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UTILIZATION OF WILD GERMPLASM IN *HEVEA* BREEDING

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Abstract

Developing high yielding clones through breeding and selection in rubber (Hevea brasiliensis *Willd. ex Adr. de Juss) Muell. Arg. has been one of the major breeding strategies of Rubber Research Institute of India, since its establishment in 1954. Over 127 Wickham clones evolved in rubber producing countries like Malaysia, Indonesia, Sri Lanka, Thailand, China and Ivory Coast have been introduced to India under clone exchange programmes. Selected clones from this collection were used in hand pollination programmes which led to the release of some very successful cultivars such as RRII 105, RRII 414, RRII 417, RRII 422 and RRII 430. In addition to the Wickham clones, a total of 4548 wild germplasm accessions collected during the 1981 IRRDB-EM BRAPA expedition to the centre of diversity in Brazil were also introduced to India during 1984-1990 and are being conserved for characterisation, evaluation and utilization in a phased manner. The wild accessions collected from three provenances in Brazil viz. Acre (AC), Rondonia (RO) and Mato Grosso (MT) are a potential source for introgression of genes required for complementing the present day cultivated clones. Since the original gene pool of* Hevea brasiliensis *utilized for the development of popular clones was narrow, hybridization was attempted using selected genetically divergent wild accessions and promising Wickham clones. The nursery evaluation of the hybrid progenies of such a breeding programme conducted in 1997 is presented in this paper.*

The parents included in the hybridization programme of 1997 were two popular Wickham clones viz. RRII 105 and RRIM 600 as female parents and nine genotypes from wild germplasm as male parents (MT 999, MT 1021, MT 1027, MT 1014, MT 1005, AC 495, AC 498, AC 817 and RO 380) which showed superior performance in certain secondary attributes. Fourteen cross combinations were attempted, a total of 3500 flowers were pollinated, 200 fruits (5.7%) obtained, and the seeds after germination were planted in a seedling nursery in August 1997. The fruit set success of the crosses ranged from 1.15 to 16.43%. Growth characters such as girth and height of plants were recorded from the next year onwards and test tapping was carried out in the second year after planting in the nursery. Mean girth in the third year ranged from10.11 cm to 15.46 cm and height ranged from 321 cm to 622 cm. Average test tap yield of the second year ranged from 0.595g/t/10t to 1.611g/t/10t. Test tap yield of the female parents RRII 105 and RRIM 600 were 1.272 and 0.902 g/t/10t respectively. Bark thickness ranged from 2.31 to 3.22 mm and number of latex vessel rows ranged from 3.5 to 6.6 in the third year. Shoot rot was observed in a few progenies with low to moderate intensity. Based on nursery evaluation, 36 progenies which showed promising yield and other secondary characters were selected for cloning and further field evaluation.

Key words: Amazonian, Brazil, Genetic divergence, *Hevea brasiliensis*, Hybridization Test tap yield, Wickham clones, Wild germplasm.

Introduction

Rubber cultivation in the tropical monsoon areas of South and South-East Asia began after the introduction of rubber in 1876 into Sri Lanka from Brazil through the Kew Gardens in UK. The original material collected by Sir Henry Wickham formed the sole basis of the genetic variability and this was further circumscribed by the genetic make-up of the particular sample received from the original 'Wickham gene pool'. During this period seed propagation was the rule. Till 1917, when bud grafting was perfected in *Hevea*, open pollinated seeds were the only means of propagation.

By the introduction of bud grafting of elite genotypes, good plantations were raised through clonal propagation. Selective hybridisation between superior clones and clonal propagation of superior hybrids were later taken up. Such an approach promoted recombination between elite genotypes thus widening the scope for further selection and thereby increasing the yield potential considerably. Most of the modern commercial clones are derived from this method of breeding. The base of available genetic variability was further broadened by frequent exchange of superior clones among rubber growing countries of Asia and Africa. In India, 127 Wickham clones evolved in countries like Malaysia, Indonesia, Sri Lanka, Thailand, China and Ivory Coast had been introduced under a bilateral clone exchange programme.

Rubber productivity in India has registered commendable progress from 200 kg/ha/yr for unselected seedlings to 4000 kg/ha/yr for modern hybrid clones(Saraswathyamma *et al.*, 2006). Rubber Research Institute of India (RRII) has also imported a total of 4548 wild *Hevea* germplasm accessions from the Malaysian centre during the period 1984-1990 and these are being conserved for characterization, evaluation and utilization in a phased manner. The wild *Hevea* genotypes (Amazonian) are a potential source for introgression of certain genes

complementing the present day cultivated clones. Since the original gene pool of *Hevea brasiliensis* utilized for the development of popular clones is narrow, breeding programmes were conducted incorporating selected wild accession with promising Wickham clones. The nursery evaluation of the progenies of such a breeding conducted in 1997 is presented in this paper.

Materials and methods

The parents selected for the crosses were two popular Wickham clones viz. RRII 105 and RRIM 600 as female parents and nine genotypes from wild germplasm as male parents (MT 999, MT 1021, MT 1027, MT 1014, MT 1005, AC 495, AC 498, AC 817 and RO 380) which showed superior performance in certain secondary attributes as male parents. The hand pollination work was conducted in the Central Experiment Station of RRII at Chethackal near Ranny in Central Kerala. The pollination work was started in the beginning of February 1997 and completed by the end of March, since these months are the peak flowering period of Hevea brasiliensis. The mature seeds were harvested by the fifth month after pollination. The seeds were carefully separated taking care to maintain the identity of the seeds from each cross combination. The seeds were sown family wise in trays filled with sand. They were kept moist for proper germination. The germinated seeds were then planted family wise in a seedling nursery along with budded plants of clone RRII 105 at the RRII campus, Kottayam in August 1997. The characters recorded from the seedlings at the age of 2 years were test tap yield, plant girth, height, number of leaf whorls, bark thickness and number of latex vessel rows. Test tapping was done on two year old trees at a height of 20 cm from the base. Tin spouts were attached to the stem bark with the collection cup placed on the ground. Latex from the first five tapping was discarded. Thereafter, latex from 10 consecutive tapping was collected, sun dried for a day and the cup lumps were extracted for oven drying. Oven drying at 55°C was done for 10 days. The dried cup lumps were then weighed to record the dry rubber yield per plant per ten tappings.

Anatomical observations were recorded from bark samples collected from two year old seedlings. The latex

Table 1. The fruit set per cent and progeny size in each cross

Sl. No	Cross combinations	Fruit set (%)	Hybrid seedlings plantd (No.)
1	RRII 105 x MT 1027	1.65	6
2	RRII 105 x MT 1005	14.45	54
3	RRII 105 x MT 999	7.8	13
4	RRII 105 x MT 1014	4.57	12
5	RRII 105 x MT 1021	1.15	5
6	RRIM 600 x MT 1027	6.8	15
7	RRIM 600 x MT 1005	8.33	45
8	RRIM 600 x MT 999	5.85	50
9	RRIM 600 x MT 1014	4.74	21
10	RRIM 600 x MT 1021	15.15	44
11	RRIM 600 x AC 495	7.33	54
12	RRIM 600 x AC 498	4.53	31
13	RRIM 600 x AC 817	7.8	23
14	RRIM 600 x RO 380	16.43	27

vessel rows were recorded by microscopic observation of radial longitudinal sections of the bark stained using Sudan IV. Superior hybrid seedlings were identified on the base of juvenile yield, vigour and other structural components such as bark thickness and number of latex vessel rows.

Results and discussion

Fourteen cross combinations were attempted among two Wickham clones and nine wild germplasm accessions. A total of 3500 flowers were pollinated, 200 fruits were obtained and the average success of fruit set was 5.7%. The per cent fruit set in each cross and progeny size is given in Table 1. The fruit set varied widely between the different crosses, and ranged from 1.15 to 16.43%. The cross combination RRIM 600 x RO 38 exhibited the highest fruit set (16.43%) followed by the cross RRIM 600 x MT 1021 (15.15%). The lowest was exhibited by the family RRII 105 x MT 1021(1.15%).

The *Hevea* tree produces flowers in abundance, but even in good flowering season, not more than three per cent of the female flowers develop into fruits. (Mass, 1919; Attanayake and Sumeda 1984). Under artificial pollination also fruit set is equally low (Mydin *et al.*, 1989). There are several references on the problem of low fruit set in *Hevea* (Warmke, 1951; Rao, 1961; Gandhimathi and Yeang, 1984). The low

Table 2. Mean girth, height and test tap yield of each family in the second year

Sl. No	Cross combinations	Mean girth (cm)	CV (%)	Mean height (cm)	CV (%)	Mean Yield (g/t/10t)	CV (%)
1.	RRII 105 x MT 1027	14.06	32.82	321	38.62	1.018	58.17
2.	RRII 105 x MT 1005	12.81	21.38	381	18.32	0.776	43.31
3.	RRII 105 x MT 999	12.81	25.52	430	32.84	0.894	55.84
4.	RRII 105 x MT 1014	11.50	19.64	575	24.21	0.618	41.93
5.	RRII 105 x MT 1021	15.00	19.45	622	11.33	0.595	53.61
6.	RRIM 600 x MT 1027	15.46	13.42	585	15.78	1.222	52.31
7.	RRIM 600 x MT 1005	12.81	23.68	563	22.25	0.741	48.04
8.	RRIM 600 x MT 999	13.99	26.63	572	26.50	1.611	47.48
9.	RRIM 600 x MT 1014	13.81	14.62	581	18.65	0.701	52.54
10.	RRIM 600 x MT 1021	14.56	23.32	563	21.84	1.345	47.78
11.	RRIM 600 x AC 495	15.23	22.24	545	20.63	0.821	38.55
12.	RRIM 600 x AC 498	10.11	25.23	533	26.86	1.384	31.72
13.	RRIM 600 x AC 817	14.59	15.75	484	13.44	1.115	39.55
14.	RRIM 600 x RO 380	13.73	24.29	553	19.85	1.425	47.03
15.	Check clone RRII 105	9.20	12.23	420	14.25	1.272	28.03
16.	Check clone RRIM 600	9.51	15.25	435	17.32	0.902	25.43

Table 3. Number of leaf whorls, bark thickness and latex vessel rows at the age of two years

Sl. No	Cross combinations	Number of leaf whorls		Bark thickness	Number of latex vessel rows
		Range	Mean	$\bar{X}$ ± SE	$\bar{X}$ ± SE
1.	RRII 105 x MT 1027	2 - 3	3.0	2.42 ± 0.24	5.6 ± 0.51
2.	RRII 105 x MT 1005	1 - 4	2.63	2.62 ± 0.24	4.8 ± 0.49
3.	RRII 105 x MT 999	2 - 5	3.8	2.35 ± 0.24	5.4 ± 0.51
4.	RRII 105 x MT 1014	2 - 5	4.0	3.22 ± 0.37	6.5 ± 0.93
5.	RRII 105 x MT 1021	2 - 3	2.25	2.65 ± 0.24	6.0 ± 0.71
6.	RRIM 600 x MT 1027	2 - 5	3.44	2.78 ± 0.37	6.3 ± 0.60
7.	RRIM 600 x MT 1005	1 - 3	1.75	3.02 ± 0.32	6.0 ± 0.71
8.	RRIM 600 x MT 999	2 - 5	3.3	3.18 ± 0.37	6.5 ± 0.51
9.	RRIM 600 x MT 1014	2 - 4	2.85	2.31 ± 0.24	3.5 ± 0.24
10.	RRIM 600 x MT 1021	1 - 4	2.5	3.21 ± 0.37	6.6 ± 0.60
11.	RRIM 600 x AC 495	1 - 4	2.70	2.65 ± 0.37	4.0 ± 0.32
12.	RRIM 600 x AC 498	2 - 3	2.38	2.81 ± 0.34	6.4 ± 0.40
13.	RRIM 600 x AC 817	1 - 4	2.30	3.03 ± 0.37	6.2 ± 0.58
14.	RRIM 600 x RO 380	1 - 3	2.2	2.35 ± 0.24	5.5 ± 0.75
15.	Check clone RRII 105	1 - 3	3.92	2.80 ± 0.32	6.5 ± 0.42
16.	Check clone RRIM 600	1 - 3	2.53	2.35 ± 0.24	5.2 ± 0.35

fruit set resulting from hand pollination programmes of *Hevea* hinders the progress of Hevea breeding (Attanayake and Sumeda, 1984). Here in the present study even though the average fruit set was 5.7%, certain combinations such as RRIM 600 x RO 380, RRIM 600 x MT 1021 and RRII 105 x MT 1005 resulted in very high fruit set to the tune of 16.42%, 15.15 % and 14.45 % respectively.

The test tap yield of the families is given in Table 2. The mean test tap yield ranged from 0.595 g/t/10tap to 1.611 g/t/10t, the highest was in the family RRIM 600 x MT 999 (1.611g/t/10t) followed by RRIM 600 x RO 380 (1.425g/t/10t). It was lowest in RRII 105 x MT 1021 (0.595g/t/10t). Test tap yield of RRII 105 was 1.272 and RRIM 600 was 0.902 g/t/10t. Four families viz. RRIM 600 x MT 999, RRIM 600 x RO 380, RRIM 600 x AC 498 and RRIM 600 x MT 1021 showed yield higher than the high yielding parental clone RRII 105. Among the five crosses incorporating RRII 105 as one of the parent, the family RRII 105 x MT 1027 exhibited the highest yield of 1.018 g/t/10t.

Early evaluation in the seedling nursery is done by test tapping and selection of seedlings with vegetative vigour. Test incision method for one year old plants is another early evaluation technique developed at RRII (Varghese *et al.*, 1989). Test tapping gives more consistent results. In the present study four families viz. RRIM 600 x MT 999, RRIM 600 x MT 1021, RRIM 600 x AC 498 and RRIM 600 x RO 380 exhibited test tap yield higher than that of the high yielding check clone RRII 105. The general mean of the test tap yield of the families was 1.019 g/t/10 t. According to Varghese et al., (1993) selection should not be confirmed to the highest juvenile yielders alone as this is likely to eliminate moderate high yielders with wider adaptability. A more rational approach would be to select all genotypes with juvenile yield above the general mean so that none of the potential high yielders are lost. Genotypes that showed yield above the general mean were thus selected for the next phase of evaluation.

Important growth and anatomical parameters are given in Table 2 & 3. Growth characters were recorded from the second year onwards. In the third year average girth ranged from 10.11cm to 15.46 cm, the highest was exhibited by the cross RRIM 600 x MT 1027 (15.46 cm) followed by RRIM 600 x AC 495 (15.23 cm) and the lowest was in RRIM 600 x AC 498 (10.11 cm). Mean plant height was highest in cross RRII 105 x MT 1021 (622cm) and lowest in RRII 105 x MT 1027 (321cm). Average number of leaf whorls in the crosses ranged from 1.75 in RRIM 600 x MT 1005 to 3.8 in RRII 105 x MT 999.

Growth vigour is genetically controlled, hence variations exist among the genotypes with regard to morphological characters.

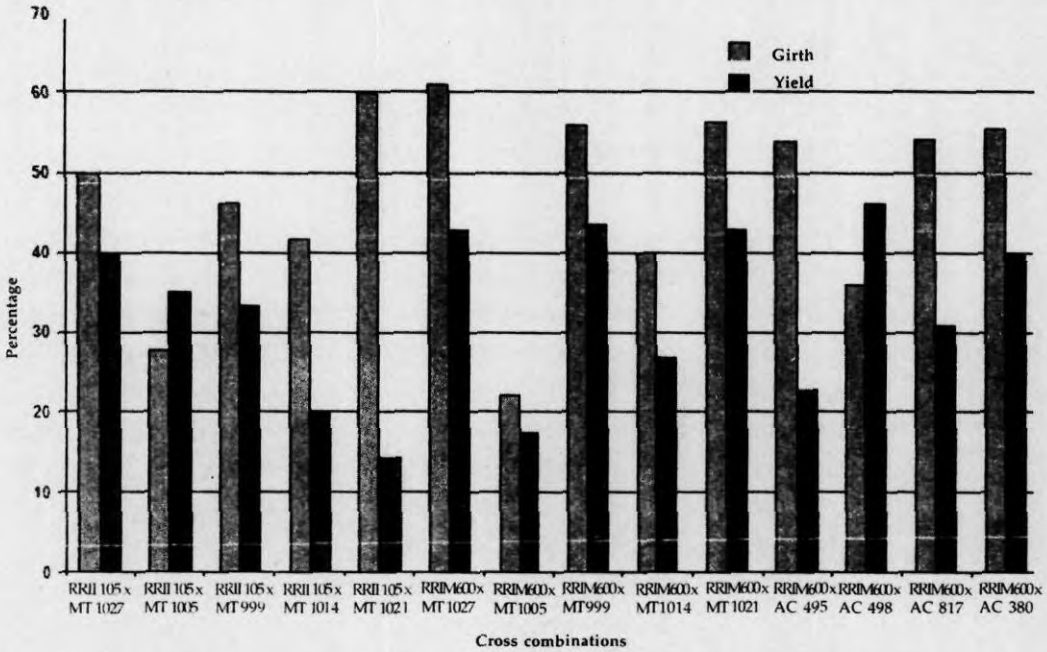

Fig 1. Percentage of hybrids within each family that were better than the check clone RRII 105 for test tap yield and girth

According to Simmonds (1989) yield and vigour in the crop are hardly separable. All the high yielding families exhibited average girth above 13cm except RRIM 600 x MT 498 (10.11cm).

Yield and growth attributes would be of great value in the selection of superior seedlings. Percentage of hybrids within each family that were better than the check clone RRII 105 for test tap yield and girth is represented in Fig. 1. Out of 400 seedlings planted in the nursery 79 seedlings showed high yield and 183 showed high girth when compared to the check clone RRII 105. Among the 14 families, in 4 families viz. RRIM 600 x MT 1027, RRIM 600 x MT 999, RRIM 600 x MT 1021 and RRIM 600 x AC 498 above 40% of the seedlings showed higher yield than the control of which the family RRIM 600 x AC 498 was superior (46.15 %). Above fifty percent of the seedlings in seven families showed girth better than the control. The highest was exhibited by RRIM 600 x MT 1027 (61.13%) followed by RRII 105 x MT 1021 (60%).

The progenies also exhibited difference with regard to bark thickness and number of latex vessel rows. Bark thickness ranged from 2.31 to 3.22mm, highest was observed in the family RRII 105 x MT 1014 (3.22mm) and lowest in RRIM 600 x MT 1014 (2.31mm). Number of latex vessel rows (LVRs) was high in the family RRIM 600 x MT 1021 (6.6) and low in RRIM 600 x MT 1014 (3.5). Most of the high yielding clones recorded above 3mm bark thickness and 6 numbers of LVRs. According to Hamzah and Gomez (1982) girth, bark thickness, latex vessel rows and vessel volume are all inter-related and these are in turn related to the total yield.

Diseases in the nursery were recorded. Nine out of fourteen families were affected by shoot rot from low to moderate intensity, it was high in the family RRII 105 x MT 1005, eight out of fifty four seedlings were affected and in RRIM 600 x MT 1021 six out of forty four plants were affected. Powdery mildew disease was observed in low to moderate intensity in the families in general.

The most important method of developing clones of desirable genetic constitution is the controlled hybridization between selected parent clones,

evaluation of F1 hybrids and selection of promising recombinants for further evaluation (Varghese *et al.*, 2006). Since the genetic base of cultivated *Hevea brasiliensis* is narrow, incorporation of wild germplasm in breeding programmes assumes great significance. The wide genetic diversity available in the wild germplasm has been established using morphological data and molecular markers (Varghese *et al.*, 2002) and scope for selection of accessions with desirable agro morphologic traits has been indicated (Mercy et al., 1995; Abraham *et al.*, 2000).

The choice of parents for crossing programmes from 1992 onwards was based on genetic divergence studies and general combining ability/ pre potency (Mydin, 1992), in addition to specific stress tolerance traits both from Wickham clones and the wild germplasm.

Sources of resistance have been located in the wild Brazilian germplasm as well as in Wickham clones (Varghese *et al.*, 2002; John *et al.*, 2001). Systematic screening programmes are essential for locating the sources of superior genes. In the context of climate change also, conservation and utilization of natural resources assumes much significance.

The present hybridization between widely divergent genotypes has released a wide range of variability for various traits. Based on the nursery evaluation, 36 hybrid seedlings of Wickham x Amazonian origin which showed promising yield and other secondary attributes such as high vigour and other structural components were selected for cloning and further field evaluation towards evolving superior hybrid clones.

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