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Juvenile growth, rubber yield and putative tolerance to foliar diseases in interspecific hybrids and half-sibs of Para rubber tree (*Hevea* spp.)

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Abstract

Introgressive hybridization was initiated using different Hevea spp. and wild germplasm accessions. High yielding H. brasiliensis clones but susceptible to foliar diseases (RRII 105, RRII 414 and RRII 430) were hybridized with other Hevea spp. (H. spruceana and H. camargoana) and wild accessions (RO 380 and RO 2871) which are putatively tolerant to important foliar diseases. In addition, half-sibs were collected from a disease tolerant interspecific hybrid clone, Fx 516. The F1 hybrids and half-sibs were evaluated for growth and yield at the age of three years. Regarding family-wise growth in terms of girth, RRII 430 x RO 380 (mean, 28.6 cm) and RRII 430 x Hevea spruceana (mean, 23.0 cm) had more girth. In contrast, RRII 105 x H. camargoana and RRII 430 x H. spruceana had progenies with low girth. With reference to family mean yields, RRII 414 x RO 380 gave a mean yield of 64.8 g/t/10t followed by RRII 430 x RO 380 (mean yield, 15.3 g/t/10t) and RRII 430 x H. camargoana (mean yield, 15.2 g/t/10t). Remaining cross-combinations produced progenies with low yield ranging from 7.1-8.5 g/t/10t. The highest yielding individual of the family RRII 414 x RO 380 gave a yield of 190.8 g/t/10t. Half-sibs of Fx 516 had a mean yield of 17 g/t/10t with maximum individual yield of 56 g/t/10t. Progenies were variably affected by powdery mildew disease caused by Oidium. After confirming segregation of disease resistance trait among progenies through detailed field evaluation in hot spots, the F1 population will be continuously used in back crossing programme to ensure introgression of disease resistance traits.

Key words: disease tolerance, Hevea spp., introgressive hybridization, juvenile growth, juvenile yield, wild germplasm

Introduction

In genetic improvement programme of *Hevea*, the Para rubber tree, rubber yield is generally considered as the prime breeding objective (Simmonds, 1989; Mydin, 2014). Since breeding programmes were mainly yield-based, most of the high-yielding rubber clones showed variable levels of susceptibility to major fungal diseases (Liyanage & Jacob, 1992; Jacob, 1997; Jayasinghe, 1999). Leaf diseases due to *Phytophthora*, *Corynespora* and *Oidium* and stem disease due to Corticium cause up to 40% economic loss in rubber yield (Jacob, 1997; Narayanan & Mydin, 2012; Narayanan & Reju, 2020). Recently, disease epidemics have been reported from rubber plantations in Cameroon, Indonesia, Malaysia, and Sri Lanka (Ngobisa, 2018). South American Leaf Blight (SALB) caused by *Pseudocercospora ulei* (= *Microcyclus ulei*) is a devastating fungal disease which almost wiped out rubber plantations in Brazil epidemic outbreaks (Hora Júnior *et al*., 2014). SALB is a looming threat to the global rubber cultivation (Chee, 1990).

In the event of more such unexpected epidemics in future, availability of clones with enhanced tolerance to diseases will be a critical factor for sustainable natural rubber production. Development and use of disease tolerant clones will considerably reduce dependence on fungicides in disease management thereby lowering costs of rubber production (Fernando, 1969). Hence, an introgressive hybridization programme was initiated for evolving high yielding clones with tolerance to different diseases. Introgression (or "introgressive hybridization") involves integration of genes usually *via* hybridization and repeated backcrossing of alleles from one species into another (Anderson & Hubricht, 1938; Anderson, 1949). In the present breeding programme, the aim was to transfer genes for disease resistance from other *Hevea* species into the high yielding clones belonging to *H. brasiliensis* through hybridization and subsequent back crossing. In the first step of introgressive breeding, hybridization was carried out between high yielding but disease susceptible *Hevea brasiliensis* clones (RRII 105, RRII 414 and RRII 430) as female parents and two *Hevea* spp. (*H. spruceana* and *H. camargoana*) and two wild germplasm accessions from Rondônia (RO 380 and RO 2871) as male parents. In addition to the above, half-sibs were collected from an interspecific hybrid Brazilian clone, Fx 516. In the first phase, the full-sib and half-sib populations recovered through the above breeding programme were assessed for juvenile growth and yield and superior selections were identified. This paper reports the findings from the first phase of the study on variability in early growth, juvenile yield, and disease resistance of the progenies and their selections.

Materials and Method

The parental clones of *H. brasiliensis* and other *Hevea* spp. were located at Central Experimental Station (CES) of Rubber Research Institute of India, situated at Ranni Dt. (Kerala State, India). Details of parents are given in Table 1. Distinctive species variation of the parents was evident from their leaf, flower, fruit and seed characters (Figs. 1 and 2). Identity of the species was further confirmed based on their unique and distinguishing reproductive characters already described (Schultes, 1990). Regarding floral characters, *H. spruceana* produced long-pedicellate flowers which are dark yellowish above and brownish to reddish purple below. The wild accession RO 380 possessed smaller and comparatively short-pedicellate flowers which are also slightly rose-coloured at the base. In contrast, *H. camargoana* produced very unique, strongly elongated, rose-coloured flower buds with inflated globose base. While the flowers of *H. brasiliensis* are creamy-yellowish and extremely pungent-aromatic, the flowers of *H. benthamiana* are comparatively smaller and yellowish. The interspecific hybrid clone Fx 516 possessed yellowish flowers and other characteristic vegetative and reproductive traits which are intermediary between *H. brasiliensis* and *H. benthamiana*. Hybridization was carried out at CES using various cross combinations during months of January-March during 2014 to 2016. Details of cross-combinations are given in Tables 2. Half-sibs were collected during 2015 (91 nos.) from clone Fx 516. The full-sib and half-sib progenies of the different years were evaluated (spacing 60 x 60 cm) in nursery trials in a contiguous area in the experimental farm of Rubber Research Institute of India, Kottayam (Kerala State, India). Progenies were assessed for growth and juvenile yield after 36 months of planting following standard protocols (Mydin & Saraswathyamma, 2005). Briefly, girth (cm) was recorded at 20 cm height and rubber yield was evaluated through test-tapping at same height. Progenies were tapped for latex by the modified Hammaker-Morris-Mann method where thin layer (1-3 mm thick) of bark was excised from outer stem portion following a downward angle using a modified tapping tool. A tin spout was attached to the end of tapping wound or channel to collect the exuding latex in a collection cup. Test-tapping was carried out following S/2.d3.6d/7 system (1/2S, half-spiral incision; d/3, collection every third day). After discarding latex from first five tappings, latex from subsequent ten tappings were accumulated, air-dried and weighed for recording individual yield performance. Since some of the rubber lumps were larger for air-drying, they were dried in a smoke house. Dry rubber yield (g/t/10t) was recorded during the months of November and December which is the period when latex yield is high in *Hevea*.

Table 1. *Details of parental clones and other Hevea spp.*

Parental clone	**Clone origin/distribution of species/Habitat**	**Remarks**
RRII 105 (Tjir 1* x Gl 1#); RRII 414, RRII 430 (RRII 105 x RRIC 100)	Hybrid of *H. brasiliensis* (Willd. ex A. Juss.) Mueller-Argoviensis evolved in India. The species naturally occurs in South of Amazon river (Brazil, Bolivia, Ecuador and Peru). Large trees	High-yielding clone classified under Category-I of India Rubber Board's Clone recommendation
Fx 516** (F 4542 x AVROS 363$)	Hybrid developed in Par΄a (Brazil). F 4542 is a selection of *H. benthamiana* Mueller-Argoviensis originating from upper Rio Negro in South America. Medium to large sized trees	Highly tolerant to leaf diseases caused by *Phytophthora* and *Corynespora*

Parental clone	Clone origin/distribution of species/Habitat	Remarks
RO 2871	Wild germplasm accession. Collected from Rondônia province of Brazil in 1981. Large trees	Highly tolerant to *Powdery mildew*
RO 380[@]	Wild germplasm accession from Rondônia. Medium to large sized trees	Variably tolerant to major diseases
H. camargoana Murca Pires	Naturally occurs in Marajo island of Amazon river delta (Brazil). Small trees of 2-2.5 m ht.	Variable tolerance to SALB
H. spruceana (Benth.) Mueller-Argoviensis	Naturally occurs in banks of Amazon, Rio Negro and lower Madeira (Brazil). Medium sized tree	Variable tolerance to SALB

[*]Primary clone of *H. brasiliensis* from Indonesia; [#]Primary clone of *H. brasiliensis* from Malaysia; ** Fx, Ford Cross; [$]AVROS, Al-gemene Verneiging Rubber planters Oostkust Sumatra, Indonesia; [@]possibly a putative interspecific hybrid of *H. brasiliensis* with *H. pauciflora* (Jayashree *et al*., 1997)

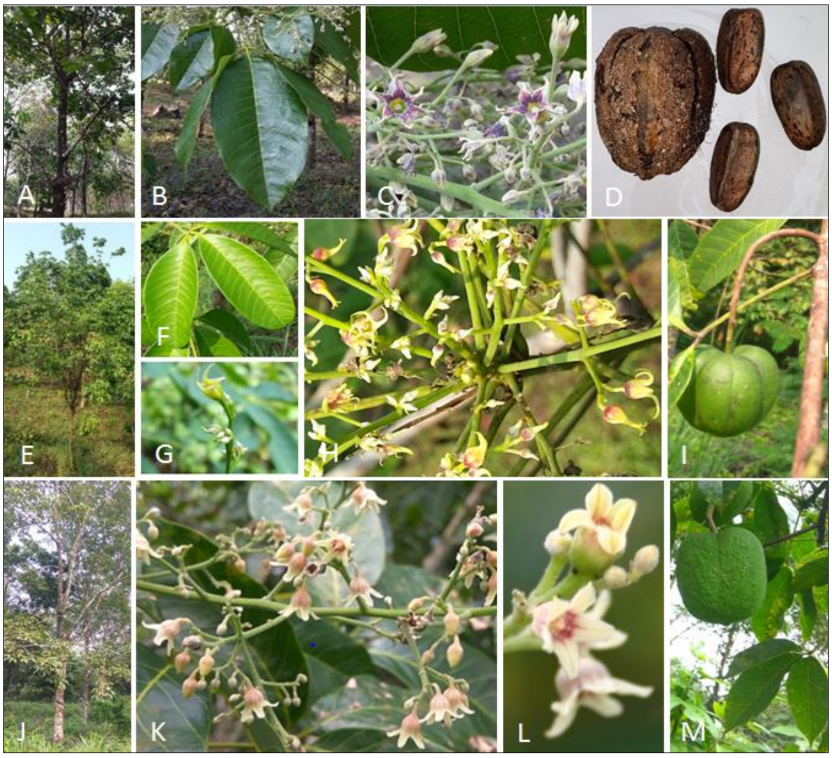

Fig. 1. A-M. Habit, leaf, floral, fruit and seed characteristics of *Hevea* spp. and wild germplasm accession used in introgressive hybridization. A-D. *H. spruceana* E-I. *H. camargoana* J-M. Rondônia accession, RO 380

Fig. 2. A-N. Habit, leaf, floral, fruit and seed characteristics of *Hevea* spp. used in introgressive hybridization. A-E. Clone RRII 430 (*H. brasiliensis*) F-I. Clone F 4542 (*H. benthamiana*) J-N. Clone Fx 516 (*H. benthamiana* x *H. brasiliensis*)

Results and Discussion

Success rate of hybridization and progenies recovered

The success of hybridization over three years ranged from 0.9 per cent to 11.4 per cent (Table 2). The average success rate over the years was estimated as 5.6% which is in agreement to earlier reports in *Hevea* (Simmonds, 1989). Family-wise, RRII 430 x RO 380 had maximum progenies while RRII 414 x RO 380 and RRII 430 x *H. camargoana* had minimum progenies.

Table 2. *Details of year-wise cross-combinations with success rate*

Year	Cross combination	Number of crosses	Hybrids recovered	Success rate (%)
2014	RRII 414 x RO 380	446	6	1.3
	RRII 105 x RO 380	371	19	5.1
	RRII 105 x *Hevea spruceana*	147	6	4.1
2015	RRII 430 x RO 380	1605	95	5.9
	RRII 430 x *Hevea camargoana*	58	6	10.3
	RRII 430 x *Hevea spruceana*	429	49	11.4
2016	RRII 430 x RO 2871	1259	11	0.9
	Total	4315	192	
	Overall success rate			5.6

Growth and yield performance of progenies

The F1 hybrids and half-sib progenies showed considerable variation for juvenile growth and yield (Table 3). Individual girth of full-sibs ranged from 10.0 to 39.0 cm. Family RRII 414 x RO 380 (mean girth, 28.6 cm) followed by RRII 105 x RO 380 (mean girth, 23.0 cm) and RRII 430 x *Hevea spruceana* (mean girth, 22.7 cm) displayed good growth vigour. Family RRII 430 x RO 2871 attained comparatively lesser girth (mean girth, 13.9 cm). In terms of individual growth performance, a progeny of RRII 414 x RO 380 achieved maximum girth (39.0 cm). Overall, progenies from cross-combination involving RO 380 as male parent possessed high girth. This indicated that RO 380 could be a good candidate for improving growth vigor with potential adoption in future breeding of vigorous latex-timber clones. Half-sibs of Fx 516 exhibited a mean girth of 20.1 cm with girth ranging from 5.0 to 40.0 cm.

There was high variation in test-tapping yield of the progenies. While highest test-tapping yield (190.8 g/t/10t) was recorded in a progeny of RRII 414 x RO 380, lowest yield (0.5 g/t/10t) was recorded in a progeny of RRII 105 x RO 380. Regarding family mean yields, RRII 414 x RO 380 gave high yield (mean, 64.8 g/t/10t) followed by RRII 430 x RO 380 (mean, 15.3 g/t/10t) and RRII 430 x *H. camargoana* (mean, 15.2 g/t/10t). Remaining cross-combinations produced progenies with low mean yield (7.1 - 8.5 g/t/10t). One progeny of RRII 430 x RO 380 recorded high yield within that family (54.4 g/t/10t). Half-sibs of Fx 516 gave a mean yield of 16.9 g/t/10t with maximum individual yield of 56.1 g/t/10t.

Among different cross combinations, RRII 430 x RO 380 produced more selections followed by RRII 430 x *Hevea spruceana* (Table 3). Considerable number of half-sib selections could also be recovered from the disease tolerant clone Fx 516.

Table 3. *Family-wise growth and yield performance of F1 hybrids and half-sibs of Fx 516*

Family	Girth (cm)		Yield (g/t/10t)		Number of selections
	Range	Mean	Range	Mean	
Year 2014					
RRII 414 x RO 380	18.5-39.0	28.6	1.0-190.8	64.8	4
RRII 105 x RO 380	10.0-35.0	23.0	0.5-33.5	7.1	7
RRII 105 x *Hevea spruceana*	10.0-24.0	17.0	1.0-16.9	8.5	3
Year 2015					
RRII 430 x RO 380	10.0-30.5	19.4	0.7-54.4	15.3	36
RRII 430 x *Hevea camargoana*	15.5-19.0	17.6	6.8-25.3	15.2	4
RRII 430 x *Hevea spruceana*	14.0-35.5	22.7	1.0-23.9	7.9	19
Year 2016					
RRII 430 x RO 2871	10.0-19.5	13.9	1.0-23.9	7.9	3
Year 2015					
Half-sibs of Fx 516	5.0-40.0	20.1	1.1-56.1	16.9	34
Total number of selections					110

Putative resistance to powdery mildew disease among progenies

Based on disease assessment during first three years of the nursery evaluation, progenies did not exhibit any visible symptoms of leaf diseases caused by *Phytophthora*, *Colletotrichum* and *Corynespora*. However, progenies were variably affected by powdery mildew disease caused by *Oidium* sp. (Table 4). Percent disease incidence (PDI) of powdery mildew ranged from 12% in RRII 105 x RO 380 to 22% in RRII 430 x RO 380 and disease severity (*DI*) ranged from 1.3 in RRII 105 x RO 380 to 1.8 in RRII 430 x *H. camargoana*. Progenies of crosses which involved *H. spruceana* and RO 2871 as male parents had progenies without the disease indicating high disease tolerance of above two parents. Half-sibs of Fx 516 were not affected by any of the above diseases indicating high disease tolerance of this clone.

Table 4. *Variation in incidence of powdery mildew*

Family	*Powdery mildew*	
	PDI (%)	*DI*
RRII 414 x RO 380	18	1.7
RRII 105 x RO 380	12	1.3
RRII 105 x *H. spruceana*	-	-
RRII 430 x RO 380	22	1.4
RRII 430 x *H. camargoana*	16	1.8
RRII 430 x *H. spruceana*	-	-
RRII 430 x RO 2871	-	-
Half-sibs of Fx 516	3	0.7

The wild accession RO 380 used in above hybridization programme is a putative interspecific hybrid of *H. brasiliensis* and *H. pauciflora* (Fig. 1; Jayashree *et al*., 1997). Since *H. pauciflora* was found to be tolerant to SALB, it was used in breeding for tolerance to the disease in Brazil (Chee & Wastie, 1980; Goncalves *et al*., 1980, 1982). Similarly, the female parent of clone Fx 516, F 4542 (*H. benthamiana*), was identified as highly resistant to SALB and was used in breeding for resistance to SALB and other major fungal diseases in Sri Lanka (Senanayake & Wijewantha, 1968; Fernando & Liyanage, 1975, 1980). Similarly Fx 516 showed high tolerance to many fungal diseases including those caused by *Phytophthora* and *Oidium* (Narayanan & Mydin, 2012). Besides, Fx 516 is possibly a potential source of genes for resistance to SALB, as it originated from the breeding programme which was aimed to combine SALB-resistance (of original SALB-resistant seedling selections from the Ford plantations, Fordlandia) with yield of high-yielding clones of Southeast Asia (Subramanian, 1970). Hence, it is hypothesized that some of the progenies might have inherited few resistance genes, which was the primary aim of the present introgressive breeding. While introgression implies transfer of a small sequence the genome from one parental (or species) to another through hybridization and repeated backcrossing, adaptive introgression refers to introgression of genomic regions with potential positive fitness (in the present case, disease resistance) in the recipient species (Bechsgaard *et al*., 2017; Suarez-Gonzalez *et al*. 2018). In order to determine whether the present breeding population is introgressive *per se* or due to adaptive introgression, there is need for more detailed investigations without which results from present study is inconclusive.

Conclusion

In the present study, progenies with vigorous growth, very high juvenile yield and putative resistance to diseases have been recovered through introgressive hybridization. In order to further confirm their disease resistance, the selected population will be screened for major diseases in hot-spots across multiple locations. Once the segregation of disease resistance trait in the progenies could be confirmed through laboratory or field screening, the F1 generation will be used in back crossing to ensure introgression of specific disease resistance traits. Simultaneously, the best selections will also be subjected to clonal evaluation for testing their viability for large-scale commercial planting.

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