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CONTROLLED POLLINATION OF HEVEA - PROBLEMS & PERSPECTIVES

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Introduction

Genetic improvement of Hevea over the years has led to tremendous yield increases. Cross combinations produced by hybridization have formed the base material for several cycles of clonal selection and evolution of a large number of the present day clones. Controlled pollination is still the most reliable means of obtaining genotypes of the desired genetic constitution. But, hindrances to the success of pollination programmes are many and efforts made to overcome them are reviewed in the present communication with emphasis on work done in India.

As listed out by Tan (1987) the three main problems confronted by Hevea breeders are (1) seasonal nature of flowering, (2) lack of synchrony in flowering among clones, and (3) low fruit set success.

Of these, low fruit set is the most serious bottleneck to the success of controlled pollination programmes, despite numerous efforts to overcome (Gandhimathi and Yeang, 1984; Leconte et al., 1984; Sedgley and Attanayake, 1986; Kavitha et al., 1989).

Seasonality of flowering

Most rubber clones flower only once a year in India and flowering is restricted to 1-2 months during January-March. The pattern, however, is different in the non-traditional rubber growing zone in north-east India, where flowering within clones was observed to be distributed over a fairly long period of 5 months from February to June (Rajeswari et al., 1989). At present, artificial hybridization efforts are concentrated in the traditional rubber growing tract of south India, where the brief and periodic nature of flowering impedes the progress of pollination programmes. Very often it becomes impossible to attempt sufficient number of artificial pollinations to obtain families large enough to conduct effective seedling selection in the nursery, partly due to the short span of time when flowers are available.

Certain clones like F 4542, Ch 2 and RRII 33 have shown off season flowering under Indian conditions. Identification of more such clones which flower more than once a year and induction of off season flowering could serve to mitigate the difficulties caused by the seasonal nature of flowering in *Hevea*.

The effect of growth regulators on the induction of flowering and their influence on the time of flower initiation have been reported in other perennial species. In rubber young buddings were induced to flower by ring barking (Campaignolle and Bouthillion, 1954; Ong, 1972; Saraswathyamma, 1975) and off season flowering was also induced by this method on mature trees (Premakumari and Nair, 1976; Majid *et al*., 1977). In their study, Premakumari and Nair (1976) tried ring barking and foliar application of different chemicals alone and in combinations on clone Gl 1. The results indicated that in *Hevea* off season flowering could be achieved by ring barking rather than by application of chemicals alone. However, ring barking in combination with application of chemicals like TIBA at 600 ppm or with potassium gibberellate at 20 ppm had an added effect and flowering could be advanced by 2-3 months. When these treatments were imposed in August, more than 50% of the branches flowered by November.

Consequent to the above study an effort was made to assess the effect of ring barking on fruit set. Twenty seven branches selected at random on three trees of clone Gl 1 were subjected to ring barking. An equal number of branches were also left untreated on the same trees. The number of fruits set per branch was observed. The mean fruit set per branch was 3.22 $\pm$ 1.40 for the ringed branches and 3.22 $\pm$ 1.28 for the control. Obviously, ring barking does not adversely affect fruiting branches and fruit set in the ringed branches and untreated ones was comparable.

Mechanical induction of off season flowering is a very attractive proposition in that flowering could be advanced to escape Oidium infection which otherwise takes a heavy toll of flowers during the normal flowering season. Off season flowering also brings about a corresponding advancement in fruit maturity and thus escape from pod rot caused by Phytophthora spp. which is yet another pathogen interfering in the success of hand pollination programmes. It would also enable breeders to conduct hand pollinations throughout the year, hence spreading the workload.

Non synchronous flowering

In any well planned hybridization programme parents are selected on the basis of their desirable attributes and their genetic potential to transmit those attributes to their progeny. Combining ability estimates have enabled *Hevea* breeders to identify suitable parental clones. But certain clones do not synchronise well in flowering time. This lack of synchrony in flowering restricts hand pollination programmes and the breeder is forced to leave a number of desirable cross combinations unattempted.

Observations on the peak period of female flower maturation were recorded from 20 clones for three consecutive years at the RRII and the pooled data are furnished in Table 1. The trees were of uniform age and were situated in the same location.

As evident from the observations, the peak flowering period ranged from the second week of January in clones IAN 45-717 and F 4542 to the last week of March in clones RRII 118 and GT 1, though the majority of the clones flowered during the month of February. The wide interval between female flower maturation period of the early flowering clones and the late flowering ones makes the chances of effecting crosses among them remote.

Pollen storage could be explored as a means to solve the problem of non synchronous flowering and efforts in that direction have been initiated at the RRII. Dijkman (1938) reported that pollen of many clones may be kept in reasonably good condition upto 19 days when the anther columns are kept over 27-35% sulphuric acid in a refrigerator at 6°c and a relative humidity of 80%. Scant attention has been paid since then to evolve methods to further prolong storage life of Hevea pollen. Techniques to prolong storage life of pollen beyond one month could enable successful completion of prescheduled pollination programmes with all desired corss combinations. Flower induction by ring barking could also be attempted to synchronise flowering among clones.

Low fruit set

Another major constraint in Hevea breeding is the low fruit set under controlled pollination. The failure of hand pollinations results in the loss of numerous potentially good cross combinations thus limiting the progress of genetic improvement of the species. Low fruit set considerably reduces the size of legitimate families on which selection is to be applied for the evolution of new clones with the desired attributes. Efforts to overcome the problem have been in vain and fruit set could not be raised to more than 5% at the RRII (Kavitha et al., 1989). In Malaysia, the mean fruit set was 3% for the main flowering season and 8% for the second season during the period 1969 to 1980 (Gandhimathi and Yeang, 1984). Equally low fruit set success under hand pollination was reported from Ivory Coast (Nicolas, 1979) and Sri Lanka (Attanayake and Sumeda, 1984). Besides being a serious impediment to *Hevea* improvement, low fruit set necessitates intensified hand pollination programmes, bringing about heavy input involvement. Failure to realise desired crosses also limits the progress of genetic studies like diallel matings which are essential to provide basic information for future crop improvement programmes.

At the RRII during the 1987 flowering season, 5 female parents were crossed in different combinations involving 15 male parents and the data are presented in Table 2.

The observations reveal wide variation in fruit set among the crosses. The success rate ranged from 0 - 12.38% within combinations involving the same female parent, as in the case of RRII 105. This indicates considerable influence of the male parent. RRIM 600 was found to be a favourable female parent, having produced high rates of fruit set success when crossed with eight different male parents of Malaysian, Sri-Lankan and Indian origin. Mean fruit set success in crosses involving RRII 105 as the female parent was also reasonably high, though it failed to set fruit in three combinations, and comparable with RRIM 600 (Table 3). For clones RRII 118, IAN 873 and PCK 1, fruit set under controlled pollination was significantly lower.

Clone RRII 118 has shown signs of incompatibility when used as the female parent. Except for its combination with PR 107 producing very low fruit set success, it failed to set fruit under controlled pollination. Further detailed investigations could help in understanding the extent and nature of incompatibility of the clone.

As suggested by Simmonds (1989), the relation between seed fertility and genetic advancement through controlled pollination would be worth investigation. Infertility would tend to be selected over generations as a correlated response to selection for the vegetative characters, growth and latex yield (Simmonds, 1979). The rubber breeder of the future may have to struggle to get desirable crosses of some outstanding parents.

Fruiting ability should therefore be considered as a criterion for selection of parents for hybridization. But, when a Hevea breeder is confronted with exceptionally good clones which are shy seeders, other means should be resorted to for ensuring the success of hand pollinations. In this context the possible factors contributing to low fruit set need to be examined.

Hormonal imbalance was explored as a possible cause of low fruit set and according to Paranjothy (1980), attempts t0 increase fruit set by application of growth regulators have generally been disappointing. However, views on this aspect differ. Leconte et al., (1984) obtained encouraging results with the use of NAA and GA_3. Investigations at the RRII showed application of GA_3 at 20 ppm to be promising but it did not bring about significant improvement in fruit set compared to that by the conventional hand pollination procedure (Kavitha et al., 1989).

The time of pollination was examined as another aspect influencing fruit set (Attanayake and Sumeda, 1984). Comparison of the standard method of controlled pollination in the morning prior to anthesis and hand pollination in the afternoon coinciding with anthesis showed that afternoon pollination may result in higher fruit set. Pollination between 12.00 and 16.00 hours gave higher pollen tube growth (Sedgley and Attanayake, 1986). Altering the time of pollination could thus increase fruit set success. Under Indian conditions, anther dehiscence was reported to occur upto 12.00 hours only (George et al., 1967) and as such, the need to change the time of pollination does not arise.

Flowering and fruit development in Hevea is accompanied by extensive refoliation creating a severe competition for assimilates. Application of complete fertilizer mixtures (Haines, 1946) and continuous soil application of ammonium sulphate to widen the N/K ratio of the leaves (Watson and Narayanan, 1965) have produced significant increase in seed production in rubber.

A preliminary study conducted at the RRII (Sasikumar et al., 1987) indicated direct application of phosphorus as foliar spray to play a considerable role in preventing premature fruit drop in rubber. A subsequent study, the results of which are presented in Table 4 also corroborates the previous report that competition for phosphorus between the developing fruits and other plant parts could be one of the causes of reduced fruit set in rubber.

Six treatments with water spray as control were imposed by foliar application on trees of clone Gl 1. Three rounds of treatment sprays were given at fortnightly intervals following refoliation. The experiment was conducted in a randomised block design with three replications and single tree per plot. In order to avoid overlapping of treatment sprays, one tree on either side of each treated tree was left unsprayed. Fruit counts were recorded 1 month and 4 months after completion of refoliation, from four branches selected at random on each tree.

All treatments were found to be statistically on par for both fruit set and retention. In terms of the mean number of fruits per branch 100 ppm spray of Borax appeared promising. Deficiency of boron has previously been suggested as a factor in reducing fruit set (Samaranayake et al., 1979). As regards retention of fruits upto maturity, 1% spray of orthophosphoric acid helped retain 82.77% of the fruits set initially.

In recognition of the competition for nutrients on fruiting branches, reduction of fruit load was considered for reducing fruit drop. Dijkman (1951) recommended hand pollination of 6-8 flowers per inflorescence. Sedgley and Attanayake (1986) studied the relationship between the number of pollinations per inflorescence and the number of initial fruits set and mature fruits harvested. Though the number of pollinations had no effect on the recovery of mature fruits, the highest initial fruit set percentage was found to result from between one and four pollinations per inflorescence. Pollination of not more than four flowers per inflorescence has been recommended by them to ensure fruit set success. According to Yeang and Gandhimathi (1984) while intra branch competition among developing fruitlets is probably not important, the possibility of inter branch competition influencing fruit set cannot be ruled out. Their observations revealed a disparity in the propensity to successful fruit set between different flowering branches, which could not be explained and could only be attributed to chance. Usually not more than 9 healthy female flowers per inflorescence are available at a time for controlled pollination. From the reports discussed, it does not seem necessary to reduce the number of pollinations per inflorescence to less than 6-8.

The effect of pathogens like *Oidium* on flowering intensity and *Phytophthora* on fruit drop is considerable in India where flowering and fruit maturation coincide with the period of rapid proliferation of the fungi. While stringent plant protection measures could alleviate the intensity of infection, escape from the pathogens through induction of off season flowering appears to be a better proposition.

Meteorological factors like evaporation and solar radiation determine fruit set success to a limited extent (Yeang and Gandhimathi, 1984). From the practical view point, adjustment of pollination programmes in accordance with weather forecasts does not appear feasible.

Yeang and Gandhimathi (1984) have expressed the view that one obvious means of improving fruit set would be to increase the quantity of pollen transferred to the stigma during hand pollination. Novel pollination procedures need to be developed for the purpose. It is important that any new procedure be less laborious and more efficient than the conventional method. It is also worth examining the possibility suggested by them that a certain proportion of pollen grains are not released with anther dehiscence within the artificially pollinated flowers. The conventional method of hand pollination, in addition to allowing for chances of mechanical injury to floral parts could restrict proper dehiscence of anthers and dispersal of pollen grains within the limited space available after the staminal column is inserted along with a wad of cotton wool and the perianth lobes fused together with latex. Recent investigations at the RRII (Kavitha _et al_., 1989) revealed injury to floral parts to be a possible cause for low fruit set success on hand pollination. Protection of hand pollinated panicles with a butter paper cover instead of sealing individual flowers with cotton wool and latex gave significantly better results than the conventional procedure. Damage to the stigma following hand pollination has been previously reported (Sedgley and Attanayake, 1988) and differences between operators in the amount of damage inflicted was observed. Damaged stigma supported reduced pollen tube growth. It was suggested that pollinators be instructed to place the staminal column and the cotton wool plug with a light touch and not to press down on the stigma during the pollination process. The modified method of protecting pollinated panicles with a butter paper cover is less laborious and reduces injury to floral parts to the minimum. While the conventional hand pollination procedure gave only 2.87% fruit set, the butter paper cover method gave a significantly higher success of 4.98% as evidenced by analysis of pooled data for the three years of study (Kavitha et al., 1989).

The inaccessibility of flowers in a tree crop like rubber needs no special mention. As suggested by Sedgley and Attanayake (1986) establishment of a breeding orchard where the trees are not tapped and the plant breeder has full control over the management of the trees would go a long way in overcoming the practical problems associated with controlled pollination. The trees could be planted at a wider spacing to facilitate maximum crown expansion as well as exposure of branches to sunlight and they could be made to branch at lower levels to enable closer supervision of hand pollination programmes by the plant breeder. In addition to easy accessibility of flowering branches this would eliminate the cost and risk involved, in erecting huge scaffolds every year for pollination purposes. Steps in this direction have already been initiated at the RRII.

Prospects for the future

In the light of the problems and perspectives discussed, the following suggestions are made for ensuring success of controlled pollination programmes in Hevea.

1. Establishment of breeding orchards where trees are planted at wide spacing and are induced to branch at lower levels.

2. Proper management of trees, with optimum doses of fertilisers administered during the period proceeding refoliation. Foliar application of necessary nutrients during the flowering and fruit maturation period may be done based on leaf sample analyses.

3. Induction of off season flowering by ring barking to advance the flowering season and synchronise flowering among clones.

4. Pollination of 6-8 flowers per inflorescence to reduce fruit load and competition for assimilates among the developing fruits and vegetative parts.

5. Adoption of the method of bagging pollinated panicles with a butter paper cover to simplify the crossing procedure and minimise injury to floral parts.

It would be desirable to undertake indepth studies on the following aspects, which could pay rich dividends in the future.

1. Storage of *Hevea* pollen for long periods.
2. In cases where female infertility or incompatibility are evident, detailed investigations to determine the extent and nature of the phenomena and adoption of *in vitro* techniques to circumvent them.

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Table 1: Peak flowering in some clones

Sl. No.	Clone	Peak period of female flower maturation
1	IAN 45-717	January 2nd week
2	F 4542	-do-
3	Ch 31	January 3rd week
4	Fx 516	February 1st week
5	PB 5/60	February 2nd week
6	Ch 2	-do-
7	Ch 4	-do-
8	RRII 6	-do-
9	Gl 1	-do-
10	RRIM 526	-do-
11	PB 86	February 3rd week
12	Tjir 1	-do-
13	RRII 105	-do-
14	RRII 102	-do-
15	RRII 106	March 1st week
16	RRII 109	-do-
17	RRII 203	March 2nd week
18	RRII 33	-do-
19	RRII 118	March last week
20	GT 1	-do-

Table 2: Fruit set success under different cross combinations

Sl. No.	Cross combination	No. of pollinations	Fruit set No.	Fruit set %
1	RRII 105 x RRII 118	325	--	
	" x PB 28/59	202	25	12.38
	" x PB 235	165	2	1.21
	" x RRIM 623	375	--	--
	" x RRIM 703	354	19	5.37
	" x RRIM 628	132	--	--
	" x RRII 208	358	12	3.35
	" x RRII 308	375	1	0.27
	" x RRIC 102	87	5	5.75
	" x PCK 1	207	33	15.94
2	RRII 118 x RRIM 600	100	--	--
	" x PB 260	32	--	--
	" x PB 235	108	--	--
	" x RRII 300	161	--	--
	" x RRII 308	95	--	--
	" x PR 107	169	1	0.59
3	RRIM 600 x RRII 118	401	11	2.74
	" x RRII 208	349	36	10.32
	" x RRIM 623	303	4	1.32
	" x RRIM 628	394	9	2.28
	" x RRIM 703	280	5	1.79
	" x PB 28/59	600	25	4.17

RRIM 600 x RRIC 102	374	25	6.68
" x PCK 1	156	9	5.77
IAN 873 x RRII 105	164	1	0.61
" x RRII 208	356	--	--
" x RRII 300	301	2	0.66
" x RRIM 623	323	4	1.24
" x RRIM 628	174	4	2.3
" x RRIM 703	231	13	5.63
" x RRIM 600	547	4	0.73
" x PB 28/59	246	11	4.47
" x PB 235	171	--	--
" x RRIC 102	385	4	1.04
" x PCK 1	414	6	1.45
PCK 1 x RRII 105	167	15	8.98
" x RRII 203	260	2	0.77
" x RRII 208	68	2	2.94
" x RRII 118	237	--	--
" x RRIM 600	352	12	3.41
" x RRIM 703	150	2	1.33
" x PB 235	64	1	1.56
" x PR 107	225	7	3.11

Table 3: Influence of female parent on mean fruit set success

Sl. No.	Female parent	No. of pollinations	Fruit set No.	Fruit set %
1	RRII 105	2580	97	3.76
2	RRII 118	665	1	0.15*
3	RRIM 600	2857	124	4.34
4	IAN 873	3312	49	1.48*
5	PCK 1	1523	41	2.69*

*Chi-square value for comparison with RRIM 600 significant at 0.05 level.

Table 4: Effect of foliar spray of nutrients on fruit retention

Treatments	Mean fruit count per branch		% Fruit retention
	Initial	Final	
1. Borax 100 ppm	28.33	19.08	67.35
2. Urea 5%	10.42	8.00	76.78
3. Urea 10%	19.42	11.67	60.09
4. Orthophosphoric acid 1%	22.75	18.83	82.77
5. Orthophosphoric acid 2%	13.17	8.75	66.44
6. Urea 10% + Ortho-phosphoric acid 1% + Borax 100 ppm	18.17	13.33	73.36
7. Water spray (control)	14.67	9.5	64.76