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REVIEW

Refinements to *Hevea* rubber breeding

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Abstract *Hevea brasiliensis* (Willd. Ex. A. de. Juss. Müell-Arg.) is the prime source of natural rubber. Domestication of rubber began since 1876 with Wickham collecting 70,000 seeds from Upper Amazon and transported them to Kew Botanic Gardens. Somehow, rubber trees covering millions of hectares are believed to be derived from "22 seedlings" of Wickham's original stock. Improving dry rubber yield is the exclusive and ultimate objective of *Hevea* breeding with consistent yield of 70 to 80 g/tree/tapping. Ultimately, in a small holding, a planter must gain an average yield of around 2200 to 2400 kg/ha from his stand (under optimal conditions), after accommodating tree-to-tree variations due to stock-scion interactions and soil heterogeneity. This is arduous, but achievable. Initial production of high-yielding clones gave 1600 kg/ha against 496 kg/ha of unselected seedlings. Adaptation and yielding potential of clones to specific environments are optimized through localized experimentation. Studies on adaptation of clones to new environments, especially to sub-optimal or marginal areas, are gaining momentum. As this extension happens, demand for new clones is on the rise. Possibilities of using rubber trees for reforestation, carbon sequestration and application of genomics in deriving climate resilient clones may come up in future, which breeders may have to take up with required priority. Five major methodologies followed are (a) primary clones and seed gardens, (b) derivation of recombinants and clone selection, (c) genetic analysis and variability management (d) early selection and estimation of genetic value and (e) application of genomics. Primary clones have immensely contributed in exploiting heterosis and production of new clones. For evaluation of recombinants, families are to be raised in closer spacing (2 or 3 m) and allowed to attain tappable girth for evaluation. While a normal breeding cycle takes 35 to 40 years, through skipping SSCTs and LSCTs, the scheme proposed can derive a clone in 17 years. Recent advances like transcriptome sequencing of bark and EST sequences generated from suppression subtractive hybridization-cDNA libraries could facilitate marker-assisted selection that could very well be used for selecting high-yielding genotypes at juvenile stage. Paternity identification can be done through breeding without breeding (BwB) in half-sibs and poly-cross (open pollinated) seedlings. Transcriptome studies have to come a long way to yield meaningful results to tag vivid genes responsible for QTLs, resistance and other quality traits, especially markers for cold/drought stress. It is opined that instead of subjecting plants for artificial cold/drought conditions, plants continuously exposed to such stress conditions must be used for analyses that can give a comprehensive indication of stress tolerance. The application of genotyping-by-sequencing (GBS) technique to simultaneously discover and delineate single nucleotide polymorphism (SNP) markers is a robust and cost-effective approach for generating a common set of genome-wide SNP data suitable for constructing integrated linkage maps from multiple populations. Studies on mitochondrial and chloroplast DNAs are welcome steps towards understanding ATP efficiency of accessions that need to be augmented further, so that clones with higher ATP efficiency can be used for breeding. Such innovative techniques shall govern breeding *Hevea* rubber in the future, only when breeders and genomic specialists are working in tandem.

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Introduction

Hevea brasiliensis (Willd. Ex. A. de. Juss. Müell-Arg.), the prime source of natural rubber, is a deciduous perennial tree of *Euphorbiaceae*. Rubber has wide utility from erasers to aviation tyres, making it an undeniable, durable commodity with great resilience. The predominant constituent of rubber derived from *Hevea* is *cis*-1,4 polyisoprene $(C_5H_8)_n$, where *n* may range from 150 to 20,00,000 (Pushparajah 2001). Otherwise unknown to the mankind for over 450 years, the scientific advancement through the discovery of vulcanization by Goodyear in 1839 adjudged rubber as a prime industrial commodity. Christopher Columbus gave the first description of rubber in the fifteenth century (Priyadarshan and Clément-Demange 2004).

Domestication of rubber began since 1876 with Wickham collecting 70,000 seeds from Upper Amazon and transported them to Kew Botanic Gardens. But by somehow, rubber trees covering millions of hectares in Southeast Asia are believed to be derived from "22 seedlings" of Wickham's original stock (Imle 1978). After reviewing the history of rubber tree domestication into East Asia, Thomas (2001, 2002) drew the conclusion that the modern clones have invariably originated from the 1911 seedlings sent to Ceylon (Sri lanka) from Kew during 1876. Moreover, if the modern clones are derived from 1911 seedlings, then the argument that they originated from a 'narrow genetic base', as believed even now, needs to be reviewed. It is pertinent to note that of the batch that arrived in Ceylon, 22 seedlings were subsequently forwarded to Singapore. Nine of these made their way north to Peninsular Malaya. Rubber plants established in Malaya were later gifted to Dutch East Indies and these two countries accounted for the bulk of rubber cultivation into the middle of the twentieth century. It is certain that Ceylon did receive many more Kew seedlings than Malaya, but it remained a minor player all this while. One cannot say with absolute certainty that the entire natural rubber industry today is based on 22 Wickham seedlings. Only historians can ascertain the truth.

The first commercial planting with bud-grafted plants was undertaken during 1918 in Sumatra's east coast. Ct3, Ct9 and Ct38 were the first clones identified by P.J.S. Cramer who developed the bud-grafting technique (Dijkman 1951; Tan et al. 1996). Progress in yield improvement in *Hevea* resulted in a gradual increment, from 496 kg/ha in unselected seedlings during 1920s to 1600 kg/ha in best clones during 1950s. The yielding potential was further enhanced to 2500 kg/ha in PB, RRIM (Malaysian), RRII (Indian), RRIC (Sri Lankan), IRCA (Côte d'Ivoire), BPM (Brazilian) RRIV (Vietnamese) and many Chinese clones during 1990s. During these 70 years of rigorous breeding and selection, notable clones like RRIM 501, RRIM 600, RRIM 712, PB 217, PB 235, PB 260, RRII 105, RRIC 100, IRCA 18, IRCA 230, IRCA 331 and BPM 24 were derived (Tan 1987; Simmonds 1989; Clément-Demange et al. 2000; Priyadarshan 2003a). Primary clones selected during the aforesaid period (PB 56, Tjir 1, Pil B84, Pil D65, Gl 1 and PB 86) became parents of improved clones. It must also be acknowledged that primary clones like GT 1 and PR 107 are still widely used although their identification traces back to 1920s.

The genus *Hevea* includes 10 species (Webster and Paardekooper 1989; Wycherley 1992) of which a few are inter-crossable (Clément-Demange et al. 2000). Consequently, the *Hevea* species can be considered as a species complex. An elaborate description of taxonomical and botanical aspects of *Hevea* is out of scope of this article, and for a narration, readers may refer to other sources (Schultes 1977, 1987; Wycherley 1992; Priyadarshan 2003a; Priyadarshan and Gonçalves 2003; Priyadarshan and Clément-Demange 2004). All species are diploids having 2n = 36 chromosomes (x = 9), with the exception of one triploid clone of *Hevea guianensis* (2n = 54) and the existence of one genotype of *Hevea pauciflora* with 18 chromosomes (Baldwin 1947). However, *H. brasiliensis* behaves as amphidiploid (Raemer 1935; Ong 1975; Wycherley 1976; Priyadarshan and Gonçalves 2003). All species were probably evolved in Amazonian forests over 100,000 years (Clément-Demange et al. 2000).

The main objective of rubber breeding is to provide genetically superior clones that yield high, multiplied through bud-grafting. (The word 'budding' meant for multiplication is colloquial).

Breeding objectives

Improving dry rubber yield is the exclusive and ultimate objective of *Hevea* breeding. Growth of the trunk during immature phase, yield per tree over a specific period, stability of the stand per unit area and resistance to stresses (tapping panel dryness, wind damage, varied diseases, low temperature, higher altitude and moisture deficit) are few of the factors that govern productivity levels. Consistently getting 70 to 80 g/tree/tapping is the prime current objective of breeding programmes. Ultimately, in a small holding, a planter must gain an average yield of around 2200 kg/ha from his stand (under optimal growing conditions) after accommodating tree-to-tree variations due to bud-grafting. This is arduous, but achievable. Breeding and selection are exclusively applied to scion and the choice of rootstock is very limited due to open pollination (flowers are monoecious) with almost 79% cross-pollination (Pawsoi et al. 2013). The possibility of cloning whole plant in vitro would allow breeding to be applied to the root system for resistance to root diseases, for better

adaptation to specific soils and for anchorage. This leads to the concept of 'compound tree' with three different genetic components, viz., roots, trunk and canopy, each selected for its own requirements (Simmonds 1985, 1989). Though high-yielding trunks with canopies resistant to SALB had been experimented by the way of crown bud-grafting, it failed commercially. In vitro multiplication of *Hevea*, on the other hand, has not been taken forward due to bottlenecks in the multiplication and acclimatization phases, though much effort in this direction was made during 1980s (Enjalric and Carron 1982; Mendanha et al. 1998). A pulsed-air temporary immersion system for enhancing embryo production, through culturing embryogenic callus under immersion in an autoclavable filtration unit RITA™ (Etienne et al. 1997) also did not revolutionize the micropropogation in *Hevea* rubber.

Adaptation and yielding potential of clones to specific environments are optimized through multi-location trials and localized experimentation. Characterization of the architecture of the trees in connection with wind risk and phenology assessed in relation to susceptibility to leaf diseases (*Colletotrichum gloeosporioides* Penz. Sacc., *Microcyclus ulei*) are vital (Priyadarshan et al. 2001). Studies on adaptation of clones to new environments, especially to sub-optimal or marginal areas, are gaining momentum (Priyadarshan 2003a, b). Almost all rubber growing countries have non-traditional zones, where rubber cultivation is getting actively expanded. In all these aspects, diversification of clones allows large plantations to mitigate risks. Among those clones, the more stable ones are identified for recommendation to small holders, since small holders represent a predominant share. A selection focused on fast-growing trees with effective competence towards weed growth, canopy adapted to multi-cropping, clones adapted to uneven and intensive tapping systems and climatic variations needs to be exercised. As rubber cultivation is getting extended to new areas, demand for new clones is on the rise.

Derivation of clones for timber has emerged as a recent objective. An estimation from RRIM shows that a hectare of rubber plantation can yield around 190 m^3 of rubber wood, and 2.7 million m^3 of *Hevea* wood would be available from Malaysia (Arshad et al. 1995). This estimate can vary with the area and country. Also, there is some interest generated among the scientists to evolve rubber as a factory producing useful chemicals, especially life saving drugs (Yeang et al. 2002). Possibilities of using rubber trees for reforestation, carbon sequestration and application of genomics in deriving climate resilient clones may come up in future, which breeders may have to take up with required priority.

Breeding methodologies

Breeding methodologies and techniques are aimed at maximizing the genetic gain offering the farmers with the best efficiency (formerly seedling varieties, now the bud-grafted clones and, in the future, clones with clonal roots derived in vitro). Those methodologies and techniques, backed by the theory of quantitative genetics, must derive clones adapted to the available growing environments (Priyadarshan, 2003a).

Bud-grafting makes possible the multiplication of the aerial part of the trees identical to mother trees evolved from controlled pollination and preliminary selection. At the end of the selection process, grafting makes possible the organization of mass multiplication of recommended clones in rather short period, with the support of nurseries and bud-wood gardens, in accordance with the needs of the planters and at reasonable cost. Conversely, this possibility induced most of breeders to practice very early selection of individual genotypes, so neglecting a more 'in depth' analysis of the agricultural value of full-sib families derived from controlled pollination.

Elements of breeding methodologies have been synthetically described in literature, with the major contributions of Dijkman (1951), Shepherd (1969), Wycherley (1969), Tan (1987), Simmonds (1989), Clément-Demange et al. (2000), Priyadarshan (2003a) and Priyadarshan and Clément-Demange (2004). Five major methodologies followed are (a) primary clones and seed gardens, (b) derivation of recombinants and clonal selection, (c) genetic analysis and variability management and (d) early selection and estimation of genetic value and (e) application of genomics.

Primary clones and seed gardens

After Whitby (1919) reported considerable variability in seedlings that lead to selection of primary clones like GT 1 and PR 107, the first clones released out of seedlings were those of Cramer's *Cultuurtuin* (Ct3, Ct9, Ct88) selected from 33 seedlings planted in Penang through Java in Indonesia (Dijkman 1951). Mixed planting of these clones gave yield over 1700 kg/ha, against unselected seedlings (496 kg/ha) (Tan et al. 1996). During 1924, Major Gough selected 618 seedlings from a population of about one million in Kajang district of Malaysia, which yielded prominent primary clones like Pil A44, Pil B84, Pil B16, PB 23, PB 25, PB 86, PB 186 and Gl 1 (Tan et al. 1996). By 1930, it was understood that the primary clones had reached a plateau of yield (Tan 1987). While recombination breeding was underway, polyclonal seed gardens with improved clones were set up to have poly-cross seedlings for ensuring supplementary planting materials. Thus, the best seedlings came from Prang Besar Isolated Gardens (PBIG-Gough Gardens GG), and Prang Besar Further Proof Trials (Tan et al. 1996). By 1970, poly-cross seedling areas extended to 7700 ha. Shepherd (1969) explains how positive were the Gough Gardens for rubber selection and how complementary the two 'vegetative' and 'generative' selections have been at that period. Both yield and secondary attributes were given deserving importance while selecting clones (Ho et al. 1979).

Final selection was on the basis of 65 and 35% scores for yield and secondary attributes respectively (Tan et al. 1996). The procedure involved field selection in the estates, nursery selection applied to seedlings, small-scale selection with 16 plants per genotype,and large-scale testing with 128 plants per genotype.

The constitution of polyclonal seed gardens involving clones with high general combining ability (GCA) is expected to provide panmictic conditions and to ensure seedlings with high genetic divergence. Selection for both vigour and high yield can be exercised in such seedlings (Simmonds, 1986). After popularization of clones in 1950s, the potentiality of extending rubber to marginal areas was understood and the concept of producing polyclonal seedlings through constituting seed gardens emerged. This seems to be an appreciable option since results on the yield of polyclonal seedlings from non-traditional areas like Tripura and Konkan (India) are encouraging (Sasikumar et al. 2001; Chandrasekhar et al. 2002). There is a contention that yield and girth variation can be largely accounted for by additive genetic variance (Gilbert et al. 1973; Nga and Subramaniam 1974; Tan 1981). However, phenotypic selection would be effective only within the Wickham population and cannot be extended directly to new combinations such as Wickham × Amazonian crosses. As per general genetic principles, selection based on genotypic values as reflected by GCA would be more reliable and desirable. GCA could be estimated by the evaluation of seedling progenies, in order to select the best parent clones. It is only here that biotechnology can contribute significantly to assess molecular diversity of parents. The number of parents is an important element in determining the constitution of polyclonal seed gardens. An optimum of nine clones with hetero-neighbours has been suggested (Simmonds 1986) (Fig. 1). For this, poly clonal-cum-breeding orchards can be constituted where the branches are induced at an appropriate height to induce flowers during season so that the breeder can conveniently undertake hand pollination (Fig. 2a, b).

The extent of self-pollination within seed gardens may reduce the vigour of the first generation population (SYN_1), since there is no evidence of self-incompatibility. A contrary contention is that such genetic material can be used as

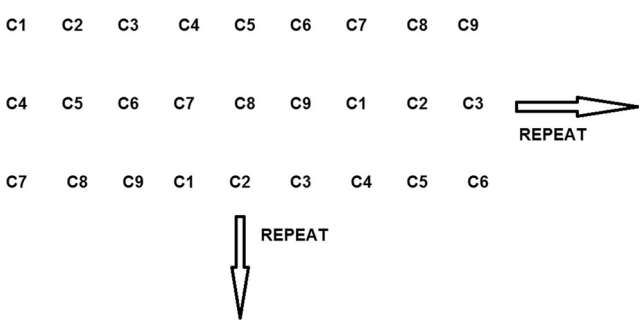


Fig. 1 Breeding orchard layout

breeding material for producing hybrids of high genetic value (Tuy et al. 2015). *Hevea* appears to be obligatory insect-pollinated (Rao 1961) and predominantly cross-fertilized (Simmonds 1982). Maas (1919) first worked on controlled pollination in rubber, and demonstrated that success rate was much higher in cross-pollination than in self-pollination. Paiva et al. (1994) indicated cross-pollination to be 64% through isozyme studies. Since many allozymes are produced at different development stages, it is always reliable to use DNA analysis as a means to spell out the proportion of cross-pollination. Dijkman (1951) argued that a clone like LCB510 (PR107) is practically self-sterile and that over 3000 self-pollinations yielded only one seed, indicating clonal variation towards self-incompatibility. The clone PB5/51 is heterozygous for a recessive yellow gene and open pollination led to an estimation of 16–28% selfing (Simmonds 1989). However, it can be presumed that the seeds produced are cross-pollinated, given the argument that zygotic inability reduces germination due to inbreeding (Simmonds, 1986). Such SYN_1 progenies were considered till recently as class I planting material in Malaysia. A methodological study was conducted on one seed garden established in Côte d'Ivoire with 50 Amazonian parents and overall 300 trees (around 6 grafted trees per parent). Paternity testing was done by using microsatellite markers over samples of seeds collected from identified mother trees within the seed garden (Blanc et al. 2001). The main result was that most of the paternal contribution to the progenies was due to a restricted number of male parents with substantial flowering. Consequently, this seed garden was very far from a panmictic status, and the GCA of parents could not be exploited fully. As biotechnology is now providing new tools that are able to identify male parents of progenies, natural pollination associated with mass selection might gain new interest in the form of what was called 'ortet' selection. The most recent method to exploit primary clones and ortet selections is to undertake breeding without breeding (BwB) (see later for details).

Controlled pollination and clonal selection

A rubber recombination breeding programme is initiated by controlled hand pollination for the production of full-sib families, followed by three selection stages, viz., Seedling Evaluation Trial (SET), Small Scale Clonal Trial (SSCT) and Large Scale Clonal Trial (LSCT). SSCT and LSCT sometimes are designated as Preliminary Proof Clone Trial and Further Proof Clone Trial respectively. The process is cyclical, with the best clones becoming candidates for recombination in the next cycle.

From around 500 kg/ha in primary clones to more than 2500 kg/ ha in the best current clones, rubber breeding has come a long way primarily due to recombination breeding and selection of clones under RRIM and PB series. RRIC 100

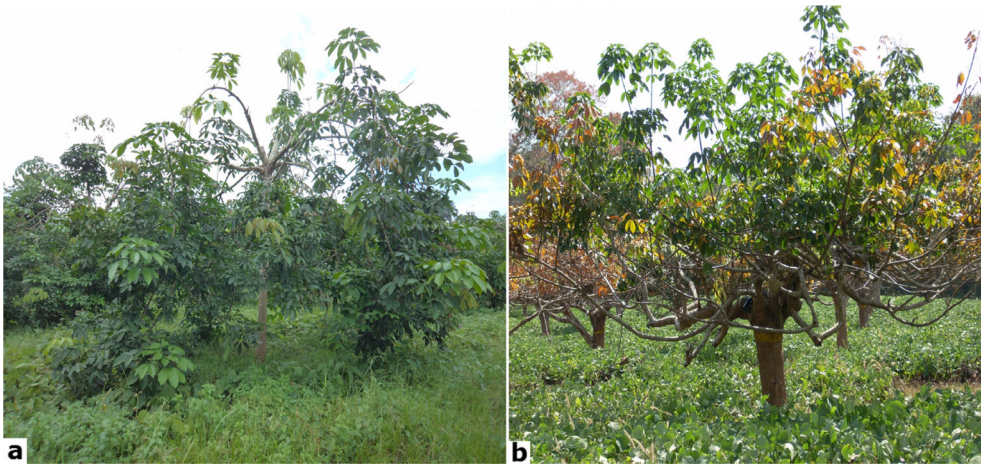


Fig. 2 **a**, **b** View of breeding orchard

series released in Sri Lanka during 1970s is yet another example. Much of the hybridization work in Malaysia (RRIM, Prang Besar), Indonesia, India, Côte d'Ivoire, Brazil, Thailand and Vietnam further strengthened the array of hybrid clones (see Priyadarshan and Clément-Demange 2004 for more details). These clones are known for their adaptability to specific hydrothermal/agro-climatic situations. At least 16 primary clones played major role and can be considered as prime progenitors of many modern clones (see Priyadarshan and Clément-Demange 2004) (Fig. 3). Many valuable recombinants must have been lost during the course of assortative mating of primary clones and of hybrid clones followed by subsequent directional selection for yield under varied geo-climates (Priyadarshan 2003a). The breeding policy has been mainly to cross 'the best with the best' (GAM, generation-wise assortative mating), with strong emphasis on precocious yield in selection within Wickham material (Wycherley 1976). But it could be considered to take more advantage of genetic analysis and of quantitative estimation procedures, especially for the assessment of clonal general combining ability (GCA) for growth and latex yield improvement. Breeding for disease resistance has to take account of specific aspects related with host-pathogen interactions.

The aforesaid breeding scheme is of the past, and lately, *Hevea* breeding has been compelled to undertake drastic reduction in the years consumed for completing a breeding cycle and for derivation of clones. SSCTs and LSCTs are well avoidable. The scheme proposed here is to start with Seedling Evaluation Trial (SETs) comprising hybrid seedlings. The course normally followed is to evaluate through test tapping and select the best yielding seedlings. Presuming that a seedling can never reflect the yielding attribute during adult stage (*see section on early selection and estimation of genetic value*), this stage needs to be suitably modified. The families of hybrid seedlings are to be raised in closer spacing (2 or 3 m) and allowed to attain tappable girth. These seedling trees are to be evaluated with a reference clone (selections from such evaluations are to be further laid under clonal nursery, only to reconfirm the yielding potential). The high-yielding seedling trees are to be made bud-wood points, only to have enough bud-grafted plants for block level commercial evaluations. Once the yielding potential of the clonal derivative is confirmed under block trials, the clone is set for recommendation. When a normal breeding cycle takes 35 to 40 years, through skipping SSCTs and LSCTs, the scheme proposed can complete a breeding cycle in 17 years (Fig.4). It takes 6 years for an LSCT to confirm yielding potential of clones (Chandrasekhar et al. 2007). However, the aforesaid scheme can confirm the yield, if the block trials are laid under multi-locations.

Genetic analysis and variability management

Different expeditions for the collection and transfer of allied species and Amazonian accessions have been organized since 1890. During 1951–1952, 1614 seedlings of five *Hevea* species (*H. brasiliensis*, *H. guianensis*, *H. benthamiana*, *H. spruceana* and *H. pauciflora*) were introduced to Malaysia (Tan 1987). Brookson (1956) has given an account of these introductions. In Sri Lanka, 11 clones of *H. brasiliensis* and *H. benthamiana* and 105 hybrid materials were imported during 1957–59, through triangular collaboration of USDA, Instituto Agronomico do Norte (IAN) (Brazil) and Liberia. Many of these clones were later given to Malaysia (Tan 1987).

During 1981, due to initiative taken by IRRDB, 63,768 seeds, 1413 m of bud wood from 194 high-yielding trees, and 1160 seedlings were collected from Brazilian Amazonia (Tan 1987; Simmonds 1989). This collection was carried out over three states, viz., Acre, Rondonia and Mato Grosso, in 16 different districts and in 60 different locations overall. Of this, 37.5% of the seeds were sent to Malaysia and 12.5% to Côte d'Ivoire. Half of the collections were maintained in Brazil. The accessions from bud-wood collection were brought to Malaysia and Côte d'Ivoire after quarantine against SALB.

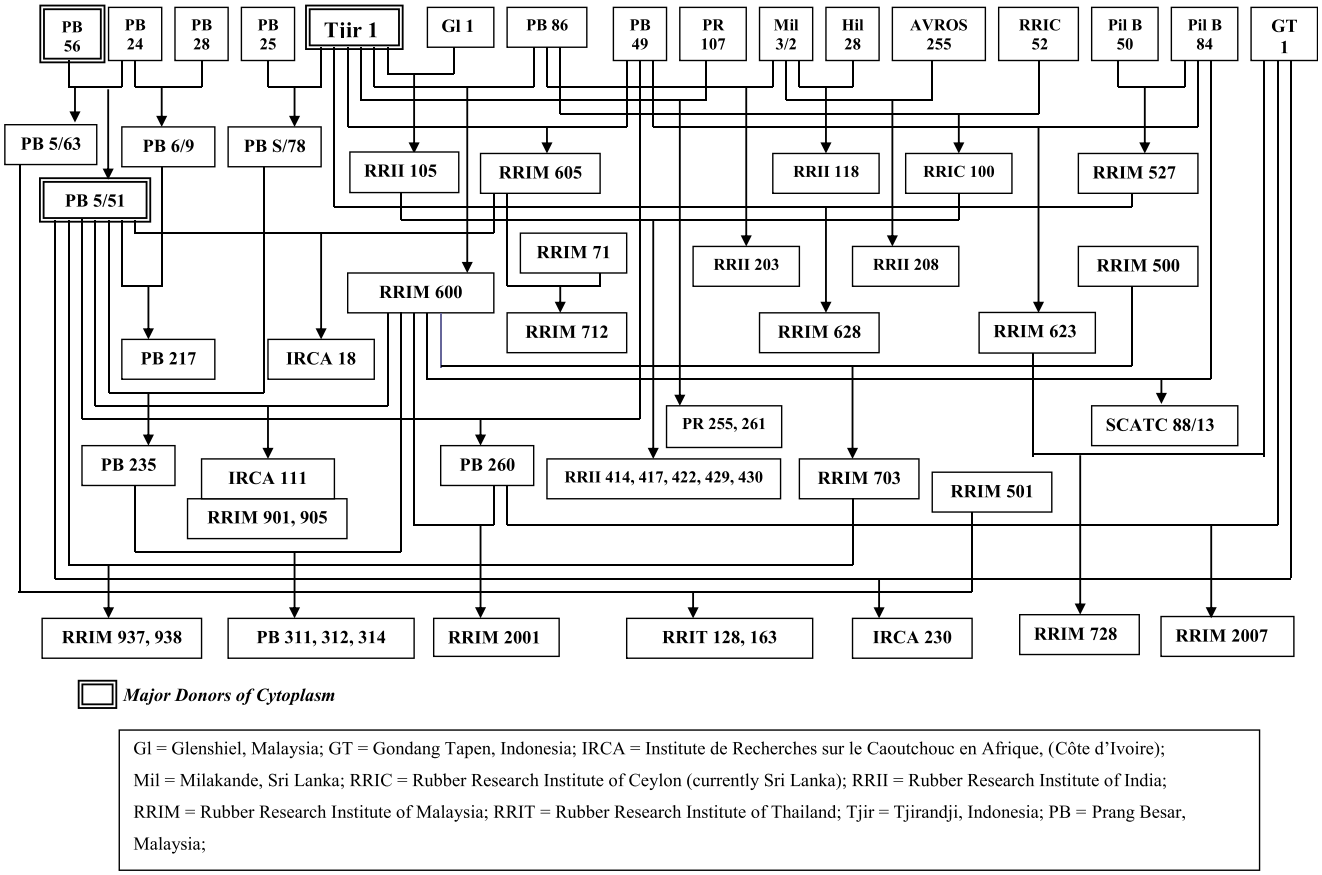
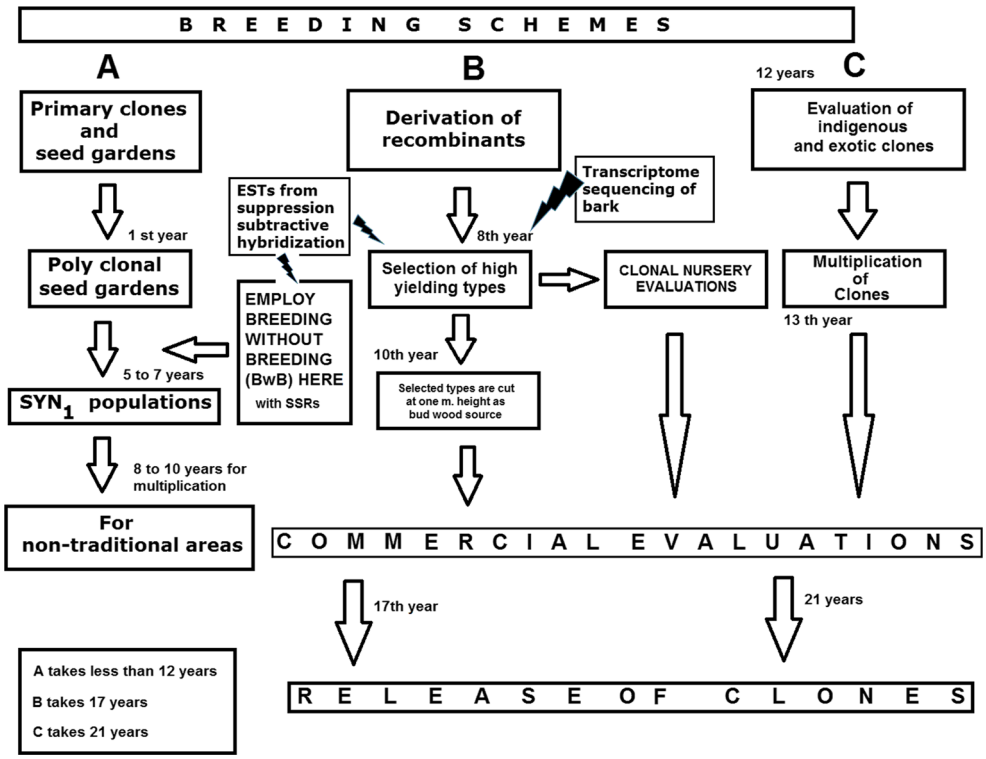


Fig. 3 Parentage of outstanding clones

Fig. 4 Various breeding schemes



After the establishment of two IRRDB Germplasm Centres in Malaysia and in Côte d'Ivoire, other IRRDB member countries were supplied with material according to their request. In 1995, an expedition was launched by RRIM to collect rubber seeds from Brazil. From this collection, about 50,231 seedlings were planted in Malaysia, including allied species (RRIM Annual Report 1997; MRB Annual Report 1999). Many of these accessions were and are being integrated into different breeding programmes in many rubber research institutes worldwide. However, a breakthrough in the form of a clone is yet to emerge.

Few results have been published about the assessment of genetic parameters of Wickham × Amazonian crosses so far. Those crosses appear as the best way to introgress the new germplasm into breeding populations, notably for the introduction of components of resistance to leaf diseases into productive clones. But most of the still unselected Amazonian genotypes from recent collections bear a large part of alleles unfavourable for yield (genetic burden in heterozygous plants). Pre-breeding appears necessary within the Amazonian groups before using Amazonian genitors in crossing with Wickham in an efficient way (Baudouin et al. 1997). Based on field experiments for yield, a working population of 287 accessions was extracted at the IRRDB African Germplasm Centre (Clément-Demange et al. 1998. This was done since a detailed evaluation of whole *Hevea* germplasm is quite impossible for preserving genetic variability. It was proposed to combine the use of field experiments and genetic molecular markers (microsatellites) for extracting a clonal population of reduced size containing maximized genetic variability, according to the concept of 'core collection' (Brown 1989; Hamon et al. 1998). Managing accessions and data on different traits raises the need for the development of *Hevea* germplasm database. One idea would be to raise clonal population of these accessions and then to allow them to cross under natural conditions along with Wickham clones. Seeds borne from these clones (half-sib) can be subjected to breeding without breeding (BwB) mentioned later.

Early selection and estimation of genetic value

Test procedures of new clones in a perennial crop like rubber are lengthy, still extending up to 20–30 years between pollination and yield assessment, distributed over three selection stages. This justifies efforts intended to improve early selection methods in order to optimize and shorten the cycle as much as possible. One component of early selection is made of identification of traits that can be measured at young age and are predictive enough of behaviour at maturity. Another is optimization of available information and of combined management of the different selection stages in order to improve the accuracy of estimation of genetic value. The relationships between yield and parameters including girth, height, bark thickness, latex vessel number, latex vessel and sieve tube diameters, and rubber hydrocarbon in bark and petiole were inconsistent (Summers 1930; Gunnery 1935). This is probably because those simple relationships were not adequate enough to explain the whole-tree functioning. According to Hénon and Nicolas (1989), the thickness of the bark cannot be considered as a reliable attribute to predict yield but the number of latex vessel rings can help to differentiate poor yielders in both Amazonian and Wickham populations. But the variability of the anatomical traits of the bark appeared too narrow for undertaking selections within the Wickham population.

A method was developed in China to predict yield potential through quantifying latex oozing out of leaflets or petiolules (Zhou et al. 1982, 1983). Physiological parameters like plugging index (Ho 1976), bursting index (Dintinger et al. 1981), photosynthetic rates (Samsuddin et al. 1987), and morphological attributes like number of stomata (Senanayake and Samaranayake 1970) were also used, but only latex vessel number showed consistent and significant correlations with yield (Huang et al., 1981). On the other hand, correlations between yield of 2–3-year-old bud-grafted plants and mature yield could be demonstrated (Ho 1976; Tan and Subramaniam 1976). Gonçalves et al. (1998) analysed selection for growth of grafted clones at different ages in order to determine the younger possible age for efficient selection.

The first stage of selection is normally applied to seedlings (Fernando and De Silva, 1971) that are full-sib progenies borne out of hand pollination and are evaluated in 'nursery' (Seedling Evaluation Trial (SET)). Information from this stage is used for selecting new clones to be evaluated as grafted trees in Small Scale Clone Trial (SSCT). Gnagne (1988) investigated different procedures for assessing yield on young seedlings at nursery stage such as the 'Mendes' test, or the 'Hammaker-Morris-Mann' test (Dijkman, 1951), and analysed the relationships (linear correlations) between SET and SSCT in order to determine some efficient selection rates at SET stage (Gnagne et al. 1990). It was found that yield measured at SET stage can be a predictor of mature yield of grafted clones evaluated at SSCT stage (with a moderate prediction ability), but growth before tapping appeared not acceptable as a predictor of growth or yield at SSCT stage. Tan (1987) confirmed those results and recommended to use mainly nursery yield as a yield predictor and to apply a mild selection at the first nursery stage.

The combination of two early stages of selection (seedlings in SET, and grafted clones in SSCT) can be viewed only at a statistical level, based on correlations between the two stages for a set of genotypes. In this framework, Simmonds (1985) presented a theoretical approach in order to assist decision about the selection thresholds which can be applied at the first stage. But the seedlings in SET usually have the property of belonging to different full-sib families. Based on the theory of quantitative genetics, Jayasekera and Hettiarachchi (1988)

underlined the importance of taking into account the 'family' value of the seedlings under selection and the rightness of 'family selection' was theoretically confirmed by Simmonds (1996). Similarly, the case of nursery selection and the relationships between the two stages of early selection in rubber were examined by Gnagne et al. (1998) in order to have the best estimation of the genetic value of genotypes under selection. A combined family × individual selection was proposed in the form of a linear combination of family value and individual values. At nursery stage, with only one seedling tree per genotype, it will almost be impossible to directly assess the environment effect, so limiting the predictive efficiency of this first stage. Nursery selection could even be limited to a selection of the best families only. With this view, early selection does not aim priority to shortening the cycle but to improving the selection efficiency. Ong et al. (1986) proposed a modified selection scheme, based on 'Promotion Plot Clone Trials', combining SSCT and LSCT at the same stage, in order to reduce the time between hand pollination and the release of new clones.

As early selection is based on measurement of latex yield, this process introduces the risk to only select clones with greater early yield. That such clones were characterized by active metabolism and low level of sucrose in laticifers was estimated by measuring inorganic phosphorus and sucrose ratios in the latex (Jacob et al. 1995). This type of latex diagnosis applied to early selection of rubber and a procedure was developed satisfactorily at the level of SSCT at CIRAD and CNRA (Gnagne et al. 1998). The structure and expression of laticifer specific genes need to be studied in detail in order to detect molecular markers having correlation with yield or other traits. Since molecular genetic markers are independent of the environment, using them as predictors can contribute to improve the accuracy of genetic value assessment according to the concept of marker-assisted selection or MAS (Lynch and Walsh 1998). This technique needs refinement towards operational level (Fig. 4). Recently, Li et al. (2012) assembled and analysed de novo transcriptome sequencing data and reported the comprehensive functional characterization of rubber tree bark. This research generated a substantial fraction of rubber tree transcriptome sequences, which are very useful resources for gene annotation and discovery, molecular markers development and microarray development in rubber tree. One hundred and ten potential marker sites were randomly selected to validate the assembly quality and to develop EST-SSR markers. The profile of filtered differentially expressed (FDE) transcripts suggest that jasmonic acid (JA) and linolenic acid (LA)-treated bark samples have a sufficient molecular basis for secondary laticifer differentiation, especially regarding secondary metabolites metabolism (Loh et al. 2016). Further, Nirapathpongporn et al. (2016) could derive a total of 10,321 EST sequences, generated from suppression subtractive hybridization-cDNA libraries of bark and latex. Such EST-SSR markers identified and developed could facilitate marker-assisted selection that can be used for selection of high-yielding genotypes at juvenile stage. Breeders can utilize such innovative techniques during the years to come.

Application of genomics

Application of molecular tools in rubber tree improvement was lagging behind because of limited knowledge of the genome. Initially, hybridization-based RFLP markers, providing co-dominant information were used to characterize *Hevea* germplasm. RFLP technique was proved to be useful for genetic diversity study in wild and cultivated *Hevea* accessions using low copy number nuclear probes (Besse et al. 1994). RFLP analysis of organelle genomes of *Hevea* was also performed for establishing evolutionary relationships as these two genomes could reflect true evolution because of their uniparental inheritance (Luo et al. 1995). Mathew et al. (2005) studied the phylogenetic relationship among three species of rubber, *H. brasiliensis*, *H. benthamiana* and *H. spruceana* employing different molecular marker techniques namely, RAPD, chloroplast DNA, PCR-RFLP and heterologous chloroplast microsatellites. RAPD analysis clearly indicated a high degree of polymorphisms among the three species. For the first time, Low et al. (1996) detected microsatellites in the *Hevea* genome through the database search of some *Hevea* gene sequences. The construction of a microsatellite-enriched library in *H. brasiliensis* was reported by Atan et al. (1996). The detection of high degree of polymorphism indicates a way to introduce desirable variation into *H. brasiliensis* either through introgression or transformation.

Genetic linkage map presents the linear order of markers (genes and other identifiable DNA sequences) in their respective linkage groups depicting the relative chromosomal locations of DNA markers by their patterns of inheritance. The linkage map allows revelation of more and more restricted segments of the genome and undoubtedly enhances our understanding in many areas of plant systematics. A genetic map for *Hevea* spp. was constructed using a population derived from an interspecific cross between PB260 (*H. brasiliensis*) and RO38 an interspecific hybrid clone (*H. brasiliensis* × *H. benthamiana*) following the pseudo-testcross strategy (Lespinasse et al. 2000a). The markers were assembled into 18 linkage groups, thus reflecting the basic chromosome number, and covered a total distance of 2144 cM. A total of 717 loci constituted the synthetic map, including 301 restriction fragment length polymorphisms, 388 amplified fragment length polymorphisms, 18 microsatellites and 10 isoenzymes. Homologous linkage groups between the two parental maps were merged using bridge loci. Average marker density was 1 per 3 cM. Lespinasse et al. (2000b) mapped Quantitative trait loci (QTLs) for resistance to South American leaf blight

(SALB), a disease of the rubber tree caused by the fungus *M. ulei* using the same cross combination (PB 260, a susceptible clone and RO 38, a SALB resistant clone). Eight QTLs for resistance were identified on the RO38 map, whereas only one QTL was detected on the PB260 map.

Past several years have witnessed major advances in our understanding of plant genomes and genomic information through whole genome sequencing. The increasing availability of data from several plant genome-sequencing projects provides a promising direction for investigating genes and their functional and sequence homologues involved in plant development (Avraham et al. 2008). Although genome-sequencing projects lead to the identification of the complete catalogue of genes of an organism, they do not consider the gene expression patterns. Large-scale end sequencing of cDNA library generates ESTs, representing genes expressed in particular tissues or under particular developmental or environmental conditions. They have also been the target of sequencing in many of the projects and found invaluable for genome assembly and annotation. Whole genome sequence information helps in many aspects of plant trait improvement through gene discovery to transgenesis and use of molecular markers in breeding. *Hevea* genome-sequencing project was first launched jointly by Tun Abdul Razak Research Centre (TARRC) of the Malaysian Rubber Board and the newly established Genome Analysis Centre at Norwich, UK, and probably RRIM 600 was sequenced and draft sequence has been made available (Rahman et al. 2013). Two more comprehensive draft genomes (Lau et al 2016 and Tang et al 2016) have been published since then. Quantum of *Hevea* genome-sequencing work is a monumental task as the haploid genome size is enormous as an estimate by Bennett and Leitch (1997) keep it as ~2.15 Gb based on Feulgen microdensitometry. However, discrepancies between these draft genomes made available in the public domain are to be addressed effectively in the days to come (see Perspectives).

Conventional rubber breeding takes more than 25 years to develop a new clone, but genetic transformation is the quick alternate method to introduce desirable genes. The first transformation report in *H. brasiliensis* was published in 1991 (Arokiaraj and Wan 1991) through *Agrobacterium*-mediated transformation. The first transgenic *Hevea* plants, using anther-derived callus as the explant of the clone Gl1 was successfully developed by Arokiaraj et al. (1994) following biolistic transformation method. Subsequently, transgenic plant was developed using *Agrobacterium*-mediated gene transfer of anther-derived calli Arokiaraj et al. 1996, 1998). Inner integument tissue of the immature fruit of the clone PB260 was used as the explant for genetic transformation (Montoro et al. 2003). Transgenic plants of *H. brasiliensis* PB260 were developed through *Agrobacterium*-mediated transformation by Blanc et al. (2005). Earlier transformation events were only with various marker genes. Later, the experiments were focused on transferring various agronomically important genes into *Hevea* with enhanced tolerance to abiotic stresses, production of recombinant proteins etc. Subsequently, attempts were made to increase the SOD enzyme activity by over-expression of the same genes in *Hevea*. Transgenic plants were developed with SOD gene under the control of CaMV 35S and FMV 34S promoters (Jayashree et al. 2003; Sobha et al. 2003a). Biochemical analysis of the transgenic embryogenic callus of *Hevea* with SOD indicated significant increase in the activity of superoxide dismutase, catalase and peroxidase as compared to the control (Sobha et al. 2003a, b). Jayashree et al. (2003) reported successful development and establishment of transgenic rubber plant with SOD gene for their further evaluation. Genetic transformation experiment to over-express *hmgr1* gene, involved in latex biosynthesis, in *Hevea* was performed by Arokiaraj et al. (1995). They could generate transgenic embryos, which failed to produce any transgenic plant. However, they showed enhanced *hmgr* activity in the transformed calli. A significant achievement towards antibiotic marker-free *Hevea* transgenic development avoiding the constraints of GMO regulations was made by Leclercq et al. (2010). They developed an efficient genetic transformation procedure for PB260 using a recombinant green fluorescent protein (GFP). They showed GFP selection is less time consuming in terms of callus subculturing and offered the possibility of producing antibiotic-resistant marker-free transgenic plant. Unfortunately, till date, none of these transformed genotypes has been taken to the planter's field for commercial utilization.

Transcriptome sequencing and development of microarrays have been undertaken recently in *Hevea* rubber (Triwitayakorn et al. 2011; Salgado et al. 2014). Sequencing of transcriptomes of bark that leads to EST-SSR markers is also of prime importance (Li et al. 2012; Cubry et al. 2014) that calls for rigorous research. Such developments are certainly welcome that elevates *Hevea* rubber research on par with other tropical tree crops. However, such innovations must help to find answers to intriguing issues like Tapping Panel Dryness (TPD), stock-scion interactions and yield differences exhibited among trees raised through bud-grafting, molecular markers for selecting of high yielders at juvenile stage, delineation of parents of open pollinated seedlings, production of natural somatic seeds and so on.

Paternity identification and breeding without breeding (BwB) Pre-breeding of the Amazonian genetic groups has been considered to be carried out by recurrent selection based on recombination through seed gardens, natural pollination, and intensive selection. For methodological purposes, one seed garden made up of 50 Amazonian genotypes and the GT 1 clone, planted at CNRA (Côte d'Ivoire), was subjected to the analysis of gene flux and paternity identification with isozymes and microsatellites (Blanc et al. 2001; Lidah, 2005).

Paternity identification with microsatellites was carried out using the Cervus software (Marshall et al. 1998). A high level of confidence was found for paternity identification carried out using eight microsatellite markers. The distribution of the contribution of the different genotypes to pollination was found to be highly unequal, with four genotypes accounting for 40%, 14 genotypes accounting for 80% and 25 genotypes accounting for 95% of the total fertilization of the seed garden. The variation of selfing rate was assessed among the genotypes with an average of 5%, and no selfing was found in GT 1, as expected for a male-sterile clone. The isolation of the seed garden was confirmed since no allele other than those belonging to the parental population was found. Garcia et al. (2011) could adjudge identification of ten clones out of 12 with four microsatellites. The efficiency in paternity identification made possible by microsatellites suggests that a new method of selection may be possible by which the best trees are selected from seedlings resulting from natural pollination and their paternity is identified a posteriori. Transcriptome analysis of bark is yet another upcoming area for marker-assisted selection during juvenile stage that can revolutionize breeding *Hevea* rubber (Li et al. 2012). Also, Mantello et al. (2014) performed RNA sequencing (RNA-seq) of bark on the Illumina GAIIx that validated 78 SNPs in 36 genotypes. This new dataset represents a powerful information source for rubber tree bark genes and will be an important tool for the development of microsatellites and SNP markers for use in future genetic analyses such as genetic linkage mapping, quantitative trait loci identification, investigations of linkage disequilibrium and marker-assisted selection. Characterization and cross-amplification of microsatellites from wild *Hevea* species has augmented the possibility of transferability of these microsatellites to *H. brasiliensis* (Mantello et al. 2012). Many of these Amazonian accessions could be interspecfic hybrids and microsatellite/transcriptome analysis of their seedlings could turn as a worthy exercise to delineate useful variation.

The classical breeding methods used by tree breeders rely on pre-determined mating designs. However, El-Kassaby et al. (2006) has introduced a scheme of breeding without breeding (BwB) that allows the assemblage of full-sib (FS) and half-sib (HS) families from seed orchards' naturally pollinated offspring without conducting any crosses. This scheme circumvents artificial mating, focusing instead on a subset of randomly sampled, maternally known but paternally unknown offspring to delineate their paternal parentage (Lstibůrek et al. 2012). This method calls for highly informative molecular markers (e.g. SSRs) for pedigree reconstruction and to unravel the parentage (El-Kassaby and Lstibůrek 2009). SSRs are now in a development stage in *Hevea* rubber (Garcia et al. 2011; Li et al. 2012; An et al. 2013). But this situation shall improve with time. In *Hevea*, well-organized breeding orchards that permit pollen from only hetero-neighbours can be subjected for raising such FS and HS families. A two dimensional hetero-neighbours' layout as proposed by Simmonds (1986) can be well suitable for such an exercise (step 2 of Fig. 5). This can be used for both breeding full-sib offspring and for collection of polyclonal seeds. For this, large polyclonal orchards are necessary that can produce thousands of seeds every year. Alternately, a clone evaluation garden laid under completely randomized design (CRD) can also be used for collecting half-sib seeds (Fig.5). CRD almost ensures a panmictic population. Such HS families shall be raised in closer spacing (3 m) that can be subjected for yield screening upon attainment of 50 cm girth. A mistake usually being committed by the breeders is to select the early yielding genotypes and reject the ones that are yet to be tapped. This exercise has indirectly culminated in the selection of clones with faster girth increment that reduces gestation period. However, a point to be remembered here is that the left over set may contain high-yielding recombinants, which may attain maturity a little late. While exercising clone selection, both early yielding and late yielding clones are a necessity, to present before the planters an array of clones with vivid attributes to choose from (Priyadarshan 2017). If SSRs that are linked to QTLs for high yield can be used, then the exercise can minimize screening process to a great extent. In this way, this method allows the capture of 75–85% of the genetic response to selection attained through conventional programmes without the need to do any controlled pollination and simplified or possibly no experimental field testing: both considered to be the most resource-demanding activities in breeding programmes. The selections borne out of these HS evaluations can be further confirmed through clonal nursery trials following line RBD with a reference clone. Simultaneously, these selections can be propagated and given for trials in government owned areas with a reference clone. In this way, a quick derivation of clone can be achieved. For all this, DNA profile of all available clones is a prerequisite to ascertain the parentage.

Genetic mapping A cross between two heterozygous parents can yield information about up to four alleles in *Hevea*, which are segregated further. The first comprehensive genetic linkage map of *H. brasiliensis* has been built with RFLPs, AFLPs, microsatellites and isozymes (Lespinasse et al. 2000a). The parents used were PB 260 (PB 5/51 × PB 49) and RO38 (F4542 × AVROS 363). F4542 is a clone of the species *H. benthamiana*. The F1 synthetic map of 717 markers was distributed in 18 linkage groups corresponding to the 18 chromosomes. These comprised 301 RFLP, 388 AFLP, 18 microsatellite and ten isozyme markers. Through the same cross, (Lespinasse et al. 2000b) developed eight QTLs, with one predominant on linkage group g13, that was identified for resistance to SALB in the RO38 map. The F1 consensus map confirmed the results obtained in the parental maps. It

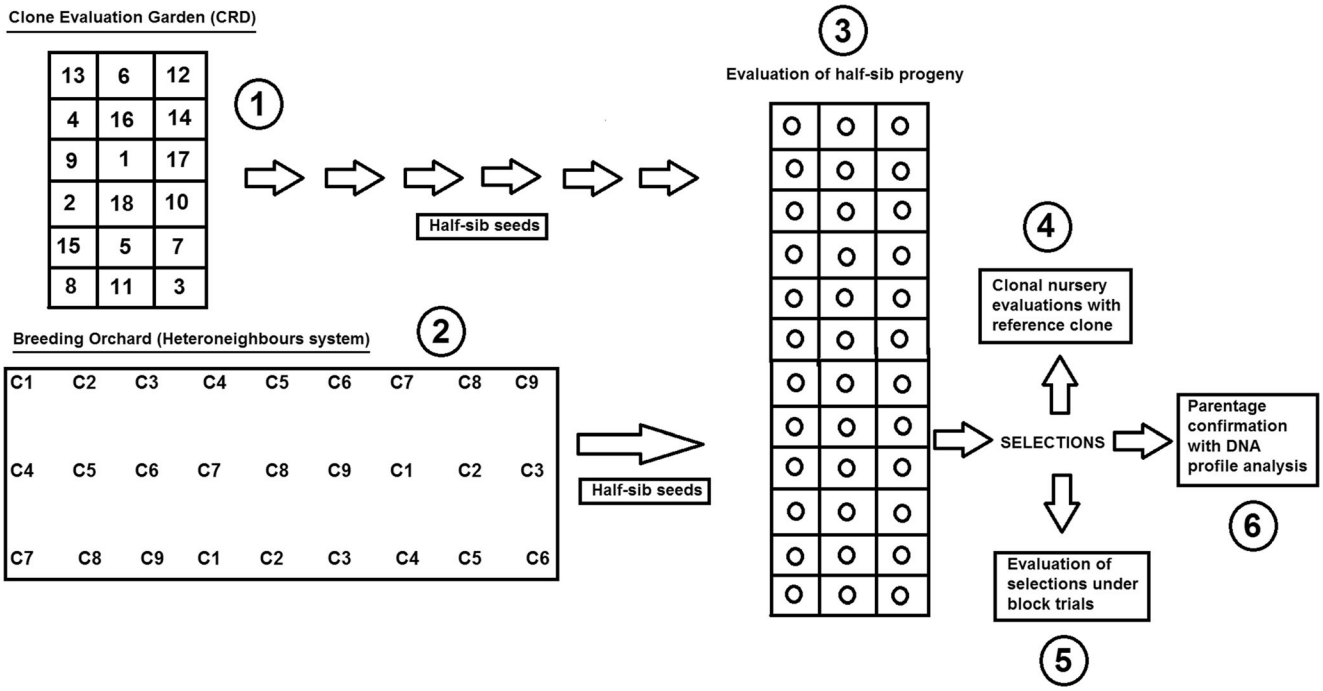


Fig. 5 Half-sib analysis

was further rationalized that the resistance alleles of RO38 (with eight QTLs) were inherited from a wild grandparent (*H. benthamiana*) and no favourable alleles came from AVROS 363, the Wickham parent. Field evaluation against the pool of *Microcyclus* strains available in French Guiana was carried out under real infestation conditions, and it confirmed the presence of the predominant QTL in g13 previously found under controlled infestation (Le Guen et al. 2003). Resistance against virulent and aggressive strains are to be ascertained through excavating more linkage groups.

Páez et al. (2015) used a secondary clone FX 3864 that was resistant to *M. ulei* isolate GCL012. They identified 86 differentially expressed genes associated with the defence response of FX 3864 to GCL012. The defence response of FX 3864 against the GCL012 isolate was associated with the antagonistic salicylic acid (SA), ethylene (ET) and jasmonic acid (JA) pathways. These responses are characteristic of plant resistance to biotrophic pathogens. Pootakham et al. (2015) further constructed a high-density integrated genetic linkage map by applying a genotyping-by-sequencing (GBS) technique to simultaneously discover and genotype single nucleotide polymorphism (SNP) markers in two rubber tree populations. A total of 21,353 single nucleotide substitutions were identified, 55% of which represented transition events. GBS-based genetic maps of populations P and C comprised 1704 and 1719 markers and encompassed 2041 and 1874 cM, respectively. An integrated linkage map consisted of 2321 markers and spanned the cumulative length of 2052 cM. The composite map showed a substantial improvement in marker density, with one SNP marker in every 0.89 cM. This is the most saturated genetic map in rubber tree to date. A draft genome sequence of *Hevea* was brought out by Rahman et al. (2013). The assembly spans ~1.1 Gb of the estimated 2.15 Gb haploid genome. Overall, ~78% of the genome was identified as repetitive DNA. Gene prediction shows 68,955 gene models, of which 12.7% are unique to *Hevea*. Most of the key genes associated with rubber biosynthesis, rubber wood formation, disease resistance and allergenicity have been identified. All these studies on genetic mapping and genome sequencing are to be furthered to have more insights into the genome.

Recent advances in next generation sequencing technologies allow for the generation of high-density linkage maps (Pootakham et al. 2015). The application of genotyping-by-sequencing (GBS) technique to simultaneously discover and genotype single nucleotide polymorphism (SNP) markers could gather 21,353 single nucleotide substitutions, 55% of which represented transition events. A composite map of two populations showed a substantial improvement in marker density, with one SNP marker in every 0.89 cM. This seems to be is the most saturated genetic map in rubber tree to date. Pootakham et al. (2015) further argued that GBS is a robust and cost-effective approach for generating a common set of genome-wide SNP data suitable for constructing integrated linkage maps from multiple populations (see section on 'Perspectives').

Genomics for changed climates Plant species are the best experimental material to study changes in climate since they express their reactions to such changes through significant

manifestations. There will be perceptible indications in terms of morphological, physiological and genomic expressions towards such changed climates. As rubber spreads to new areas where drought and cold are conspicuous stress factors, employing genomics tools can largely help in advancing knowledge about the ability of rubber to adjust such environments. The work of Silva et al. (2014) stems promise in this direction. They studied leaf, panel and latex ESTs under cold-stressed conditions. For panel and latex libraries, samples were collected from 16-year-old tree clones of PB 217, PR 255, GT 1, PB 235, RRIM 701 and IAN 873, and leaves of the same clones were collected from the rubber tree germplasm. PB 217, PR 255, GT 1 and IAN 873 were subjected to a 24-h cold treatment in a growth chamber and maintained at 8^0 C with a 12-h photoperiod. This treatment was performed to promote the expression of genes involved in cold response and for the development of molecular markers related to this stress condition. A total of 8263 reads were assembled, generating 5025 unigenes that were analysed, 912 expressed sequence tags (ESTs) represented new transcripts, and two sequences were highly up-regulated by cold stress. These unigenes were scanned for microsatellite (SSR) regions and single nucleotide polymorphisms (SNPs). In total, 169 novel EST-SSR markers were developed of which, 138 loci were polymorphic. Nearly 98% of this presented transferability to six other *Hevea* species. Locus duplication was observed in *H. brasiliensis* and other species. Additionally, 43 SNP markers in 13 sequences that showed similarity to proteins involved in stress response, latex biosynthesis and developmental processes were characterized. cDNA libraries are a rich source of SSR and SNP markers and enable the identification of new transcripts. Transcriptome analysis is one of the main approaches for identifying the complete set of active genes in a cell or tissue for a specific developmental stage or physiological condition. Salgado et al. 2014 reported the sequencing, assembling, annotation and screening for molecular markers from a pool of *H. brasiliensis* tissues. A total of 17,166 contigs were successfully annotated. Then, 2191 single nucleotide variation (SNV) and 1.397 simple sequence repeat (SSR) loci were discriminated from the sequences. This is the first study of the *Hevea* transcriptome, covering a wide range of tissues and organs, leading to the production of the first developed SNP markers. Transcriptome studies have to come a long way to yield meaningful results to tag such gene expression studies to vivid genes responsible for QTLs, resistance and other quality traits, especially markers for cold/drought stress. It is opined that instead of subjecting plants for artificial cold/drought conditions, plants continuously exposed to such stress conditions must be used for analyses that can give a comprehensive indication of the stress tolerance.

Mitochondrial and chloroplast DNAs As explained earlier, nuclear diversity and polymorphism has been studied in some detail in *Hevea* rubber. Mitochondrial DNA (mtDNA) was also analysed over 395 genotypes with heterologous probes from broad bean (Luo et al. 1995; Luo and Boutry 1995). A high mt DNA polymorphism was found in Amazonian accessions; by contrast, the diversity of mt DNA in the Wickham population is almost nil as only GT 1, a male-sterile clone, exhibited a different type from that of 49 other Wickham clones analysed. Sequencing of a highly polymorphic mt DNA fragment from 23 genotypes showed real potential for phylogenetic analysis in *Hevea* (Luo and Boutry 1995). Shearman et al. (2014) constructed the mitochondrial genome of a cytoplasmic male-sterile variety, BPM 24, and annotated the genome with the aid of Illumina RNA-seq data and performed comparative analysis. They then compared the sequence of BPM 24 to the contigs of the published rubber tree, variety RRIM 600, and identified a rearrangement that is unique to BPM 24 resulting in a novel transcript containing a portion of atp9. Cytoplasmic donors for most of the improved clones are either PB56 or Tjir1. Obviously, this is the reason for the mt DNA profile showing only two clusters (Priyadarshan and Gonçalves 2003). A possible explanation for greater polymorphism in mt DNA of wild accessions is that many might have been evolved through interspecific hybridization. Chloroplast (cpDNA) analysis was carried out over 217 accessions representing 126 mitochondrial genotypes; only two cp DNA RFLP profiles were found, thereby showing a much lower polymorphism and indicating the high level of conservation of this chloroplast genome (Luo 1995). Tangphatsornruang et al. (2011) reported the complete chloroplast genome sequence of rubber tree as being 161,191 bp in length including a pair of inverted repeats of 26,810 bp separated by a small single copy region of 18,362 bp and a large single copy region of 89,209 bp. Such studies are welcome step towards understanding ATP efficiency of accessions that need to be augmented further, so that clones with higher ATP efficiency can be used for breeding programmes.

Perspectives

Hevea breeding needs to be made more precise to significantly reduce the time taken to derive a clone. There are two strategies for this: (a) to cut short the breeding steps being followed by conventional means and (b) to inculcate genomics into breeding programmes specially to identify high-yielding genotypes in half-sibs, full-sibs and poly-cross seedlings during juvenile stage that can minimize both space and time. Both these steps are succinctly explained in this review. As a tree and a perennial crop, rubber exhibits new aspects and differences between clones over time and location. Of this, location effects are more important and conspicuous that needs to be handled effectively by the breeders. Confirmation through multi-location trials is the only option. The tapping schedule to be followed and the stimulation to be applied are two major

issues that call for multidisciplinary approaches. Resistance to diseases and addressing it through QTLs/markers is much more important than ever before, because as rubber spreads to new environments, the chances of exposing it to new pathogens are much more, in addition to the threat posed by SALB, *Phytophthora*, *Corynespora* and *Oidium* in the traditional areas. QTLs identified for resistance to SALB are to be transformed to high-yielding clones. The recently developed genotyping-by-sequencing (GBS) technology for high-density mapping is a welcome sign for genome characterization. Gene constructs for apomixis are expected to be available in the public domain sooner than later. Tailored genes for apomixis are to be introduced into popular clones through genetic transformation. True somatic seeds borne out of apomictic genes are a boon to rubber cultivation that ensures uniform yield. The technologies enumerated under genomics are to be effectively utilized by the breeder to take a clear road map with milestones. For instance, the characterization and cross-amplification of microsatellites from wild *Hevea* species and their transferability to *H. brasiliensis* is a notable achievement that can be translated into practical utility. The essential point to be noted here is that every investigation involving genomics has the prospective narration 'studies presented here can invigorate variation into existing *Hevea* germplasm that can nurture a great boon to future breeding programmes'. In fact, after such studies, concerted efforts by both breeder and the genomics specialist to demonstrate these abilities in terms of higher yield are always meagre. If this co-ordination is made possible, then, *Hevea* breeding can achieve explosive advancements. Here, the much acclaimed political will power is a prerequisite for addressing the so called 'environmental issues' that are anticipated while implementing such scientific advancements.

Derivation of draft genome is the 'trendy research' of late. Rahman et al. (2013) do not offer the newest *Hevea* genome, and the honour goes to the RIKEN team of Japan, working in collaboration with Lau et al. (2016) at Universiti Sains Malaysia for a more comprehensive genome analysis. However, the most comprehensive genome to date comes from Chinese Academy of Tropical Agricultural Sciences (CATAS) group (Tang et al. 2016). As *Hevea* genome has now been published three times, yet not everyone comes up with the same findings. CATAS declares the genome size as 1.46 Gb, whereas USM and RIKEN/USM both give the figure of 2.15 Gb. Despite this discrepancy, it is noteworthy that the CATAS assembly captures practically all the USM sequences contained in a purportedly larger draft genome. It appears that CATAS has done a better job of fitting contiguous sequences into a smaller number of scaffolds. Thus, while USM has a scaffold N50 of only 3 kb, CATAS weighs in with a massive scaffold N50 of 1280 kb. The RIKEN scaffold N50 comes in at 67 kb. Despite three published *Hevea* genomes now in the public domain, the discrepancies between the reports keep the last word waiting.

With the advent of the genome sequence, many functions of erstwhile genetic maps are superseded. Hence, the implications of the claims made by Pootakham et al. (2015) that the GBS-based genetic map as the most efficient and cost-effective needs to be viewed cautiously. Having said that, this does not necessarily mean that older genetic maps have immediately lost their relevance. After all, the complete genome in its early stage is a vast wilderness of sequences with limited landmarks to point the way. The transcribed and non-transcribed sequences are to be sorted out. While its potential in rubber tree breeding is tremendous, useful application of the genome will take time to materialize. On the other hand, purpose-constructed genetic maps, especially those with markers linked to known agronomic traits (QTLs), could validate their usefulness in the shorter term.

Most DNA markers used in breeding are linked to specific genes—basically one gene to one marker. This is problematic in traditional rubber tree improvement where, the yield output from the rubber tree is dependent on the volume of latex exuded from laticifers upon tapping. Duration of latex flow is the decisive factor which in turn depends on a large extent to the rate the laticifers that are plugged after tapping. Another aspect that also contributes to the yield is the dry rubber content (drc). Whether it is drc or plugging rate, it is pertinent that both variables are controlled by multiple genes. What makes a tree high yielding is therefore very complex, defying explanations by single genes. Hence, it would be no easy task to improve yield productivity by using individual gene markers.

If the aim is to select for drought or cold resistance, there is no short-cut substitute to actually subjecting the plants to the targeted conditions and then observe how they thrive in the face of adversity. Transcriptome analysis highlighted can be useful but not adequate enough to tell the whole story. When a gene responds to a stress stimulus (e.g. cold), it is not known if the plant has the means of overcoming the stress concerned. Hence, high expression of a gene in reaction to a stress may simply be a distress signal for which the tree may or may not have resistance. As mentioned earlier, instead of subjecting plants for artificial cold/drought conditions, plants continuously exposed to such stress conditions can give a comprehensive indication of stress tolerance.

Setting aside gene selection, one approach to DNA-breeding that is due for evaluation is gene insertion. Subsequent to successful transformation, any number of transgenic clones can be replicated by conventional bud-grafting to form an experimental population. The real test is on how well the transgenic plant actually resists tapping panel dryness by increased SOD neutralizing 'toxic oxygen' in the laticifer system. High SOD-expressing transgenic plants were produced in 2003, and 13 years is sufficient time for transgenic and

control trees of the same clone to be compared head-to-head in the field. Unfortunately, till date, none of these transformed genotypes have been taken to the planter's field for commercial evaluation citing *environmental issues* as the real bottle neck.

Rubber-producing countries have developed their own procedures for the recommendation and dissemination of new clones. Within each producing country, direct contacts are established for clone testing and for communication about clones between breeders/extension services and planters. Even if the selection process officially ends with full-sibs, half-sibs, poly-cross seedlings or on-farm trials, studies on clones continue long after recommendations, in relation to wind damage, tapping panel dryness, biotic or abiotic stresses and their adaptation to specific tapping systems, farming systems and new areas. It is at this juncture the production of new clones of *Hevea* rubber gains prominence. The identification/derivation of new clones must be a continuous process that amply employs methods of both conventional breeding and genomics in pragmatic ways. Recommendation of a new clone must be accompanied with advice on tapping schedule, stimulation, cultural practices and so on. And for this, concurrent investigations on these aspects are a must while deriving new clones. Bilateral exchange of clones between countries is welcome to enrich the genetic resource, but those clones bred under a given environment shall be a better candidate clone. The aforesaid aspects are worthy to make *Hevea* rubber commercially more lucrative, that helps small holders all over the world.

Data archiving statement This is a review, and hence, no accession numbers are involved.

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