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GENETIC IMPROVEMENT IN HEVEA: ACHIEVEMENTS, PROBLEMS AND PERSPECTIVES.

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The original genetic material of the para rubber tree of commerce, Hevea brasiliensis (willd. ex. Adr. de Juss.) Muell. Arg. is the "Wickham gene pool" introduced to South East Asia by Sir Henry Wickham in 1876 from the Amazon river basin in Brazil, the centre of diversity of the genus. From this original material with an average yield of less than 300 kg ha^{-1} yr^{-1}, substantial improvement in productivity, to over 2500 ka ha^{-1} yr^{-1} for modern clones has been achieved over a limited period mainly through genetic improvement of the species.

However, there are certain problems associated with breeding and selection and quick release of cultivars. These include seasonal flowering pattern, non-synchronous flowering in potential parents, low fruit set following hand pollination, lack of reliable early selection parameters, long breeding and selection cycle, etc. In addition, from the original narrow Wickham base and a cyclical generation wise assortative mating, further dramatic improvement in productivity is not expected. Attempts are, therefore, under way to identify and formulate future strategies to solve the problems impeding rapid progress in crop improvement.

Breeding methods

The conventional methods of tree improvement adopted in Hevea are ortet selection and hybridization. The flowers though monoecious, the plant is both cross and self pollinated, the former condition being predominant. Allogamy coupled with seed propagation offers much variation through genetic recombination in the seedling population.

Ortet selection

The occurrence of considerable variability in yield in seedling populations observed by Dutch workers in Java and Sumatra in the second decade of the present century led to the earliest attempts for genetic improvement of this crop. 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 or mother tree selection, the oldest breeding method, involves systematic screening of seedling plantations for superior mother trees. Rapid progress with mother tree selection was achieved in Indonesia and Malaysia, which resulted in a large number of early primary clones like Tjir 1, PR 107, GT 1, Gl 1, PB 86, Pil B 84, Pil D 65 etc. In India, the earlier ortet selection programmes resulted in 46 clones. In the RRII 1 series - RRII 1, 2, 3, 5 and 6 appear promising selections for yield. RRII 33 is a clone resistant to abnormal leaf fall caused by Phytophthora spp.

Over 150 recent selections have been established in field for preliminary evaluation. So far, over four lakhs seedling trees have been screened. Since the seedling areas are increasingly being replaced with modern clones, further extensive screening for yield, resistance to diseases, drought etc in traditional as well as non-traditional areas is necessary.

Seedlings, though not comparable with high yielding clones in production potential, have special agricultural merits that there is a need for superior polycross progenies from special polyclonal seed gardens as planting materials. The evaluation of such polycross population can be considered as selective breeding. Such 'multiparent', first generation synthetic varieties (Syn-1 - Simmonds, 1986) have been economically successful for many decades, predominantly due to additive genetic control of vigour and yield as well as high general combining ability (Tan, 1987; Simmonds, 1986). Such large seedling populations constitute a valuable genetic resource for ortet selection.

Hybridization and selection

Artificial pollination between selected parent clones, evaluation of F_1 hybrids and selection of promising recombinants for commercial planting has been and still is the most important method of developing clones of desirable genetic constitution. Systematic breeding efforts in different rubber research institutes have resulted in the development of a series of high yielding clones of commercial significance. The early primary clones were used as parents for the first hybridization series and resulted in hybrid clones like RRIM 500, RRIM 600, RRII 100, RRII 200 and RRII 300 series. The best primary and hybrid clones are used in further breeding programmes. Conventionally clones resultant of ortet selection and hybridization are evaluated in three consecutive phases viz., small scale trials, large scale trials and block trials.

In India, breeding work was initiated with the inception of RRII in 1954. So far, over 1.6 lakhs artificial pollinations have been attempted; around 5700 hybrid seedlings produced and around 2300 clones established in small scale trials. The earlier selections recommended for planting include clones from RRII 100, RRII 200 and RRII 300 series. The later selections are in different stages of experimental evaluation. The programme is continuous and annually around 10,000 hand pollinations are attempted. Among the RRII 100 series clones, RRII 105 is a very promising selection (Nair and George, 1969; Nazeer et al., 1986; Annamma et al., 1990), popular in the plantation sector, while RRII 116 and RRII 118 are outstanding for growth vigour. RRII 203 and RRII 208 (Saraswathya Amma, 1987) and RRII 300 and RRII 308 (Premakumari et al., 1984) are the best selections among 200 series and 300 series respectively.

In addition, the RRII has got a programme of introduction of promising clones from other rubber producing countries, with a view to evaluating them under local agroclimatic conditions. So far, 114 clones have been introduced during different periods. At present, 105 clones of indigenous and exotic origin are under evaluation in 20 large scale trials covering an area of 28 ha. Similarly, a total of 44 clones in153 blocks (153 ha) in 25 different trials are under final evaluation. The performance of certain promising clones is furnished in Table 1.

Special techniques in breeding

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

The chromosome constitution of *Hevea brasiliensis* is $2n = 2x = 36$. Induction of polyploidy and mutations have been attempted in a limited scale in different countries. The Indian scientists have developed tetraploids with chromosome complement $2n = 4x = 72$. By crossing diploids and tetraploids, a triploid has been synthesized in the clone RRII 105 (Saraswathy Amma, et al., 1980, 1988). A spontaneous triploid (Nazeer and Saraswathy Amma, 1987) and a genetic variant with dwarf stature (Markose et al., 1981) have also been identified. Induction of mutations using ionizing radiations as well as chemical mutagens have also been attempted.

While these attempts are of significance, progress in these fields is rather slow. The frequency of desirable genetic variations is in general low which necessitate screening of very large populations for identification of useful genetic variants. Similarly the problem of chimera formation when multicellular meristems are treated with

Table 1. Performance of certain promising *Hevea* clones

Clone	Country of Origin	Parentage	Small scale trial g $tree^{-1}$ tap^{-1}	Large scale trial kg ha^{-1} yr^{-1}	Commercial yield kg ha^{-1} yr^{-1}
RRII 5	India	Primary	89 (10)	2942 (10)	--
RRII 105	India	Tjir 1 x Gl 1	81 (20)	2480 (10)	1653 (7)
RRII 118	India	Mil 3/2 x Hil 28	50 (4)	--	1117 (6)
RRII 203	India	PB 86 x Mil 3/2	85 (15)	2487 (8)	1142* (5)
RRII 208	India	Mil 3/2 x AVROS 255	87 (15)	2625 (8)	1226* (6)
RRII 300	India	Tjir 1 x PR 107	105 (16)	--	--
RRII 308	India	Gl 1 x PB 6/50	72 (16)	--	--
RRIM 600	Malaysia	Tjir 1 x PB 86	--	2199 (15) M 2200 (16) I	1692 (10) M 1317 (10) I
PB 217	Malaysia	PB 5/51 x PB 6/9	--	1778 (13) M 2209 (10) I	-- 1258 (10) I
PB 235	Malaysia	PB 5/51 x PB S.78	--	2458 (15) M	1258 (10) I
GT 1	Indonesia	Primary	--	1723 (13) M 1484 (10) I	-- 1279 (8) I

Figures in paranthesis indicate the period of evaluation in years.

M : Yield in Malaysia. I : Yield in India.

* : Block trial yield.

mutagenic agents as reported in other crops (Annamma and Roebellen, 1984), complicate isolation and selection of mutants. However, further attempts in these fields may yield useful results.

The use of *in vitro* culture techniques offers various possibilities for creation of genetic variability in addition to being a propagation system, like exploitation of somaclonal variability and production of haploids. Reports on micropropagation studies in different countries reveal varying degrees of success. At RRII, scientists have succeeded in perfecting a tissue culture system, utilising shoot tips of some of the popular clones and the plants have been established in a field trial (Ashokan *et al*., 1988). While micropropagation facilitates propagation of clones with their own root system, thus eliminating the chances of possible stock-scion interaction, somaclonal variations associated with mass multiplication 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.

Constraints in breeding

Several problems have been identified which hamper quick release of cultivars as well as further genetic improvement in *Hevea*, as in the case of most of the perennial crops.

Nature of flowering and fruit set

In the traditional rubber growing tract in India, flowering is restricted to only 1-2 months during February to March. This short period and also non-synchronization of flowering in certain potential clones, limits the production of sufficient legitimate families for making effective selection as well as attempting all the desired cross combinations. Pollen storage and induction of off-season flowering have been suggested to overcome these.

The low percentage of fruit set following controlled pollinations is a serious bottleneck in hybridization programmes. The average fruit set resultant of controlled pollinations is generally less than 5%, even under best climatic conditions. The success rate varies widely with clones and seasons from less than one to twelve per cent. The factors leading to low fruit set include:

(1) Severe Oidium infection during the flowering season.

(2) Fruit drop caused by Phytophthora spp.

(3) Clonal difference in female fertility.

(4) Injury caused to the female flowers consequent to conventional technique.

Studies on fruit set are under way to investigate other possible reasons and to find out methods to increase fruit set in hybridization programmes. Recent investigations at 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 to yield relatively higher fruit set (Kavitha et al., 1989). However, the extend of fruit set is even then too low and inadequate, that further investigations are necessary.

Early selection methods

Other factors which hamper quick release of cultivars include lack of reliable early selection methods and the long breeding and selection cycle. Although studies on early evaluation carried out in different Rubber Research Institutes have resulted in techniques for rejection of low yielders, no fully reliable characters, which can be used for selection of the best types from hybrid progenies has so far been identified. Realising the significance of juvenile selection, early workers developed a number of techniques like testatex method (Cramer, 1938), use of a perforated wheel (Meyer, 1950), a single half spiral sloping cut (Ferrand, 1939), needle prick test (Waidyanatha and Fernando, 1972), Hamaker - Morris - Mann Method (HMM method, Hamaker and Morris Mann, 1914). Among these different techniques, the HMM method, the widely adopted one consists of successive tappings of 2-3 years old plants and quantifying the latex yield. A test incision method developed at RRII

(Annamma et al., 1989), facilitates quantification of juvenile yield still earlier i.e., at an age of one year.

However, studies on the correlation between nursery and mature yield revealed only low to moderate association between the two parameters (Ong et al., 1986). Thus, with the available early prediction methods, nursery yield can only be considered as a fair indicator of mature yield. Therefore, based on nursery yield only mild selection should be adopted to ensure minimum loss of potential high yielders of hybrid seedling populations. Further, investigations are required for development of fully reliable parameters for early prediction of yield.

Breeding and selection cycle

The conventional breeding and selection cycle which is well established is elaborate and require 30-32 years from seedling nursery till final release of a clone. This sort of three phase selection through small scale, large scale and block trials impedes quick release of cultivars, though necessary for systematic exploration of large number of progenies. The process can be supplemented by promotion plot trials introduced by the RRIM in 1972, which saves a period of ten years in comparison to conventional method. In this method, a few high yielding progeny selected based on nursery evaluation is tested straight from the nursery to a kind of large scale trial in two replications with a plot size of 0.2 ha per clone. Early results reveal that a 'fair proportion' of the tested clones is promising. But the main drawback is that only a very small proportion is selected based on nursery yield prediction which, by itself, is not fully reliable. Many potential yielders as well as useful recombinants for secondary characters are most likely to be lost.

Proposals have also been made for shortening the testing cycle by bye-passing one of the testing stages (Senanayake and Wijewantha, 1968; Subramaniam, 1980). Markose and Panikkar (1984) suggested establishment of replicated field trials during the third year after hand pollination and task wise trials, if possible, in different locations during the 12th year. This would enable a planting recommendation in 24-25 years.

At RRII, the present strategy is to exercise only mild selection of hybrid progenies (ie., around 20%) based on nursery evaluation, layout small scale trials employing suitable statistical designs, record pre-mature yield for one year before commencement of normal tapping and similar procedure adopted for large scale trial.

Conventional method	Year	Modified procedure suggested
Hybrid seedlings in nursery	0	Hybrid seedlings in bursery.
	1	Recording juvenile yield and growth vigour.
Laying out small scale trial	3	Selection (around 20%) Laying out small scale trial along with parents employing suitable statistical designs.
	8	Recording premature yield (1 year).
Tapping small scale trial	9	Selection around (10%) Laying out large scale trial.
Selection, laying out large scale trial	12	
	14	Recording premature yield (1 year) and secondary characters, selection (around 5%).
	15	Laying out block trial.
Tapping large scale trial (3 years)	18	
Selection	21	Tapping block trial (3 years)
Laying out block trial	22	
	24	Final selection and recommendation.
Tapping block trial	28	
Selection and release	32	

Thus based on the performance in the early clonal phase, the best clones are selected for further evaluation, in effect, shortening the testing period by 6-7 years (Licy et al., 1990).

Simultaneous with the selection process, progeny testing and genetic analysis for combining ability, heritability, genetic advance etc can also be done from the small scale and/or large scale trial as suggested by Markose and Panikkar (1984). Such approaches can be valuable adjuncts to conventional procedure in designing hybridization programmes in future, especially in the context of land being a limiting factor for field experimentation.

Narrow genetic base

The genetic base of Hevea in the east is very narrow, limited to 20-22 seedlings collected from a minuscule of the genetic range (Wycherley, 1968; Schultes, 1977; Allen, 1984). Added to this constraint, wider adaptation of clonal propagation system and a cyclical breeding pattern have been factors limiting enrichment of genetic diversity.

Budgrafting is an established commercial practice of clonal propagation and results in more or less uniform plantations of high yielding clones. At the same time, clonal reproduction reduces natural genetic heterogenity available in seedling populations. The cyclical assortative mating pattern followed in Hevea resulted in a limited number of high yielding clones, most of which have originated from a few dominant parents. Considering the Wickham base generation as G_0 and the early primary clones as G_1, the current clones are mostly at G_2 or G_3. In general, the genetic advance from G_0 to G_1 and G_2 is remarkable while that from G_2 to G_3, G_4 etc show a diminishing trend (Simmonds, 1989). The yield level of certain high yielding clones and their respective parents in India indicate this trend. RRII 105 (G_2) and RRIM 600 (G_2), two secondary selections for yield recorded considerable yield increase over the parents (G_1), while an increase at a similar proposition was not observed in PB 217 a tertiary clone (G_3) with two secondary clones as parents (Fig. 1), indicating a plateau in performance at least in certain specific cases. Genetic studies, in general also indicate inbreeding depression and unpredictable

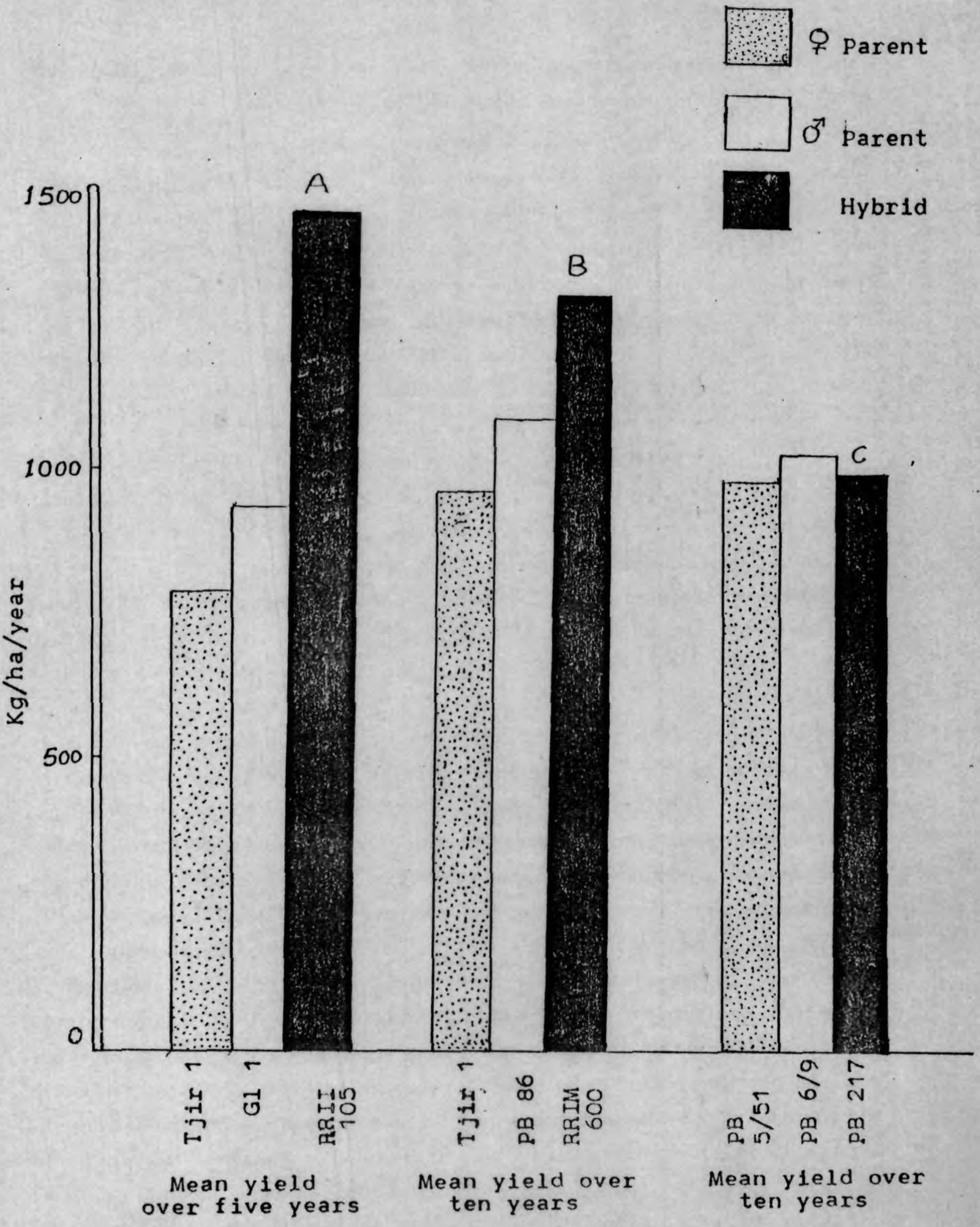


Fig.1: Difference in yield niveau of three high yielding selections and their respective parents in India. A and B secondary clones; C tertiary clone.

interaction when related parents are used in breeding (Gilbert *et al*., 1973; Nga and Subramaniam, 1974).

Considering the urgent need for broadening the genetic base, fresh, wild germplasm has already been collected from the centre of diversity in Brazil, as the result of the joint expedition of member research institutes of the IRRDB and Brazilian Government. Over 60,000 seeds and budwood from 194 presumably high yielding mother trees were collected from the states of Acre, Mato Grosso and the territory of Rondonia. The Malaysian and Ivory Coast germplasm centres received 37.5% and 12.5% seeds respectively for establishment and distribution to member countries. So far, over 7000 genotypes have been introduced to India, from the Malaysian centre, which are being established in nurseries for conservation, evaluation and utilization in breeding programmes. Preliminary evaluation of certain genotypes belonging to the early consignments revealed wide variability for juvenile yield and growth characters (Annamma *et al*., 1988).

Yield gap

In spite of the creditable improvement in productivity within a short span of time, theoretical conclusions indicate more potential in terms of yield (Sethuraj, 1981). There exists a wide gap between experimental yield and commercial yield and also between theoretical maximum and maximum experimental yield. The possible factors responsible for the gap between experimental yield and actual commercial productivity need to be studied and documented for each level of management. Because of the occurrence of significant Genotype Environment Interaction (Paardekooper, 1964; Jayasekera *et al*., 1977) trials need to be conducted in diverse environments for proper evaluation. At RRIM, clones are tested in different environs and recommendations made (enviromax recommendation - Ho *et al*, 1974).

Future prospects

Latex yield *per se* was the main objective in the early breeding phases and a ten fold increase over the original material has already been achieved. However, the genetic advance in the recent breeding phases seems to have slowed down, which is attributed to different reasons. With a view to exploiting the genetic variability at yield component level emphasis is being given for selection of parent clones with complementary yield components, which would result in higher yielding hybrid clones. Due to the directional selection for yield, genetic improvement of many secondary characters influencing yield and overall performance was ignored. Lack of resistance to South American Leaf Blight in the oriental cultivars (Baptiste, 1960) absence of major genes for resistance to *Oidium* and indications of eroded resistance to *Gloeosporium* (Wycherley, 1977) are specific examples. Efforts for incorporation of resistance to high yielding cultivars, therefore, need to be intensified in future programmes.

Specific priorities in breeding objectives vary from country to country and also between different locations with distinct agro-ecologic situations. Pressure on land and socio-economic constraints necessitates the expansion of rubber cultivation to marginal lands. In India, rubber cultivation has already been extended to non-traditional areas exposed to different stress situations like severe drought, wind, cold, high elevation etc. Clones capable of with-standing such stress situations need to be bred for maximising productivity in these areas. It is in this context that location specific research and development activities assume great importance and breeding efforts should receive due significance.

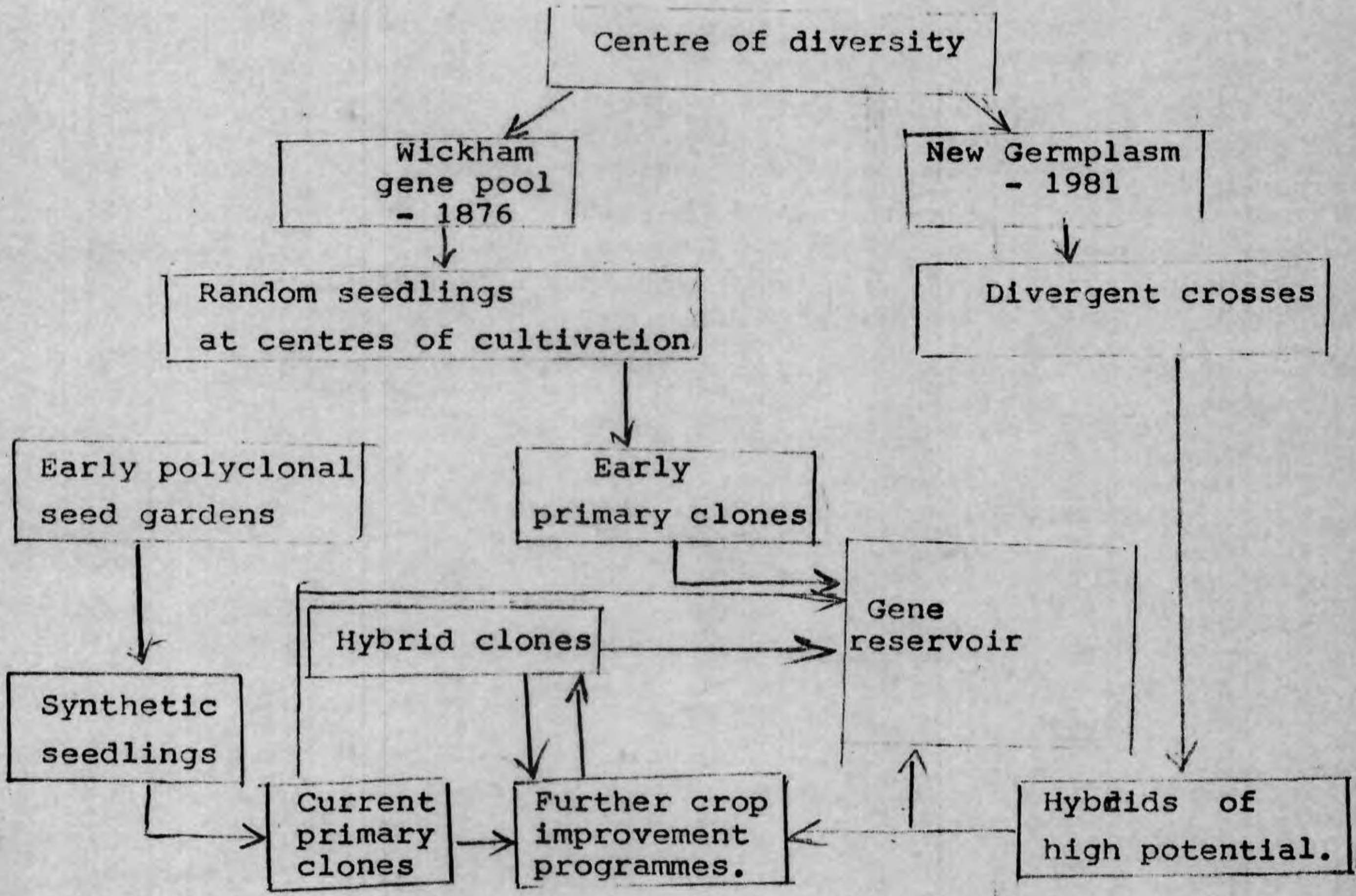
At present extensive areas are cultivated with a handful of high yielding clones which are more or less closely related. Therefore, more parallel clones should be evolved and made available for large scale planting. The rubber breeder has to be cautious about the possible danger of monoculture-vulnerability to diseases and pests. Similarly, in order to maintain genetic variability in the population, planting of more advanced generation polyclonal seedlings of high production potential should also be advocated. There is a continuing need for high quality seed gardens to produce planting materials, rootstocks and sources of continued ortet selection. Shortening the breeding cycle in Hevea is an essential pre-requisite in evolving planting materials and making them available to the planting community in shorter spells. The situation becomes complicated as the breeder has to ensure the quality and reliability of the newer materials rigorously as instances are there where some of the materials, even after prolonged testing, later showed specific disadvantages.

All the primary, and hybrid clones of importance bred in Asian countries have originated from the narrow Wickham base (Fig. 2). Valuable wild genes from the new germplasm, when identified and incorporated into modern high yielding clones, would result in clones suitable for specific locations. It is also expected that the new genotypes when utilized for hybridizations with Wickham clones would lead to better heterosis at least for certain characters of importance. With this in view, divergent crosses incorporating popular clones and new Brazilian germplasm have already been initiated at RRII.

Latex yield is primarily determined by the genetic potential of the planting materials used. But expression of these genes is determined by several heritable and environmental factors as well as agromanagement practices. This demands future inter-disciplinary approaches for development of stable, high yielding clones with initial high yield.

Rubber becoming more and more a small holders' crop, breeding objectives should take care of their specific needs like early high yields, resistance to high intensity tapping, availability of stable high yielders etc. Similarly, the future breeding objectives should be more flexible to accommodate possible techno-

Figure 2 - Breeding Scheme



logical advances, socio-economic demands as well as the likely demands of the industry.

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