.
16. DIJKMAN, M.J. (1951) *Hevea: Thirty years of Research in the Far East*. Florida: University of Miami Press.

17. POLHAMUS, L.G. (1962) *Rubber: Botany, Production and Cultivation*. London: Leonard Hill (Books) Ltd.
18. PILLAY, P.N.R. (1980) *Handbook of Natural Rubber Production in India*. Kottayam, Kerala, India: Rubber Research Institute of India.
19. SETHURAJ, M.R. AND MATHEW, N.M. (1992) *Natural Rubber: Biology, Cultivation and Technology*. The Netherlands: Elsevier Science Publishers BV.
20. GEORGE, P.J. AND JACOB, C.K. (2000) *Natural Rubber: Agromanagement and Processing*. Kottayam, India: Rubber Research Institute of India.
21. SARASWATHYAMMA, C.K. AND BHASKARAN NAIR, V.K. (1976) Relationship of Seed Weight and Seedling Vigour in *Hevea*. *Rubber Board Bulletin*, **13**, 28–29.
22. SARASWATHYAMMA, C.K. AND PANIKKER, A.O.N. (1989) Evaluation of Seedling Progenies of Male Sterile Clones of *Hevea brasiliensis* (Willd. Ex. Adr. de. Juss.) Muell. Arg. at the Nursery Stage. *Indian J. nat. Rubb. Res.* **2**, 99–104.
23. MYDIN, K.K. (1992) Genetic Divergence, Prepotency and Inbreeding Depression in Para Rubber (*Hevea brasiliensis* Muell. Arg.). Ph. D. Thesis. Kerala Agricultural University, India.
24. MERCYKUTTY, V.C., PREMAKUMARI, D., VINOTH THOMAS, AND SARASWATHYAMMA, C.K. (1996) Effects of Water Storage on Seed Germination and Seedling Growth of Rubber (*Hevea brasiliensis*, Muell. Arg.). *The Planter*, **72**, 367–373.
25. ONG, S.H., GHANI, M.N.B.A., TAN, A.M. AND TAN, H. (1983) New *Hevea* Germplasm: Its Introduction and Potential. *Proc. Rubb. Res. Inst. Malaysia Plrs' Conf.*, 1983, Kuala Lumpur, 3–17.
26. SARASWATHYAMMA, C.K., LICY, J. AND MARATTUKALAM, J.G. (2000). Planting Materials, *Natural Rubber: Agromanagement and Crop Processing* (George, P.J. and Jacob, C.K. eds.), pp. 66. Kottayam: Rubber Research Institute of India.
27. KARUNAICHAMY, K., VIJAYAKUMAR, K.R., THOMAS, K.U., RAJAGOPAL, R., AND KUMAR, D.A. (2001) Response of Rubber Trees (*Hevea brasiliensis* Muell. Arg., clone RRII 105) to Low Frequency Tapping (LFT) Systems. *Indian J. nat. Rubb. Res.*, **14**(2), 79–87.
28. LICY, J., SARASWATHYAMMA, C.K., PREMAKUMARI, D., MEENAKUMARI, T., RAJESWARI MEENATTOOR, J. AND NAZEER, M.A. (2003) Genetic Parameters and Heterosis in Rubber (*Hevea brasiliensis*) Muell Arg. V. Hybrid Vigour for Yield and Yield Components Among the RRII 400 Series Clones in Small-scale Evaluation. *Indian J. nat. Rubb. Res.*, **16**(1&2), 75–80.
29. ANG, B.B. AND SHEPHERD, R. (1979) Promising New Prang Besar Rubber Clones. *Proc. RRIM Plrs' Conf.*, 1979, 219–240.
30. PANIKKAR, A.O.N., BHASKARAN NAIR, V.K. AND MARCOSE, V.C. (1980) Breeding and Tree Improvement, *Handbook of Natural Rubber Production in India* (Radhakrishna Pillay P.N. ed.). Kottayam: Rubber Research Institute of India.
31. PILLAY, P.N.R., GEORGE, M.K. AND RAJALAKSHMI, V.K. (1980) Leaf and Shoot Diseases, *Handbook of Natural Rubber Production in India* (Radhakrishna Pillay P.N. ed.). Kottayam: Rubber Research Institute of India.

32. IHAKA, R. AND GENTLEMAN, R. (1996) R: A language for Data Analysis and Graphics. *J. Comput. Graph. Statis.*, **5**, 299–314. (<http://www.r-project.org>)
33. STEEL, R.G.D. AND TORRIE, J.H. (1980) *Principles and Procedures of Statistics — A Biometrical Approach*, pp. 477–479. Singapore: McGraw-Hill Book Company.
34. HAMZAH, S., CHAN, J.L. AND YEANG, H.Y. (2002) Pollen Tube Growth and Fruit-set Success in *Hevea brasiliensis* Hand Pollination Influenced by the Choice of Clones and Female flower. *Euphytica*, **123**, 1–8.
35. VIJAYAKUMAR, K.R., CHANDRASHEKAR, T.R. AND PHILIP, V. (2000) Agroclimate, *Natural Rubber: Agromanagement and Crop Processing* (George P.J., Jacob C.K. eds.), 97–116. Kottayam: Rubber Research Institute of India.
36. HESLOP-HARRISON, Y. AND SHIVANNA, K.R. (1977) The Receptive Surface of the Angiosperm Stigma. *Annl. Bot.*, **41**, 1233–1258.
37. KNOX, R.B., WILLIAMS, E.G. AND DUMAS, C. (1986) Pollen, Pistil and Reproductive Function in Crop Plants. *Plant Breed. Rev.*, **4**, 9–79.
38. ROACH, S.A. AND WULFF, R.D. (1987) Maternal Effects in Plants. *Ann. Rev. Ecol. Syst.*, **18**, 209–235.
39. SHIVANNA, K.R., CREST, M. AND CIAMPOLINI, F. (1997) Pollen Development and Pollen-pistil Interactions. *Pollen Biotechnology for Crop Production and Improvement* (Shivanna, K.R. and Sawhney, V.K. eds.), 15–39. Cambridge: Cambridge University Press.
40. YEANG, H.Y. AND ONG, S.H. (1988) Low Fruit Set from *Hevea* Hand Pollination: Fruit Load and Propensity of Floral Shoots to Fruit Bearing. *J. nat. Rubb. Res.*, **3**(3), 143–154.
41. HUSIN, S.B.M., CHIN, H.F. AND HOR, Y.L. (1981) Fruit and Seed Development in *Hevea* (Clone RRIM 600) in Malaysia. *J. Rubb. Res. Inst. Malaysia*, **29**(2), 101–109.