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Chapter 4

Genetic improvement

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1. Introduction
2. Objectives of breeding
3. Conventional breeding methods
   3.1 Introduction of exotic clones
   3.2 Ortet selection
   3.3 Hybridization and selection
      3.3.1 Technique of hybridization
      3.3.2 Clone evaluation
4. Special techniques in breeding
   4.1 Mutation and polyploidy breeding
   4.2 *In vitro* culture technique
5. Constraints in breeding
   5.1 Nature of flowering and fruit set
   5.2 Breeding and selection cycle
6. Early evaluation methods
7. Recent approaches
   References

1. INTRODUCTION

The original genetic material of the Para rubber tree, *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. referred to as the 'Wickham gene pool' introduced to South East Asia by Sir Henry Wickham in 1876 was reported to have an average yield of 200 to 300 kg per ha per year (Panikkar *et al.*, 1980). Now there are clones with a production potential of 3500 kg per ha per year (Licy *et al.*, 1997). This substantial improvement in productivity has been achieved mainly through genetic improvement of the species.

Genetic improvement of *Hevea* is very elaborate and time consuming as in many other perennial species. The major limitations are the very narrow genetic base, non-synchronous flowering, low fruit set, long gestation period, heterozygous nature, insufficient availability of land for field experimentation and absence of fully reliable early selection parameters. In spite of these, efforts on genetic improvement so far have paid rich dividends and a good number of high yielding clones is available for commercial planting.

2. OBJECTIVES OF BREEDING

The ultimate objective of *Hevea* breeding is to synthesize ideal clones with high production potential combined with desirable secondary attributes. High initial vigour, smooth and thick bark with a good latex vessel system, good bark renewal, high growth rate after opening and tolerance to major diseases and wind are considered to be good secondary characters (Annamma *et al.*, 1990; Varghese *et al.*, 1992). In addition, good response to stimulation and low incidence of tapping panel dryness (brown bast) are also important selection criteria. When discounted cash flow is considered, clones with early attainment of tappable girth and high initial yields are preferred to clones with higher yields in later phases of exploitation (Lim *et al.*, 1973).

Specific objectives, however, may vary depending on agroclimatic and socio-economic requirements. In countries where labour is relatively cheap and/or smallholder sector is predominant, clones capable of withstanding high tapping intensities are required. On the other hand, clones responsive to low intensity tapping are needed in countries with labour shortage. Similarly, for situations where rubber cultivation is extended to non-traditional areas, planting materials suitable for withstanding stress conditions like drought, cold and high elevation need to be developed. Rubber is now becoming predominantly a small growers' crop. In India, 86 per cent of the total area under rubber is occupied by the small growers who in turn contribute 86 per cent of the total production. Hence it is essential that breeding objectives should cater to the requirements of the small grower. Genotypes suitable for high density planting and poor soil fertility could also be aimed at. Higher timber output is another desirable attribute.

In *Hevea* cultivation, both seedlings and clones are used as planting materials, the latter being popular and very widely adopted. The former are resultant of generative reproduction (pollination) and the latter of vegetative methods (budding/tissue culture). Being predominantly cross-pollinated (mostly by midges and thrips), seedlings are genetically heterogenous. On the other hand, a clone developed from a single mother plant by vegetative multiplication is genetically homogenous and results in uniformity within a plantation.

Seedlings are either monoclonal or polyclonal depending on whether they originate from a plantation of a single clone or from one with a number of clones respectively. In the strict sense of the term, the latter includes seeds from specially laid out isolated polyclonal seed gardens. Monoclonal seedlings, as a result of selfing or inbreeding show inbreeding depression (a decrease in fitness and vigour) and are not recommended as planting materials. Polyclonal seedlings resultant of cross-pollination on the other hand express heterosis or hybrid vigour (superior performance over the parents). 'Polycross' or 'synthetic' seedling populations from polyclonal seed gardens of good clones have been successfully used as planting materials. Allogamy (cross-pollination) coupled with seed propagation increases variation through genetic recombination in the seedling population. Thus seedling plantations have special agricultural merits in maintaining the genetic variability and adaptability of the population (Mydin, 1990; Varghese, 1992).

Clones are either primary clones or hybrid clones, the former developed through ortet or mother tree selection and the latter through controlled hybridization and selection programmes.

3. CONVENTIONAL BREEDING METHODS

The conventional methods of genetic improvement are introduction, selection and hybridization.

3.1 Introduction of exotic clones

Clones introduced from other rubber producing countries form the base material for genetic improvement programmes in India. So far a total of 127 domesticated clones has been introduced from different countries. This comprises clones from Malaysia (80), Indonesia (13), Sri Lanka (17), China (3), Ivory Coast (5), Brazil (5), Thailand (3) and Liberia (1). Clones introduced from South America are considered to be a valuable genetic resource for incorporating disease resistance in the breeding programme.

Nowadays introductions are effected under bilateral and multilateral clone exchange programmes. The exotic materials introduced so far also include two other species, *Hevea benthamiana* (F 4542) and *Hevea spruceana* and four inter-specific hybrids FX 516, IAN 45-713, 45-717 and 45-873. Popular clones introduced from other countries are evaluated under local agroclimatic situations and the promising ones are recommended for large-scale planting. Selected clones from among these introductions have been used as parents in hybridization programmes which have resulted in the evolution of some very successful cultivars like RRII 105. Polyclonal seeds from Prang Besar Isolated Gardens (PBIG series), specifically from the Gough Garden (GG 1, GG 2, GG 3, GG 4, GG 5, GG 6 and GG 7) have also been imported and used as planting materials in the 1950s and 1960s. These seedling trees are still being used as source material for ortet selection.

3.2 Ortet selection

Considerable variability in yield in seedling populations was first observed by Dutch workers in Java and Sumatra in the second decade of the present century (Whitby, 1919). Simultaneously, the technique of budgrafting was perfected which facilitated the fixation of desired characters and the development of early primary clones through ortet selection.

Ortet selection, mother tree selection or plus tree selection is the oldest selection method adopted in *H. brasiliensis*. The term ortet is derived from the Latin word 'ortus' meaning origin. Ortet is, therefore, the original tree from which members of a clone have descended.

Ortet selection is aimed at selection of elite seedling genotypes resultant of natural genetic recombination. The procedure is quite elaborate. As the first step, seedling plantations of polyclonal origin, recording good average performance are selected. The area is then systematically screened for individual trees recording high yield and/or secondary characters. Such trees are observed for growth form, volume yield and dry rubber content recorded at intervals of three to four months representing different seasons. Secondary characters are also recorded. Based on the data, selected mother trees are cloned (branches are cut back for generating budwood which is used for budding). The resultant clones are subjected to field evaluation in small-scale, large-scale and block trials and the promising ones are released for commercial cultivation.

Rapid progress in plus tree selection between 1919 and 1926 in Indonesia resulted in such classical primary clones as Tjir 1, PR 107 and GT 1. Parallel efforts in Malaysia resulted in clones like PB 23, PB 25, PB 86, PB 186, Gl 1, Pil A 44, Pil B 16, Pil B 84, *etc.* In India, ortet selection in the early years has resulted in 46 primary clones designated as the RRII 1 series. Of these, RRII 1, 2, 3, 5 and 6 appear promising selections for yield (Marattukalam *et al.*, 1980). RRII 33 is a clone resistant to abnormal leaf fall disease caused by *Phytophthora* spp. Since seedling areas are increasingly being replaced with modern clones, further extensive screening for yield, resistance to disease, drought, *etc.* in traditional as well as non-traditional areas is one of the priority areas of research.

3.3 Hybridization and selection

Hybridization programmes are aimed at combining genes controlling characters like high yield, resistance to diseases, drought, canopy characteristics, *etc.*, found scattered in different clones. Further, exploitation of hybrid vigour is also possible. Artificial pollination between selected parent clones, evaluation of F1 hybrids and selection of promising recombinants from the progeny have been, and are still, the most important methods of conventional breeding.

3.3.1 Technique of hybridization

As the first step, parent clones possessing desirable characters (depending on the breeding objective) are selected, marked and special protection against powdery mildew disease is provided so as to ensure availability of healthy flowers. When flowers are mature, healthy and well exposed, flowering branches from the female parent clones are selected for hand pollination. All the male flowers, immature female flowers and those already opened are removed retaining only the mature female flower buds due to open on that particular day. Staminal columns from healthy mature unopened male flowers of the selected male parent are used as the source of pollen. The female flower to be pollinated is held lightly between the thumb and the forefinger and the perianth lobes carefully forced apart. The staminal column from the male flower is then inserted within the perianth of the female flower and placed on top of the stigma (Plate 6. a,b). The perianth lobes are then sealed using a small cotton plug and a drop of latex, to check entry of foreign pollen. The pollinated twig is finally tagged using tin/plastic labels containing relevant information like male and female parents, date and time of pollination, *etc.* (Plate 6. c).

In successful cases, fertilization takes place within 24 h and the cotton plug and perianth lobes fall off in about a week. The ovary grows into a fruit, which matures in about five months. Fruits are enclosed in net-bags to avoid loss of seeds when they dehisce on maturation (Plate 6. d).

The mature fruits are harvested and germinated seeds are planted in a seedling nursery maintaining their identity. The seedlings are subjected to juvenile evaluation by test tapping based on which the promising hybrids are cloned and subjected to further evaluation.

3.3.2 Clone evaluation

Conventionally, evaluation of the selections is quite elaborate and is done in three stages. The first stage is the preliminary evaluation known as small-scale trials (SST) in which the preliminary selections are cloned and planted in statistically laid out experiments of small plots of five trees each. Based on the data on growth and yield for three to five years and also secondary characters, promising clones are selected. These are tested in the second stage of evaluation *viz.* large-scale trials (LST), of larger plot size (usually 75 plants per clone) in three replications in a statistical layout. In LST also the best clones are selected based on growth and yield for three years. These selections are subjected to final evaluation in block trials of one tapping block of each clone, preferably in different agroclimatic situations. The clones which perform well in all the three evaluation trials are considered genetically superior and are released for large-scale cultivation. The whole process requires 30 to 34 years from the nursery stage to final release of a clone (Table 1).

Table 1. Scheme for evaluation and release of clones

Year	Steps involved
0	Hand pollination and seedling nursery (in the case of hybridization and clone selection), or mother tree selection and insurance budding (in ortet selection)
3	Small-scale trial
10	Tapping SST (for 3 years)
13	Selection, multiplication
14	Large-scale trial
21	Tapping large-scale trial (for 3 years)
24	Selection, multiplication
25	Blockwise trial; recommended for limited planting
32	Tapping block trials (for 3 years)
34	Final selection, recommendation and release

Controlled pollination was started in Malaysia in 1918, in Sumatra in 1920, in Brazil in 1937, in Sri Lanka in 1939 and in Indo-China in 1948 (Panikkar *et al.*, 1980). In India hybridization programmes were initiated in 1954. The early clones were used as parents for the first hybridization series and resulted in hybrid clones of commercial significance. These clones, in general, produced higher yields than the primary clones. Breeding in *Hevea* involves generationwise assortative mating, where the best clones in one cycle are used as parents for the next, and so on (Simmonds, 1985; 1989).

In the RRII, a total of 612 hybrid clones and 195 ortet clones are under evaluation in small-scale trials, 120 clones of indigenous and exotic origin are in large-scale trials and 36 clones in 136 blocks spread over 19 estates in different locations are under on-farm trials. Comparative performance of eight popular clones evaluated in 10 large estates (Mercykutty *et al.*, 1995) is given in Table 2.

In India, the early hybrid clones developed by the RRII include RRII 100, 200 and 300 series (Annamma *et al.*, 1990). Among clones of the RRII 100 series, RRII 105 is a highly successful and popular one (Nair and George, 1969; George *et al.*, 1980;

Table 2. Yield of popular clones in India

Clone	Yield (kg/ha/year)									
	Year of tapping									
	1	2	3	4	5	6	7	8	9	10
RRII 105	926	1262	1531	1646	1614	1848	2158	1965	1593	–
PB 217	709	1103	1414	1454	1652	1637	1720	1327	1809	2437
PB 235	670	1093	1598	1816	1668	1581	2752	1552	–	–
PB 260	639	1439	1524	1665	2826	2413	2826	2280	–	–
PB 311	880	1086	1504	1953	1833	–	–	–	–	–
PB 28/59	645	1257	1472	1753	1904	1681	1540	2034	1925	1187
RRIM 600	580	785	1457	1569	1680	1490	1867	1887	1384	1795
GT 1	623	997	1228	1431	1477	1627	2044	1971	1763	2039

Nazeer *et al.*, 1986; Mydin *et al.*, 1994). The clones RRII 116 and RRII 118 are outstanding for growth vigour but have only medium yield. Clones RRII 203 and RRII 208 (Saraswathyamma *et al.*, 1987) and RRII 300 and RRII 308 (Premakumari *et al.*, 1984) are the best selections in the 200 and 300 series respectively. RRII 203 and RRII 308 are also very vigorous in growth. Many other selections are in various stages of experimental evaluation. In a recent study on 23 hybrid clones resultant of a bi-parental cross of RRII 105 x RRIC 100, nine clones revealed marked heterotic increase for yield over the first three years of tapping in a small-scale trial (Licy *et al.*, 1998). Another set of 50 hybrid clones resultant of various cross combinations have been identified as having better potential for yield improvement based on early growth and yield. In a recent report on 24 hybrid clones, evaluated at the early stage of 44 months after field planting, 12 clones exhibited a heterotic increase of over 20 per cent for yield, which offers much scope for exploitation of hybrid vigour. (Varghese *et al.*, 1997a). In the hybridization programmes, parent clones are selected based on the available genetic variability for various components contributing to yield (Varghese *et al.*, 1990; Mydin *et al.*, 1996).

4. SPECIAL TECHNIQUES IN BREEDING

In addition to ortet selection and hybridization, special techniques in breeding like polyploidy, mutation and *in vitro* culture have also been attempted in *Hevea*.

4.1 Mutation and polyploidy breeding

The diploid chromosome number (two sets of chromosomes) in *Hevea* is 2n = 2x = 36. Induction of polyploidy (more than two sets of chromosomes) and mutation (sudden heritable changes) have been attempted on a limited scale (Saraswathyamma *et al.*, 1984). By crossing diploids and tetraploids, a triploid (with 3 sets of chromosomes) has been synthesized from the clone RRII 105 (Saraswathyamma *et al.*, 1980). A spontaneous triploid has also been identified (Nazeer and Saraswathyamma, 1987). Induction of mutations using ionizing radiations as well as chemical mutagens has also been attempted. Studies on clones resultant of gamma irradiation have identified two clones *viz.* RRII 50 and RRII 51 with growth comparable to RRII 105 (Licy *et al.*, 1997).

4.2 *In vitro* culture technique

The use of *in vitro* culture techniques (*e.g.* tissue culture) offers various possibilities for creation of genetic variability like somaclonal variability (variability in plants derived from callus cultures) and production of haploids (plants with one set of chromosomes). It is expected that somaclonal variations (Larkin and Scowcroft, 1981) occurring in populations raised through tissue culture can add to the genetic variability in *Hevea*. Further advancements in the field of biotechnology like development of haploid lines through anther culture will prove useful adjuncts to conventional breeding.

5. CONSTRAINTS IN BREEDING

Several problems which hamper breeding and quick release of cultivars as in the case of most of the perennial crops, have been identified in *Hevea*. These include seasonal nature of flowering and low fruit set, long breeding and selection cycle, lack of fully reliable early selection methods, *etc.* A slow down of genetic advance in the recent breeding phases when compared to that in the early phases is attributed mainly to a narrow genetic base.

5.1 Nature of flowering and fruit set

In the traditional rubber growing tract in India, flowering is restricted to a short period of two to three months from January to March. However, all clones do not flower simultaneously (George *et al.*, 1967). This non-synchronization of flowering in some of the parent clones selected, limits the possibility of attempting all possible cross combinations. Pollen storage and induction of off-season flowering have been suggested to overcome these.

Pollination on mature trees is cumbersome and is an elaborate process. In order to carry out hand pollinations easily and efficiently, breeding orchards are helpful. A total of 50 selected clones has been established in two breeding orchards at *Hevea* Breeding Substation, Paraliar, Kanyakumari district, during 1986-87. Here clones are planted at a wide spacing of 3.6 x 3.6 m and low branching has been induced so as to facilitate sufficient spread of the canopy at convenient height. This can simplify the pollination process and also the pre- and post-pollination prophylactic protection measures against diseases such as powdery mildew during flowering season and later, fruit rot caused by *Phytophthora*.

The low percentage of fruit set following controlled pollinations is another serious bottleneck which limits the progeny size in hybridization programmes. The average fruit set resultant of controlled pollinations is generally less than five per cent, even under the best conditions (Mydin *et al.*, 1989). The success rate varies widely with clones and seasons from less than one per cent to 12 per cent, even within combinations involving the same female parent, indicating the influence of the male parent (Mydin *et al.*, 1990).

Investigations at the RRII revealed the application of a solution of boric acid and sucrose prior to pollination and enclosing the panicles in butter paper cover after pollination yielded relatively higher fruit set (Mydin *et al.*, 1989). However, the extent of fruit set is still low and further improvements are required.

5.2 Breeding and selection cycle

The conventional breeding and selection cycle, though well established, is elaborate and requires 30 to 34 years for the final release of a clone. With a view to shortening

the breeding cycle, Subramaniam (1980) suggested an accelerated method called promotion plot trials. In this, the best one per cent of hand-pollinated progeny is selected during the fifth year based on juvenile yield. These are then tested in regionwise trials of 50 plants per clone in two replications. Early results reveal that a fair proportion of the selections is promising. But the main reservation is that only a very small proportion of the progeny selected based on juvenile yield is tested further and the chances of many potential high yielders being lost are high. Markose and Panikkar (1984) suggested establishment of replicated field trials during the third year after hand pollination and taskwise trials, if possible, in different locations during the 12th year. This would enable a planting recommendation in 24 to 25 years.

6. EARLY EVALUATION METHODS

Realizing the significance of juvenile selection, early workers studied a number of parameters for early prediction of yield. The parameters like girth, height, bark thickness, latex vessel number, latex vessel and sieve tube diameters and rubber hydrocarbon in bark and petiole showed poor and/or inconsistent results (Tan, 1987). Techniques tried for early yield prediction include 'testatex' method using a special knife of four V-shaped blades (Cramer, 1938), use of a perforated wheel (Meyer, 1950) and the needle prick test (Waidyanatha and Fernando, 1972). The modified Hamaker-Morris-Mann method (Tan and Subramaniam, 1976) is the widely adopted one. In this method two to three year old plants are tapped successively for quantifying latex yield. A test incision method developed at the RRII (Annamma *et al.*, 1989) facilitates quantification of juvenile yield at the age of one year.

Studies on correlation between nursery and mature yield revealed only low to moderate association between the two parameters (Ong *et al.*, 1985). Thus with the available early prediction methods, nursery yield can be considered as only a fair indicator of mature yield. Performance index based on yield and related juvenile traits (Mydin *et al.*, 1990) at an age of two years was found to be a good criterion for selection of clones at an early age based on a study on early evaluation of clones with high, medium and low yield potential (Varghese *et al.*, 1992). The present strategy adopted at the RRII is to exercise only mild selection pressure (around 20%) on the hybrid progenies based on juvenile characters for further testing, so that minimum loss of the potential high yielders is ensured. Further investigations are required for development of fully reliable parameters for early prediction of yield.

7. RECENT APPROACHES

At present, more than 98 per cent of the rubber production in India is from the traditional rubber growing tract of Kerala, parts of Tamil Nadu and Karnataka, where there is no further scope for expansion of area under rubber. Hence cultivation has been extended to non-traditional areas like Tripura, Assam, Meghalaya, Mizoram, West Bengal, Madhya Pradesh, Orissa, Goa, Maharashtra and Andaman and Nicobar Islands. Consequently, location-specific breeding programmes are being prioritized.

The performance of clones varies across locations and efforts are on to study the extent of interaction between the genotype and environment so as to pick out the ideal

clone for each specific location. The north-eastern region represents the most important non-traditional tract for growing rubber. However, the region faces environmental constraints like low winter temperature, high velocity winds, occasional hailstorms and high elevation conditions. Yield in BO-1 and BO-2 panels has placed clones PB 235, RRIM 600, RRII 203 and RRII 105 in this order of performance. The performance of RRII 105 is not on par with that in the traditional region. Clones RRIM 703, RRII 118, RRIC 105, RRII 203, RRII 105, PB 235 and RRIM 600 have been found promising in terms of early yield and stability in Tripura (Vinod *et al.*, 1996). The production of good quality seed material from polyclonal seed gardens established with newer clone combinations based on genetic divergence and prepotency (ability of a parent to produce uniformly superior offspring) is another recent attempt for evolving planting materials with better stability.

A morphological variant having compact crown isolated from a PBIG population was confirmed to be a genetic variant which segregated into four morphotypes *viz.* dwarf, semi-dwarf, intermediate and normal (Markose *et al.*, 1982; John *et al.*, 1995). They are being incorporated in breeding programmes for altering the tree architecture so as to combine yield and compact canopy, which in turn would be useful for high density planting.

Molecular techniques like random amplified polymorphic DNA (RAPD) analysis (Varghese *et al.*, 1997b), isozyme studies (Chevallier, 1988) and restriction fragment length polymorphism (RFLP) analysis (Besse *et al.*, 1994) have been introduced into *Hevea* breeding programmes as a means of assessing genetic variability and for identifying potential parents for breeding. Such markers also hold promise for the early identification of superior clones. DNA markers for tolerance to *Oidium* infection in *Hevea* have been reported (Shoucai *et al.*, 1994). The incorporation of genotypes from the wild Brazilian germplasm into the breeding pool is another recent priority in *Hevea* breeding.

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