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JUVENILE SELECTION IN PROGENIES FROM CONTROLLED AND OPEN-POLLINATIONS OF NEW GENERATION CLONES OF *HEVEA BRASILIENSIS*

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Juvenile evaluation of recombinants (F_1 generation) is the first stage of evaluation in the breeding and selection cycle of *Hevea brasiliensis*. Six full-sib families and three half-sib families consisting of 465 seedlings were subjected to detailed evaluation. Seedling progenies exhibited wide variability in terms of growth, test tap yield and other morphological characters. Thirtyfive progenies were selected based on growth and vigour in the early stage for further evaluation. Selection of medium to high-growth seedlings could maximize genetic gain. The present study could also identify potential parents for repeated recombination to generate more productive progenies. Recovery of high frequency of elite progenies from the half-sib progeny evaluation underlines the importance of this approach which can be repeated over the years to consolidate productive seedlings. Half-sib progeny evaluation is found to be a relatively inexpensive way of developing new productive clones in *Hevea*.

Keywords: Full-sibs, Half-sibs, *Hevea* breeding, Juvenile selection.

INTRODUCTION

The Para rubber tree [*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg.] is one of the most recently domesticated crop species. The genetic gain obtained during the last few decades in *H. brasiliensis* in terms of rubber yield is noteworthy. In India, genetic improvement efforts on the base material revolutionised rubber production during the last 50 years, with a manifold improvement in productivity. Early generation seedling plantations with a marginal yield of about 250 kg/ha/year were replaced with improved hybrid clones. The present day cultivars have a yield potential of up to 3500 kg/ha/year (Licy *et al.*, 1997). Rubber breeding procedures are often lengthy and laborious (Tan, 1987; Simmonds, 1989; Varghese and Mydin, 2000). Cyclic breeding and selection of elite genotypes have resulted in the generation of hybrids with high rubber yield. Nursery evaluation of progenies is one of the most crucial steps in this process.

In artificial pollinations, fruit set is generally low (3-5%), except in cases of higher level of female fecundity as in clones like PB 330 (Chandrasekhar *et al.*, 2004). It is expected that the repeated use of such clones would help to increase the recombination frequency and variability, facilitating efficient selection of high yielding and fast growing progenies from the population. In

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hand-pollination programmes, the number of progenies obtainable is extremely variable. Therefore statistically laid out evaluation trials are not feasible at this stage (Chandrasekhar and Gireesh, 2009). After exerting moderate selection pressure in the nursery stage, desirable progenies are clonally multiplied and evaluated in replicated clonal nurseries, and further in large-scale trials. Superior clones are then selected and commercialized based on the long-term field performance. An appreciable amount of heterosis has been realised in rubber tree breeding programmes for high yield but breeding programmes using new generation hybrid clones are meagre. The present study was undertaken to evaluate the nursery performance of seedling progenies

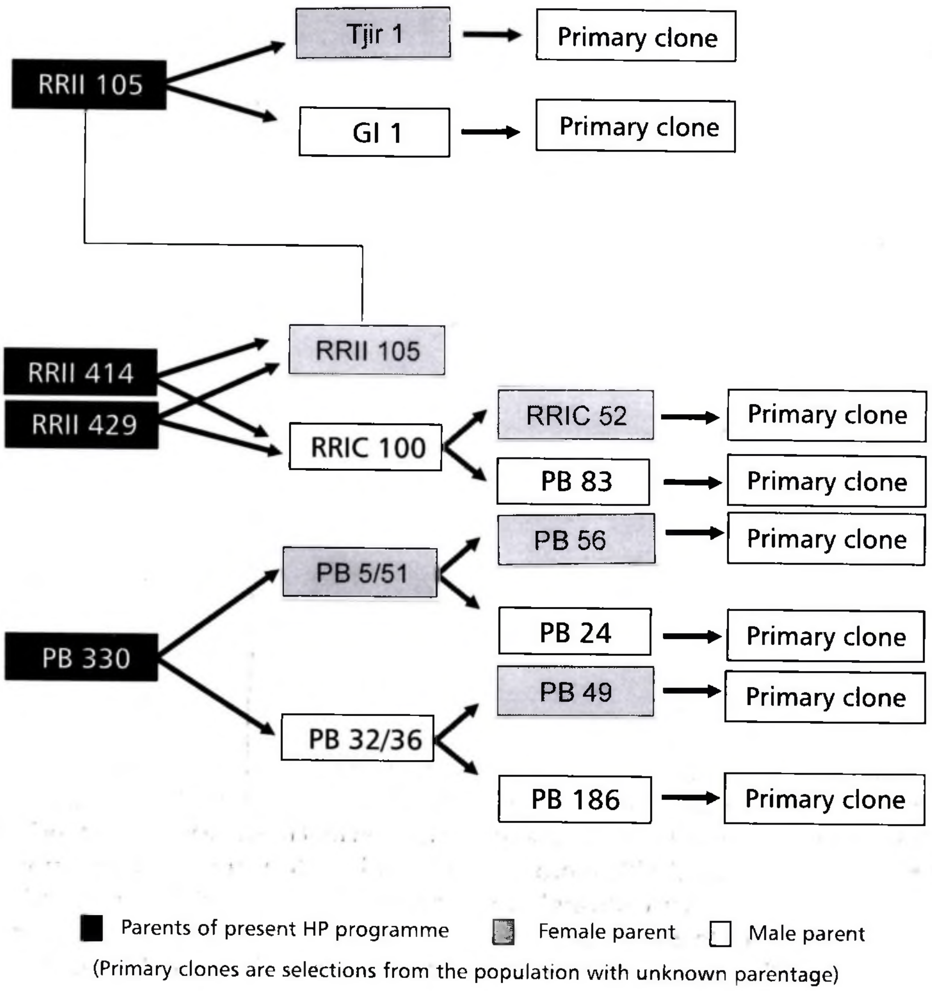

Fig. 1. Pedigree of parent clones

of a hand pollination programme done in the year 2005, involving newly developed vigorous and high yielding clones such as RRII 414, RRII 429 with PB 330 and RRII 105.

MATERIALS AND METHODS

Parental combinations

The pedigrees of the four clones involved in the crosses *viz.*, RRII 105, RRII 414, RRII 429 and PB 330 can be traced back to eight primary clones of diverse origin (Fig. 1.) These four clones were selected based on traits such as earliness in tappability, rubber yield, and good secondary features like straightness of bole. Rubber production of these parents in the traditional rubber growing areas ranges from moderate to high. RRII 105, the most popular hybrid clone of Indian origin (resultant of the 1954 HP programme) is the ruling check variety. Mean yield of RRII 105 is 1500 to 2000 kg/ha/year (Saraswathyamma *et al.*, 2000; Varghese and Abraham, 2005). Clones RRII 414 and RRII 429 are recently developed high yielding clones of secondary origin, with parentage RRII 105 x RRIC 100. Mean yield of RRII 414 was about 20-40 per cent higher than that of RRII 105 in a small-scale trial; while that of RRII 429 was about 48 per cent more than that of RRII 105 (Licy *et al.*, 2003). In addition, these clones have also proven earliness in tappability. The secondary clone, PB 330, was evolved from a PB 5/51 x PB 32/36 cross in Malaysia (Ang and Shepherd, 1979).

Seedling nursery

Hybridization between the four clones was carried out in 2005, and six full-sib families were generated (Table 1). In addition, half-sibs (open-pollinated seeds) were collected from RRII 105, RRII 414 and RRII 429. A total of 635 seedlings were established at a spacing of 40 x 40 cm, in the nursery of the Rubber Research Institute of India, Kottayam during July-August 2005. These seedlings were maintained by adopting standard practices. At the end of third year, out of the 465 seedlings that survived, 55 seedlings which attained a girth of 10 cm and above (based on the population mean) at a height of 30 cm, were selected for detailed evaluation. Data were recorded on plant height, branching height, number of leaves, number of whorls, anatomical features and test tap yield. Test tapping (S/2 d2 6d/7) was done using modified Michie-Golledge tapping knife. Dry rubber yield from 35 tappings were assessed separately each day. Seedlings were finally selected for further evaluation on the basis of the population mean for test tap yield. Family means and population means of all the characters were computed for the finally selected seedlings (Table 1). Details of recovery of half-sibs and full-sibs from the year of establishment to the third year are illustrated in the flow chart (Fig. 2).

Bark anatomy

Bark samples of 1.5 cm size were collected from the seedlings at a height of 30 cm using a bark sampler and preserved in formalin-acetic acid-alcohol fixative (Johansen, 1940) solution until sectioning and observation. Radial longitudinal sections of 30 µm thickness were taken using sledge microtome. These sections were stained with Oil Red O (Omman and Reghu, 2003), and examined using a microscope. Images were analyzed using image analysis system Motic Image Plus 2.0 software. Bark thickness and number of latex vessel rows (LVR) from multiple observations were recorded.

Table 1. **Growth and yield of selected seedlings**

			Traits of selected seedlings					
Family/cross combinations	No. of selections*		Plant height (m)	Branching height (m)	Girth (cm)	No. of leaves/ whorls	No. of whorls	Test tap yield (g/t/t)
PB 330 x RRII 414	9 (149)	Mean	5.37	3.19	11.49	15.44	14.33	0.16
		Min	4.74	-	10.30	10.00	10.00	0.04
		Max	6.00	4.50	13.40	19.00	17.00	0.36
		SD	0.44	1.26	1.00	2.96	2.18	0.10
		CV (%)	7.44	40.80	8.70	19.43	15.35	60.89
PB 330 x RRII 105	3 (37)	Mean	5.00	2.63	11.13	16.67	14.67	0.21
		Min	4.60	2.00	10.40	11.00	13.00	0.05
		Max	5.65	3.15	12.20	21.00	16.00	0.50
		SD	0.57	0.58	0.94	5.13	1.53	0.25
		CV (%)	11.40	22.05	8.45	30.77	10.42	120.00
RRII 429 x PB 330	3 (28)	Mean	5.59	3.81	11.47	17.33	14.67	0.47
		Min	4.62	2.72	10.40	15.00	10.00	0.09
		Max	6.80	4.80	12.80	20.00	18.00	1.08
		SD	1.11	1.04	1.22	2.51	4.16	0.53
		CV (%)	19.85	27.29	10.64	14.48	28.35	113.61
RRII 414 x PB 330	0 (14)				None selected			
RRII 105 x RRII 414	0 (2)				None selected			
RRII 105 x PB 330	0 (4)				None selected			
RRII 105 OP	0 (9)				None selected			
RRII 414 OP	4 (65)	Mean	4.70	2.50	12.00	18.00	14.50	0.31
		Min	2.76	2.00	10.20	16.00	10.00	0.17
		Max	5.83	2.90	14.30	21.00	17.00	0.66
		SD	1.30	0.40	1.70	2.40	3.10	0.23
		CV (%)	27.67	16.00	14.10	13.30	21.40	75.16
RRII 429 OP	16 (157)	Mean	5.77	3.99	13.51	14.31	16.38	0.34
		Min	5.20	2.30	10.80	10.00	13.00	0.04
		Max	6.87	6.00	16.30	19.00	19.00	1.98
		SD	0.47	0.88	2.07	2.41	1.78	0.45
		CV (%)	8.15	22.05	15.32	16.84	10.87	131.76
Total selections	35 (465)#							

* Family - size is shown in parentheses; # Total population; OP- Open pollinated

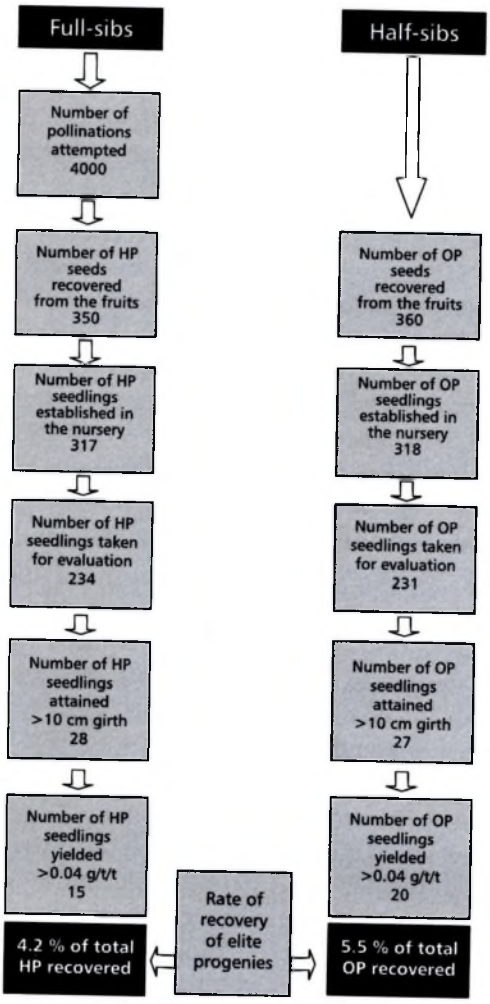

Fig. 2. Rate of recovery of productive seedlings: HP - Hand pollinated; OP - Open pollinated

Statistical analysis

The data on dry rubber yield (g), plant height (m), branching height (m), girth (cm), number of leaves per whorl and number of whorls were analysed statistically. The mean, standard deviation (SD) and coefficient of variation (CV) were worked out according to Panse and Sukhatme (1961).

RESULTS AND DISCUSSION

In the six full-sib and three half-sib families generated, only 55 out of the total of 465 seedlings attained a girth of at least 10 cm at the end of third year, and were used for evaluation of traits such as plant height, branching height, girth, number of whorls, number of leaves per whorl and test tap yield. The population mean for test tap yield of the 55 seedlings was 0.04 g/t/t, on the basis of which 35 seedlings were finally selected for further evaluation. Mean values of these 35 selections for all the traits are presented in Table 1, along with familywise pooled mean, range and CV. Family mean and population mean have been used earlier for preliminary selection of progenies (Mydin *et al.*, 1990), to exercise very moderate selection pressure by culling out the weaklings. In the absence of a true-to-type control at this stage, this is the best way to select elite progenies.

None of the seedlings was selected from the families RRII 414 x PB 330, RRII 105 x RRII 414, RRII 105 x PB 330 and RRII 105 OP since these were all weaklings. Half-sib progenies of RRII 429 and full-sib progenies of PB 330 x RRII 414 constituted the largest family size of 157 and 149 seedlings, respectively.

Among the 149 progenies of PB 330 x RRII 414, nine were finally selected on the basis of test tap yield. The height of these seedlings ranged from 4.74 to 6.00 m, girth ranged from 10.30 to 13.40 cm and test tap yield from 0.04 to 0.36 g/t/t. The highest test tap yield (0.36 g/t/t) was observed in seedling progeny 166, which recorded a height of 6.0 m, girth of 12.0 cm, and high number of leaves per whorl (19.0). The family mean test tap yield was (0.16 g/t/t). Overall variability (CV) for test tap yield within the family was worked out to be 60.89 per cent. In general, the progenies of this combination showed high vigour, test tap yield and other secondary traits. PB 330 was also earlier reported to be one of the best female parents for generating potential recombinants (Chandrasekhar *et al.*, 2004).

Of the 37 progenies in the cross PB 330 x RRII 105, three were selected on the basis of test tap yield, *viz.* seedling numbers 75 (0.50 g/t/t), 90 (0.08 g/t/t) and 116 (0.05 g/t/t). Plant height ranged from 4.60 to 5.65 m and seedlings showed branching at a low height of 2.63 m. Girth of the seedlings ranged from 10.40 to 12.20 cm. The number of leaves per whorl registered a mean of 16.67. Family mean test tap yield was 0.21 g/t/t with high variability (CV 120 %). Seedling number 75 recorded highest test tap yield (0.50 g/t/t). The mean yield of the selected progenies of this combination was also better (0.21 g/t/t) than that of the progenies of PB 330 x RRII 414 (0.16 g/t/t), but girth was almost same.

Of the 28 progenies evaluated in the family RRII 429 x PB 330, three progenies (seedlings 101, 484, 489) recorded a test tap yield of more than 0.04 g/t/t and were selected for further evaluation. These three clones showed vigorous growth (10.40 -12.80 cm). The mean height of the selected plants ranged from 4.62 to 6.80 m with a mean of 5.59. Branching height ranged from 2.72 to 4.80 m. Seedling number 484 registered the highest girth (12.8 cm). Higher yield (1.08 g/t/t) was recorded from the seedling number 489 when compared to the family mean (0.47 g/t/t). High degree of variation (CV 114%) for this trait was observed in the family. Seedling number 484 was the second highest yielder (0.24 g/t/t) in the family. This seedling also exhibited significant vigour in height, number of leaves and number of whorls. The female parent RRII 429 is a hybrid (RRII 105 x RRIC 100), and is a high yielding clone despite its susceptibility to pink disease (Mydin and Mercykutty, 2007).

Fourteen seedlings were generated from cross RRII 414 x PB 330. The family size was comparatively low and, after screening the seedlings, none of the progenies could be selected on the basis of girth. Similarly, RRII 105 x RRII 414 and RRII 105 x PB 330 also generated very few progenies (2 and 4 respectively), all of which had girth less than 10 cm and hence were not considered for the further observations. Nine half-sibs of RRII 105 were obtained, but none was selected due to insufficient growth.

Of the 65 half-sibs of RRII 414, four seedlings were selected based on test tap yield, for further evaluation. The plant height ranged from 2.76 to 5.83 m (CV of 27.67%). The relatively high girth of these seedlings (range 10.20-14.30 cm) indicated hybrid vigour of the plants. The highest girth (14.3 cm) was recorded by the seedling number 139, while the seedling number 601 showed high test tap yield (0.66 g/t/t) compared to the family mean (0.31 g/t/t). Number of leaves per whorl (21) was also high in seedling 139 when compared to the family mean (18).

The half-sib family of RRII 429 comprised 157 progenies, which was one of the largest families in the population. Sixteen progenies were selected based on girth. All these progenies also had test tap yield of more than 0.04 g/t/t ranging from 0.04 to 1.98 g/t/t. Seedling number 405 recorded the highest yield (1.98 g/t/t) and highest girth (16.3 cm). Family mean for test tap yield was lower (0.34 g/t/t) than that of full-sibs of RRII 429 (0.47 g/t/t). Girth ranged from 10.80 to 16.30 cm with a family mean of 13.51 cm.

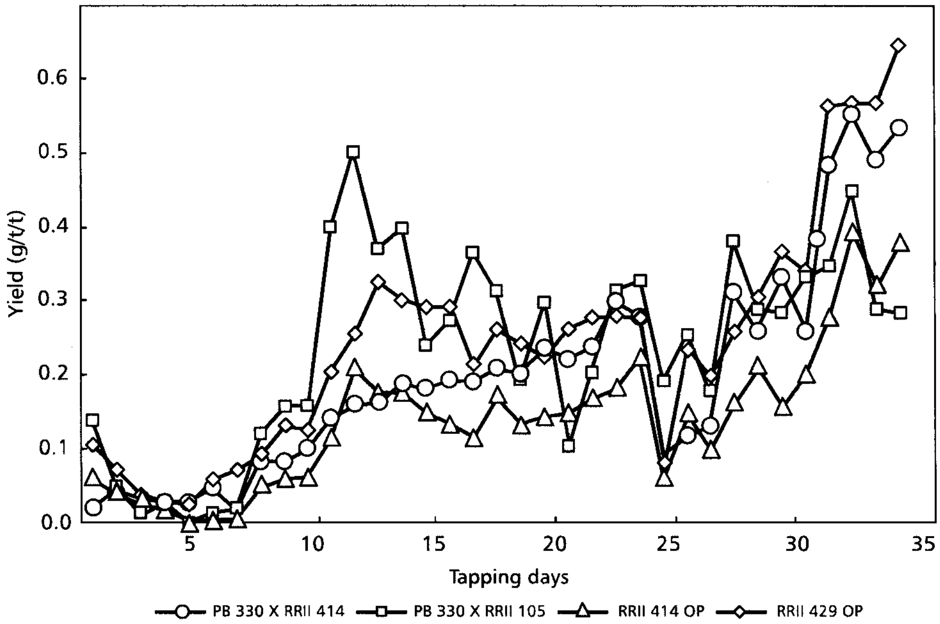

Fig. 3. Familywise rubber yield during test tapping

Seedling number 309 recorded a test tap yield of 0.34 g/t/t and girth of 15 cm. Higher number of leaves per whorl (19.0) was recorded for progeny number 405 when compared to the family mean (14.31). Seedling 405 was one of the tallest among the half-sibs, with a height of 6.87 m, in contrast to family mean (5.77 m). High level of variability for test tap yield (131.76 %) and moderate variation for growth (CV 15.32%) were observed.

The variation in familywise test tap yield over 35 days is shown in Figure 3. PB 330 x RRII 105, PB 330 x RRII 414 and half-sibs of RRII 429 recorded higher values, followed by half-sibs of RRII 414. The results of this study indicate that dry rubber yield obtained from each seedling varied during test tapping. The day-to-day fluctuations in the test tap yield data could be due to variation in meteorological factors like rainfall, minimum and maximum temperature and sunshine duration. Individual seedling variation in dry rubber yield was attributed to the differences in genotypic potential (Tan and Subramaniam, 1976; Varghese *et al.*, 1989; Saraswathyamma and Panikkar, 1989).

Vigour in terms of seedling girth is an important juvenile trait as far as reduction in immaturity period is concerned. Seedlings with high vigour reach tappability early (Simmonds, 1989). High branching was observed in some of the progenies like seedling number 355 (6.0 m). High branching tendency leads to higher timber (clear bole) volume and longer duration of tapping by including exploitation of the high panel, thereby extending its economic life. The high yield potential combined with vigorous nature and clear bole volume of parent clones

(PB 330, RRII 429 and RRII 414) has been established (Mydin and Mercykutty, 2007). The elite features could be due to the parental combination and heterotic vigour. RRII 429 could have transferred the high yielding characters largely to its half-sibs as it was evolved from the cross RRII 105 x RRIC 100, a combination known for its higher combining ability for yield and yield-related characters (Licy *et al.*, 1992).

Test-tapped progenies were categorized into different groups based on their girth (Table 2). Most of the selections (49) belonged to the high girth (10.1- 15 cm) category, whereas only four selections fell into the very high (> 15 cm) category. Only two progenies were identified from moderate growth category (5.1 to 10.0 cm).

The latex production system plays a major role in rubber yield. Therefore, anatomical parameters such as bark thickness and number of LVR at the juvenile stage were recorded. Anatomical parameters of the 35 seedlings are presented in Table 3. Seedling 36 (PB 330 x RRII 414) recorded the highest bark thickness of 6.62 mm. The number of LVR was also high (6.6). In the family of RRII 429 OP, seedling number 435 recorded maximum bark thickness (5.78 mm) and registered an average of six LVR. Bark thickness of the highest test tap yielder (seedling number 405) was 4.62 mm and the mean LVR was 6.8, whereas the second ranked seedling (489) registered bark thickness of 4.56 mm and 8.8 LVR. Significant positive correlations between rubber yield, bark thickness and LVR with mature rubber yield have been reported (Goncalves *et al.*, 2005).

Selected seedlings were ranked based on the test tap yield (Table 3). Seedling number 405 (RRII 429 OP) recorded highest test tap yield (1.98 g/t/t). In cross RRII 429 x PB 330, seedling 489 recorded an yield of 1.08 g/t/t and girth of 10.4 cm. Progeny number 241 registered the lowest yield among the selections (RRII 429 OP family). Based on the baseline girth of 10 cm, 55 seedlings were selected for detailed evaluation. These seedlings account for 11 per cent of the total seedlings raised (465). Selected seedlings were categorized into HP and OP, and among the selected 35 HP seedlings, seedling number 489 recorded the highest test tap yield (1.08 g/t/t) and a girth of 10.4 cm.

Table 2. **Distribution of different girth classes of seedlings**

Girth class (cm)	Growth quality	Total no. of seedlings	No. of selections in the class based on girth
< 2.0	Very Low	105	0
2.1 - 5.0	Low	110	0
5.1 - 10.0	Moderate	197	2
10.1 - 15.0	High	49	49
> 15.0	Very High	4	4
Total	—	465	55*

*Selection based on the girth, 20 seedlings were rejected due to very low test tap yield

Table 3. **Test tap yield, growth and anatomical features of selected seedlings**

Parentage	Seedling No.	Girth (cm)	Mean rubber yield* (g/t/t)	Bark thickness (mm)		No. of LVR	
				Mean	± SD	Mean	± SD
RRII 429 OP	405	16.3	1.98	4.62	±0.36	6.8	±0.83
RRII 429 x PB 330	489	10.4	1.08	4.56	±0.18	8.8	±1.92
RRII 414 OP	601	11.3	0.66	4.78	±0.30	3.8	±0.44
PB 330 x RRII 105	75	12.2	0.50	5.02	±0.62	5.8	±0.83
PB 330 x RRII 414	166	12.0	0.36	5.94	±0.27	5.4	±1.94
RRII 429 OP	350	15.3	0.37	4.64	±0.13	7.4	±0.54
RRII 429 OP	309	15.1	0.34	4.14	±0.15	6.8	±0.83
RRII 429 OP	265	12.3	0.33	4.84	±0.16	7.4	±0.89
RRII 429 OP	337	17. 8	0.32	5.30	±0.29	5.0	±1.00
PB 330 x RRII 414	21	10.8	0.29	4.62	±0.22	8.2	±0.48
RRII 429 OP	436	14.3	0.28	5.16	±0.44	7.0	±0.70
RRII 429 OP	355	11.0	0.26	4.80	±0.15	7.2	±0.83
RRII 429 OP	432	14.8	0.26	4.50	±0.18	5.0	±0.70
RRII 429 X PB330	484	12.8	0.24	4.40	±0.15	6.6	±1.14
RRII 414 OP	139	14.3	0.23	4.48	±0.33	4.6	±0.54
RRII 429 OP	288	13.3	0.23	5.32	±0.16	6.2	±1.09
PB 330 x RRII 414	163	11.6	0.21	4.52	±0.27	6.4	±1.34
RRII 429 OP	296	11.8	0.21	3.78	±0.17	4.8	±0.83
RRII 414 OP	11	12.0	0.19	5.18	±0.39	7.8	±0.83
RRII 429 OP	314	14.8	0.19	5.28	±0.16	6.4	±0.54
RRII 429 OP	435	12.3	0.18	5.78	±0.28	6.0	±0.70
RRII 414 OP	126	10.2	0.17	3.44	±0.41	5.2	±0.83
RRII 429 OP	276	13.3	0.16	3.86	±0.18	6.6	±0.89
PB 330 x RRII 414	40	10.3	0.15	4.40	±0.15	8.2	±1.92
RRII 429 OP	295	11.1	0.14	5.94	±0.22	7.8	±0.83
PB 330 x RRII 414	178	12.2	0.13	4.36	±0.27	5.0	±0.70
PB 330 x RRII 414	36	11.8	0.12	6.62	±1.18	6.6	±1.67
PB 330 x RRII 414	64	11.0	0.11	4.68	±0.41	6.2	±0.83
PB 330 x RRII 414	56	13.4	0.10	4.44	±0.42	8.4	±1.14
PB 330 x RRII 414	35	10.3	0.09	5.50	±0.62	4.6	±0.54
PB 330 x RRII 414	101	11.2	0.09	4.60	±0.29	6.8	±0.83
RRII 429 OP	376	11.8	0.09	5.54	±0.27	7.6	±0.54
PB 330 x RRII 105	90	10.4	0.08	4.06	±0.20	5.4	±1.14
PB 330 x RRII 105	116	10.8	0.05	4.62	±0.19	5.8	±0.83
RRII 429 OP	241	10.8	0.04	4.10	±0.18	9.6	±1.51

*Based on 35 test tapping; LVR- Latex vessel rows

The high growth rate observed in a few seedlings is the indication of possible heterotic vigour in the progenies. Early maturing clones will be preferred in future to reduce the unproductive period of the tree and further enable an early return from the plantations. Very high variability exists in the selected seedlings with respect to test tap yield. A selection index based on yield and growth indicators would be a useful parameter for the selection of superior clones. Efficiency of clonal selection largely depends on the genetic variability and heritability of traits. In rubber, yield and early maturity are considered as important agronomic traits. Broad sense heritability can be used to predict the gain in a clonally-propagated species like rubber. Studies (Simmonds, 1989; Mydin, 1992; Licy *et al.*, 1993) show that rubber yield is highly heritable and is a useful parameter for improving the efficiency of selection (Mydin and Mecykutty, 2007). Tan and Subramaniam (1976), Varghese *et al.* (1989) and Sarswathyamma and Panikkar (1989) have also reported test tap yield of rubber seedlings. The lower values reported here might be due to the genetic potential of the seedlings, spacing adopted (0.4 x 0.4 m) and tapping system (S/2 d2 6d/7). Samsuddin *et al.* (1986) have reported even higher values ranging from 21.8 to 58.9 g/t/10t for two and half-year old seedlings, where the tapping system was S/2 d3 6d/7 and spacing was 1.8 x 1.8 m.

The flow chart (Fig. 2) shows various stages involved in the recovery of seedlings during the course of juvenile screening right from crossing to progeny evaluation. In the case of HP seedling evaluation, 317 seedlings were raised in the nursery, of which 234 seedlings (74%) remained after three years. The number of seedlings which attained a girth more than 10 cm was 28 (9% of initial stand), from which 15 (5%) progenies were selected based on the test tap yield. Very similar results were obtained using half-sibs. 360 open-pollinated seeds were collected from mother trees *viz.* RRII 105, RRII 429 and RRII 414, of which 318 were established in the nursery initially. After three years, 231 seedlings (73%) remained and 27 seedlings (8%) were selected for test tapping on the basis of girth. Finally, the population was reduced to 20 seedlings (6%), considering the test tap yield of at 0.04 g/t/t and above. From harvest of seeds to selection phase, the difference in the net percentage recovery between full-sib and half-sib progenies was found to be meagre (4.2 and 5.5, respectively). Production of HP seedling population needs large resources and expertise, due to the low fruit set and seedling recovery. Considering the cost and effort involved in full sib progeny production, though the recovery of high yielding clones was on a slightly higher side when compared to the half-sib (OP) seedling evaluation, the lesser cost involved for generating the OP seedling population should encourage breeders to choose this approach as the recombination occurs at the expense of nature.

Hevea brasiliensis is known to be a natural cross breeder, producing seeds at a low rate (3 to 5% fruit set) compared to other tree crops. However, certain clones like PB 330 exhibit high fecundity (Chandrasekhar *et al.*, 2004). Use of such clones could improve the recombination frequency and create high variability in the F_1 population leading to effective selection. Therefore, it can be seen that evaluation of half-sib progeny of certain specific parents could improve the efficiency of breeding in rubber.

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