

THE 1981 IRRDB WILD *HEVEA* GERMPLASM COLLECTION IN INDIA: PRESENT STATUS AND FUTURE PROSPECTS

Varghese, Y. A., J. Madhavan, S. T. Abraham, M.A. Mercy,
G. P. Rao, C. P. Reghu, and P. J. George

Rubber Research Institute of India, Kottayam- 686009 Kerala, India

Abstract Recognizing the significance of broadening the original narrow genetic base of *Hevea* in the East, India has also been associated with the international action for collection of wild genotypes from the center of diversity in Brazil during 1981. The pedigree of present day cultivated clones in India can be traced back to only a handful of Wickham clones, indicating the lack of sufficient variability in the breeding pool. Rubber cultivation having been extended to non-traditional regions confronted with various agro-climatic constraints limiting plant growth and productivity, development of location specific clones has become essential. Recent reports of minor diseases like *Corynespora* leaf spot becoming major have also caused serious concern. Wild germplasm is a rich source of many valuable genes conferring resistance to various biotic and abiotic stresses.

From the 1981 IRRDB collection of wild germplasm, India has established 4967 accessions in two centers: 3617 in the traditional zone and 1350 in the non-traditional rubber growing area. This fresh germplasm, conserved in source bush nurseries, is being subjected to field evaluation in a phased manner. Characterization of all the wild accessions on the basis of a descriptor prepared for the purpose is being carried out. Computerization of the data bank for documentation of the data generated from the various evaluation trials has been initiated. Results so far revealed wide variability for growth form, floral and fruit characteristics, girth, number of latex vessel rows, bark thickness, juvenile yield, etc. Though wild genotypes in general are poor yielders, a few potential high yielders have been identified based on juvenile yield. Several accessions that are promising for individual desirable traits have been observed. Preliminary screening for diseases caused by *Phytophthora*, *Oidium*, etc. and for drought has revealed the existence of sufficient variability for resistance to these biotic and abiotic factors, indicating the potential for developing location specific clones. Hot spot screening for drought and cold resistance is underway. Hybridization incorporating selected wild accessions with popular Wickham clones is also being carried out with the twin objectives of evolving superior clones and broadening the genetic base.

Studies at the molecular level have been initiated to supplement the elaborate conventional procedures. Estimation of genetic relationships among the wild accessions using RAPD markers is in progress. Further studies envisaged using DNA markers are expected to provide reliable genetic information for molecular characterization, evaluation and utilization, and thereby a more efficient management of the available genetic resources in *Hevea*.

Key words: *Hevea* germplasm; Wickham clone; disease; genetic; molecular characterization; RAPD;

INTRODUCTION

The original narrow genetic base of cultivated *Hevea* in the East has been further narrowed down due to different reasons like directional selection for yield alone, cyclical generation-wise assortative mating (GAM) and large scale cultivation of a handful of high yielding clones propagated by budding. Cyclical GAM, with the best genotypes in one breeding cycle used as parents for the next, has led to selection and release of clones which are more or less related. Similarly, as a result of the emphasis on yield alone, a certain amount of genetic erosion for characters like disease resistance has taken place. Thus the pedigree of present day popular clones bred in Malaysia (Tan, 1987) and India (Varghese, 1992) can be traced back to a handful of parent clones. Hence, expansion of the breeding pool is essential for further genetic advance through breeding. Coupled with this is the increasing need for a new array of genes to meet the demands created by expansion of rubber cultivation into non - traditional areas in India, where severe abiotic stresses like drought and cold prevail. In the traditional areas too, several hitherto minor diseases like *Corynespora* leaf spot are becoming serious, for which sources of resistance have to be found. The timely action taken by the IRRDB in 1981, in collaboration with EMBRAPA of Brazil, to collect and conserve the fast diminishing genetic resources of *Hevea* from the Amazon forests, resulted in a huge quantity of germplasm collected from the Brazilian states of Acre (AC), Rondonia (RO) and Mato Grosso (MT). The material consisted of 64723 seeds and budwood of 194 high yielding trees (ortets) (IRRDB, 1982), which was shared between IRRDB and Brazil. IRRDB member countries received their shares from the African and Malaysian distribution centers in phases over the ensuing years. India has also received a share of this fresh germplasm, which is being conserved, characterized and evaluated, with the objective of broadening the gene pool for further genetic advance and also for achieving yield stability and sustainability.

CONSERVATION

India received the wild germplasm from the Malaysian distribution center during the period 1984 -1990. A total of 4967 accessions has been successfully established in two centers: one in the traditional rubber growing belt of Kerala and the other in the non-traditional regions of North East India in Assam and Tripura. In Kerala, the total number was 3617 including 97 ortets, while the figure in the North-East was 1350. Fig.1 shows the relative proportion from the three provenances. Out of the total 4967 wild genotypes established, 1885 belong to Acre, 1795 to Rondonia, 768 to Mato Grosso, while 47 are of mixed origin (including the accessions collected near Manaus). This material was established in different conservation/source bush nurseries, 6-16 plants per genotype, at a spacing of 1m x 1m, inter-planted with suitable controls ensuring proper identity. In these nurseries, one plant per genotype is allowed to grow without pruning in order to gather early information on tree habit, flowering pattern, floral characteristics and seed morphology.

CHARACTERIZATION, EVALUATION AND DOCUMENTATION

Characterization, evaluation and documentation precede utilization of wild germplasm. Characterization forms an important component of any germplasm conservation and evaluation

program. This is carried out in the preliminary evaluation stage, using a set of descriptors developed for the purpose. Abraham *et al.* (1994) reported morphological characterization of the wild germplasm at the juvenile phase (Table 1).

Evaluation of this large number of accessions is done in two stages, viz. a preliminary evaluation where all accessions are put in a field trial in a statistical design, and a further evaluation stage, where selections from the preliminary stage are subjected to a more detailed study. Due to shortage of land initially the preliminary evaluation trials were laid out adopting a close spacing of 2.5 m × 2.5 m, in a simple lattice design with four replications and RRII 105 as control. The ortets, being preliminary selections and few in number, are planted at normal spacing in an RBD or lattice design. Currently preliminary evaluation trials are planned employing augmented RBD with suitable controls so as to accommodate a large number of genotypes at a time. Data from source bush nurseries are taken into consideration for selection of genotypes for field evaluation rather than random selection. The promising materials identified from the preliminary evaluation trials are passed on to the further evaluation trials, adopting normal spacing.

Being the national agency dealing with *Hevea* germplasm research activities, Rubber Research Institute of India maintains a National Accession Register with all the basic data. RRII has initiated steps to prepare an extensive and user friendly data base of *Hevea* germplasm, where all the data generated over the different phases of evaluation, can be stored and retrieved as and when necessary. Preparation of a herbarium of the wild accessions is in progress.

EVALUATION AT THE NURSERY LEVEL

Data on several morphological and anatomical traits in the early growth phase were recorded from random samples of the wild germplasm in the different nurseries, with a view to studying the extent of variability for these traits and to identify promising materials at the particular growth stage. The morphological traits observed include height, girth, number of leaf whorls and leaves, leaf size, specific leaf weight, leaf area index and aerial biomass. The anatomical traits include bark thickness, number of latex vessel rows -both in the soft and in the hard bast, density of latex vessels per row, diameter of latex vessels, average distance between latex vessel rows and laticifer cross sectional area. Juvenile yield was also recorded by test tapping the samples under observation.

Considerable variability was observed for most of the traits in all the studies (Annamma *et al.*, 1988, 1989; Abraham *et al.*, 1992; Mercy *et al.*, 1992, 1993; Madhavan *et al.*, 1993; Reghu *et al.*, 1996; Rao *et al.*, 1996). Test tap yield showed the highest variability. In general, the wild genotypes recorded poor yield, as anticipated, when compared to the domesticated clones. However, a few promising genotypes were identified with respect to yield, the performance of which has to be verified in the evaluation trials.

Tables 2 & 3 give the range of variability observed in different samples of wild accessions for some morphological and anatomical traits. Provenance-wise analysis of the data from one study involving 75 accessions at an age of 18 months showed that genotypes from Mato Grosso were superior for number of flushes, leaf area index, total leaf weight and leaf area ratio, while those from Rondonia were superior for dry matter accumulation. In another set of 100 accessions evaluated at the age of two years (Abraham *et al.*, 1992), it was observed that though in general

the Mato Grosso accessions were the highest yielders, the maximum variability for this trait was recorded for Rondonia. Mato Grosso genotypes also showed the highest variability as well as the highest mean for bark thickness, number of latex vessel rows and laticifer area index. However maximum variability as well as the highest means for vigour in terms of girth, height and leaf size were exhibited by Acre genotypes. Alwi and Suhendry (1992) also reported similar trends from Indonesia. It was also seen that yield, leaf size and number of latex vessel rows had high heritability values. Rao *et al.* (1996) observed high variability for the first branching height among the wild accessions, with genotypes from Mato Grosso showing the maximum variability for this trait.

The direct and indirect effects of the important morphological and anatomical traits on juvenile yield have been worked out (Table 4). Number of latex vessel rows and girth were seen to have the highest direct effects on test tap yield (Madhavan *et al.*, 1996). In another study, aerial biomass was found to have a relatively high correlation with juvenile yield (Mercy *et al.*, 1993).

Based on D² analysis for five morphological parameters, viz. girth, height, bark thickness, single leaf area and juvenile yield, a set of genotypes was clustered into five groups of 76, 9, 3, 4, and 3 accessions respectively (Fig.2). Acre genotypes had a wider distribution in the clusters compared to the other two provenances, indicating the existence of greater genetic diversity among them. Plant height, leaf size and yield contributed the maximum to the diversity observed (Abraham *et al.*, 1995). Several potentially superior accessions for desirable traits like girth (55 accessions), number of latex vessel rows (29), bark thickness (21), yield (15), have been located among the accessions that have been studied so far.

Observations were initiated on floral and fruit morphology with the objectives of characterization and examining the extent of variation present for these traits. Five accessions which differed from typical *H. brasiliensis* in traits of taxonomical significance like the growth pattern, colour of flowers, presence of staminal disc in the male flowers and morphology of fruits (Figs.3 a-d) were identified from one of the nurseries. The taxonomical status of the material is being investigated. Madhavan *et al.* (1997) have discussed the possibility of such variants being infra-specific variants or inter-specific hybrids. Though flowers with anthocynated bases in earlier collections (Nicolas, 1992) were reported, no such observation has been made from this collection so far.

Variability observed for leaf whorl characteristics in the wild germplasm is shown in Figs.4a & b. Preliminary observations carried out in the nurseries indicate that the Mato Grosso genotypes had better leaf retention during the *Phytophthora* leaf fall season (Figs.5a & b) and hence are likely to be more tolerant to this disease than those of other provenances (Mercy *et al.*, 1995). Another survey carried out in all the nurseries at the peak *Oidium* season revealed considerable variability for disease incidence, indicating the possibility of selection of an array of genotypes with different levels of resistance. However, further studies are required for confirmation of the disease reaction.

PRELIMINARY EVALUATION TRIALS

Selected accessions from the nursery studies are being subjected to field evaluation, in a phased manner, for their performance in terms of growth characters, structural parameters, rubber yield,

incidence of major diseases and other desired secondary parameters. The micro-morphological and histological parameters also supplement the selection of potential genotypes for hot spot screening for resistance to various abiotic factors. So far a total of 612 accessions have been established in eight preliminary evaluation trials planted during 1990 - 1999. Plant height, girth and other growth characteristics recorded in all the trials in the first two years of growth revealed that many of the accessions surpassed the control vigour. Accessions from Acre were in general more vigorous than those from the other two provenances. Among 50 ortets evaluated for yield at the immature phase, three showed encouraging trends, though most of the clones were poor yielders. Similarly, in another set of clones, test tapping in the early years of growth has revealed that most of the wild accessions gave much lower yields compared to the agronomically superior control clones. Similar results have been reported from Malaysia (IRRDB, 1996), Ivory Coast (Clement-Demange *et al.*, 1997) and Vietnam (Lam *et al.*, 1997). The first preliminary evaluation trial was opened for regular tapping in 1998, and three of the accessions were found to be promising. Characterization of the accessions is being done in the preliminary evaluation trials following a descriptor prepared for the purpose.

FURTHER EVALUATION TRIALS

The further evaluation is carried out in the traditional zones for yield and yield related traits, while hotspot screening for biotic and abiotic stresses is carried out at specific locations. Selections from these different trials are valuable materials for direct use and / or utilization in breeding programs in order to fulfill the initial objectives of the collection, viz., widening the genetic base and utilizing the desirable attributes present in the wild germplasm.

Based on preliminary data collected on traits like girth, juvenile yield, number of latex vessel rows, bark thickness, drop in summer girth increment, *etc.* in comparison with RR11 105, 80 accessions were selected. These were planted in a further evaluation trial at the Regional Research Station in North Kerala in 1995. The design adopted was simple lattice with four replications, at a spacing of 5m x 5m and the control clone was RR11 105. Wide variability for growth form and morphological parameters like leaf size and shape, orientation, *etc.* were observed. A good number of genotypes exhibited vigorous growth in terms of girth, number of whorls and leaves at the early growth period. Further observations are in progress. Test tapping and bark anatomical observations are underway to confirm the potential of these materials.

TIMBER- LATEX STUDIES

Identification of desirable genotypes for qualitative and quantitative timber traits from the wild germplasm has been recognized as a priority area since rubber wood has been accepted as an alternate source of commercial hardwood. Screening of the wild genotypes for timber-latex traits has been envisaged and initiated in India. Among the three provenances, Acre genotypes were observed to be more vigorous with long, straight trunks and less branching, which is a desirable feature for timber clones. On the other hand, the growth habit of genotypes from Mato Grosso resembled that of Wickham clones, with short and stout trunks and profuse branching. Based on yield, vigour, trunk and branching habits, 31 wild genotypes from different states of Acre, Rondonia and Mato Grosso were selected for screening for timber-latex traits.

DISEASE SCREENING

In India, the two major leaf diseases causing considerable crop loss are abnormal leaf fall caused by *Phytophthora spp.* and powdery mildew caused by *Oidium hevea*. However, there are recent reports that the incidence of *Corynespora* leaf spot has become severe in the popular clone RR11 105 in the rubber growing tracts of Karnataka and certain parts of Kerala. Screening of all available sources of germplasm for resistance to various diseases has been recognized as a priority. Visual inspection of the source bush nurseries indicated encouraging results regarding field tolerance of the wild genotypes to major leaf diseases, indicating the chances of selection of potential sources of resistance. Laboratory screening for resistance to *Phytophthora spp.* is under way. Field screening for *Oidium* resistance has also revealed wide variability and the results are being analyzed.

DROUGHT SCREENING

Screening for drought tolerance has been initiated in the wild germplasm. On the basis of drop in summer girth in one of the preliminary evaluation trials a set of 99 wild and one Wickham clone (RR11 105) was identified to examine the extent of variability for cellular membrane stability, as part of the screening programme for drought. Leaf samples subjected to heat and drought stresses in the laboratory were analyzed for the degree of injury to the cell membranes. Variability was observed for membrane thermo-stability of leaf tissues, based on which these clones were graded for drought tolerance (Fig 6). Of the 99 wild accessions examined, 17 showed high resistance to injury. The heritability for this trait was also found to be high.

Hotspot screening of a set of 36 genotypes is being carried out at Regional Research Station, Sukma, in the state of Madhya Pradesh, which is a drought prone area.

MOLECULAR LEVEL STUDIES

Studies on molecular markers have been initiated in India. Variation in RAPD profile between TPD affected and normal plants (Thulaseedharan *et al.*, 1994), and for *Phytophthora* leaf fall (Jacob, 1996) were observed. Varghese *et al.* (1997) reported the potential of RAPD markers for genetic analysis in *Hevea*. Currently work is in progress to establish the genetic relationships in the germplasm based on genetic distance using RAPD analysis. 60 wild accessions representing the three proveniences were selected based on their superior performance over control RR11 105, for girth, bark thickness, number of latex vessel rows and dry rubber yield. Molecular characterization of all the accessions is proposed in a phased manner.

UTILIZATION

Accessions showing superiority over the control for individual traits like girth, number of latex vessel rows, density of latex vessels, disease tolerance, drought tolerance (based on drop in summer girth), and nursery yield, have been incorporated in hybridization programs with the objectives of broadening the genetic base as well as evolving superior clones. Among 12 cross combinations involving wild genotypes and two popular clones, juvenile yield at two years growth in the nursery was highest for the combination RR11 105 X RO/JP/3/6 (RR11, 1994). Selections from these are under field evaluation in a small-scale trial adopting a suitable

statistical design. A total of 489 progeny from another set of crosses made in 1997 involving 9 wild accessions, and RR11 105 and RRIM 600 as the female parents, are under nursery evaluation. Field evaluation of accessions having potential for development of timber latex clones is being initiated.

FUTURE PROSPECTS

Presently the germplasm accessions are conserved *ex situ*, which, however, are prone to natural calamities. Hence feasibility studies on alternate *in vitro* methods, viz., cryo-preservation, are envisaged.

Available reports reveal the positive response of *Hevea* embryonic axes to cryopreservation protocols (Normah and Chin, 1995; Engelmann *et al.*, 1997). However, the effect on long-term storage and regeneration are yet to be established. Hence further studies are required to establish the feasibility of this *in vitro* technique as a convenient and secure alternative to the present *ex situ* method of conservation of the large collection of *Hevea* germplasm.

Similarly, for characterization and evaluation, an optimum number of descriptors, which are highly heritable, should be utilized. A set of descriptors for *Hevea* has already been suggested which has to be updated/modified from time to time so as to accommodate all relevant traits of importance (Varghese and Abraham, 1999).

The success of all crop improvement programmes depends on proper evaluation and efficient utilization of all the available genetic resources. In *Hevea*, hybridization between widely divergent genotypes can result in greater range of variability especially in view of the highly heterozygous nature of the crop. Hence there are greater chances of heterosis and superiority of the resultant progeny in crosses involving cultivated clones and potential wild germplasm. Early performance of the progeny of such crosses indicates promising results. However, further systematic studies are required for the identification of wild genotypes with desired traits for introgression into cultivated clones.

The real potential of the wild germplasm lies in its ability to supply genes for individual traits like tolerance to biotic and abiotic stresses, as well as individual yield contributing traits like high number of latex vessels, increased photosynthetic capacity, *etc.* Considering the vast area and different eco-geographical conditions to which the *Hevea* plants have been adapted in the wild, this collection must be a repository of a wide range of valuable genes scattered in the different accessions. In the context of rubber cultivation having been extended to non traditional areas, development of location specific clones capable of withstanding different constraints, like low winter temperature, high summer temperature and prolonged drought, high velocity winds and occasional hailstorms, high altitudes, depleted soils, *etc.* is a priority. Wild germplasm is definitely a source of many valuable genes capable of withstanding such stress situations. The challenge before the breeder lies in evolving strategies for identifying these genes and incorporating into high yielding clones.

Anticipating the scenario of wide spread cultivation of rubber for quality timber production in addition to latex yield, there is a vast scope to develop clones combining high timber production with superior quality and durability along with high latex yield. The quality and durability of rubber timber can be improved by enhancing the lignin biosynthesis in wood

fibers and metabolite conversion into polyphenolic compounds.

In *Hevea*, molecular techniques have already been initiated in different countries for the analysis of the extent and distribution of genetic diversity among Wickham clones and wild germplasm (Chevallier *et al.*, 1988; Besse *et al.*, 1994; Clement-Demange *et al.*, 1997). Data on genetic distance is required for identification of diverse sub populations. The molecular techniques provide reliable genetic information for molecular characterization, evaluation and utilization of germplasm resources (Kresovich *et al.*, 1992; Karp and Edwards., 1997). Among the different techniques, RAPDs have wide application in germplasm evaluation since they are easier and faster for screening large numbers of genotypes and assay of genetic variability at the whole genome level (Varghese, 1998).

Marker Assisted Selection (MAS) offers much scope for selection of genotypes resistant to various diseases, cold, drought, tapping panel dryness syndrome, *etc.* Once molecular markers linked to such targeted genes are located, screening of germplasm and segregating progeny for the desired traits will become a very reliable, fast and routine process saving much time, money and effort. Regular use of such techniques will be of great help for molecular characterization, identification and evaluation of germplasm, construction of molecular maps and identification of markers linked to economically important traits.

Further systematic studies incorporating conventional and molecular techniques are required for yield stability and sustainability in *Hevea*. There is no doubt that the wild germplasm is the best raw material not only to realize the current objectives like development of location specific clones capable of withstanding various diseases, cold, drought, high elevation *etc.* but also to meet the future demands of the industry.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support and encouragement from Dr. N.M. Mathew, Director, RRII, for the preparation of this paper. Thanks are also due to Mr. Francis Mathew, Res. Scholar for the help in the preparation of the manuscript.

REFERENCES

- Abraham S.T., C.P. Reghu, J. Madhavan, P.J. George, S.N. Potty and A.O.N. Panikkar. 1992. Evaluation of *Hevea* germplasm: Variability in early growth phase. Indian Journal of Natural Rubber Research, 5(1 & 2): 195-198.
- Abraham, S.T., C.P. Reghu, A.O.N. Panikkar, S.N. Potty and P.J. George. 1994. Juvenile characterization of wild *Hevea* germplasm. Indian Journal of Plant Genetic Resources, 7(2): 157-164.
- Abraham, S.T., C.P. Reghu, P. J. George, S.N. Potty and P. Saraswathi. 1995. Evaluation of *Hevea* germplasm: III. Genetic divergence in certain genotypes of *Hevea*. Paper presented in the 82nd Indian Science Congress, Calcutta, 1995.
- Alwi, N. and I. Suhendry. 1992. Country reports on collection, conservation and evaluation of *Hevea* germplasm in Indonesia. Proc. IRRDB Joint Meetings of Breeders, Pathology and Physiology and Exploitation groups, Indonesia, October 1992, pp.1-5.

- Annamma, Y., J.G. Marattukalam, D. Premakumari and A.O.N. Panikkar. 1988. Nursery evaluation of 100 Brazilian genotypes of *Hevea* in India. Colloque *Hevea* 88, IRRDB, Paris, pp.353-364.
- Annamma, Y., J.G. Marattukalam, P.J. George and A.O.N. Panikkar. 1989. Nursery evaluation of some exotic genotypes of *Hevea brasiliensis* Muell. Arg. Journal of Plantation Crops, 16 (supplement): 335-342.
- Besse, P., M. Seguin, P. Lebrum, M.H. Chevalier, D. Nicolas and Lanaud. 1994. Genetic diversity among wild and cultivated populations of *Hevea brasiliensis* assessed by nuclear RFLP analysis. Theoretical and Applied Genetics, 88:199-207.
- Chevallier, M.H., P. Lebrum, and F. Normand. 1988. Approach to the genetic variability of germplasm using enzymatic markers. Colloque *Hevea*, 88: 365 - 376.
- Clement-Demange, A., H. Legnate, T. Chapuset, F. Pinard and M. Seguin. 1997. Characterization and use of the IRRDB germplasm in Ivory Coast and French Guiana: Status in 1997. Proc. IRRDB Symposium on Natural Rubber, Vol 1: General, Soils and Fertilizations and Breeding and Selection session, Ho Chi Minh City, 14th and 15th October 1997, pp. 71-88.
- Englemann, F., M. Lartaud, N. Chabrillange, M. P. Carron and H. Etienne. 1997. Cryopreservation of embryogenic calluses of two commercial clones of *Hevea brasiliensis*. Cryoletters, 18:107-116.
- IRRDB. 1982. Status report of Primary nursery. Manaus. In: The 1981 Germplasm Project: Reports from the three centers and on the meeting of Senior Plant Breeders, Brazil, 18th June 1982.
- IRRDB. 1996. Quarterly Report.
- Jacob, C.K. 1996. *Phytophthora* diagnosis. IRRDB information quarterly, 5:14.
- Karp, A. and K.J. Edwards. 1997. Techniques for the analysis, characterization and conservation of plant genetic resources. Report on IPGRI Workshop. 9-11 October 1995, Rome.
- Kresovich, S., J. G. K. Williams, B. A. Schaal, J. R. McFerson and E. Routman. 1992. Characterization of genetic identities and relationships of *Brassica oleracea* via. random polymorph DNA assay. Theoretical and Applied Genetics, 85: 190-196.
- Lam, L.V., T. T. T. Ha, V. T. T. Ha and T. Hong. 1997. Studies of *Hevea* genetic resources in Vietnam: Results of evaluation and utilization. Proc. IRRDB Symposium on Natural Rubber. Vol 1: General, Soils and Fertilization and Breeding and Selection Sessions. Ho Chi Minh City. 14th and 15th October 1997, pp. 89-100
- Madhavan, J., C.P. Reghu, S.T. Abraham, P.J. George and S.N. Potty. 1993. World *Hevea* germplasm. Paper presented in the ISPGR Dialogue on Plant Genetic Resources: Developing National Policy, NBPGR, New Delhi, 1993.
- Madhavan, J., C.P. Reghu, S.T. Abraham, P.J. George and S.N. Potty. 1996. Evaluation of *Hevea* germplasm: VII. Association analysis in wild *Hevea* germplasm. Journal of Plantation Crops, 24(Suppl.): 453-457.
- Madhavan, J., S.T. Abraham, C.P. Reghu and P.J. George. 1997. A preliminary report on two floral variants in the 1981 wild *Hevea* germplasm collection. Indian Journal of Natural

Rubber Research. (In press).

- Mercy, M.A., S.T. Abraham, P.J. George, S.N. Potty, M.R. Sethuraj and P. Saraswathi. 1992. Preliminary observations of the 1981 IRRDB *Hevea* germplasm. II. Variability, dry matter and morphological characters. *Journal of Plantation Crops*, 21(Supplement): 268-274.
- Mercy, M.A., S.T. Abraham, S.N. Potty, P.J. George and P. Saraswathi. 1993. Evaluation of *Hevea* germplasm V: Metroglyph and index score analysis. Paper presented in Golden Jubilee Symposium on Horticultural Research: Changing Scenario, Bangalore, 1993.
- Mercy, M.A., S.T. Abraham, P.J. George and S.N. Potty. 1995. Evaluation of *Hevea* germplasm: Observation on certain prominent traits in an observatory. *Indian Journal of Plant Genetic Resources*, 8(1): 35-39.
- Nicolas, D. 1992. IRRDB *Hevea* germplasm African Center: Status of the 1981 collection in July 1992. Proc. IRRDB Joint Meeting - Breeding Pathology and Physiology and Exploitation groups. 27 - 28 October, 1992, Jakarta, pp. 15-35.
- Normah, M.N., and H.F. Chin. 1995. Cryopreservation of germplasm of rubber (*Hevea brasiliensis*). *Biotechnology in Agriculture and Forestry*, Vol. 32. Cryopreservation of Plant Germplasm (Ed. Y.P.S. Bajaj), Springer Verlag Berlin Heidelberg.
- Rao, G.P., C.P. Reghu and P.J. George. 1996. Evaluation of *Hevea* germplasm VIII: Variability in certain juvenile characters of wild *Hevea* germplasm. Paper presented in 6th All India Conference on Cytology and Genetics, Rohtak, March 1996.
- Reghu, C.P., S.T. Abraham, J. Madhavan, P.J. George, S.N. Potty and K.P. Leelamma. 1996. Evaluation of *Hevea* germplasm: Variability in bark structure of wild Brazilian germplasm. *Indian Journal of Natural Rubber Research*, 9(1): 28-31.
- RRII. 1994. Annual Report, 1992-93. RRII, Kottayam, p. 6.
- Tan, H. 1987. Strategies in rubber tree breeding. In: Abbot and Atkin (Eds). *Improving vegetatively propagated crops*. Academic Press, London, pp. 27-62.
- Thulaseedharan, A., B.B. Chattoo, M.P. Asokhan and M.R. Sethuraj. 1994. Preliminary investigations on variations in RAPD profiles between TPD affected and normal plants from a seed propagated rubber plantation. Proc. Workshop on Tapping Panel Dryness of *Hevea*, Chinese Academy of Tropical Agricultural Sciences, Hainan, China. pp. 26-28.
- Varghese, Y.A. 1992. Germplasm resources and genetic improvement. In: Sethuraj, M.R. and N.M. Mathew (Eds). *Natural Rubber: Biology, Cultivation and Technology*. El Sevier, Amsterdam, pp. 88-115.
- Varghese, Y.A. 1998. Random Amplified Polymorphic DNA (RAPD) Technique and its application. In: Varghese, Ed. *Molecular Approaches to Crop Improvement*, Department of Botany, CMS College, Kottayam, pp. 53-70.
- Varghese, Y.A. and S.T. Abraham. 1999. Germplasm conservation, utilisation and improvement in rubber. In: *Crop Improvement in Plantation Crops*. Central Plantation Crops Research Institute, Kasaragod. (In press)
- Varghese, Y.A., C. Knaak, M.R. Sethuraj and W. Ecke. 1997. Evaluation of random amplified polymorphic DNA (RAPD) markers in *Hevea brasiliensis*. *Plant Breeding*, 116:47-52.

Table 1. Cataloguing of a group of wild accessions based on a set of morphological descriptors

Sl.no	Characters	AC	RO	MT	Sl.no	Characters	AC	RO	MT
1	Habit of the plants				5.3	External appearance			
1.1	Tall and stout	4	4	1	5.3.1	Open	25	25	28
1.2	Tall and lean	4	18	10	5.3.2	Close	0	2	0
1.3	Medium stout	2	0	1	5.3.3	Intermediate	5	11	7
1.4	Medium and lean	14	4	15	6	Leaves			
1.5	Dwarf and stout	0	0	0	6.1	Pulvinus			
1.6	Dwarf and lean	1	1	1	6.1.1	Swollen	24	26	25
2	Girth of the plants				6.1.2	Normal	1	1	3
2.1	Above average	3	11	8	6.2	Petiole			
2.2	Average	14	13	13	6.2.1	Shape			
2.3	Below average	8	3	7	6.2.1.1	Arched		1	1
3	Branching				6.2.1.2	Concave	17	18	8
3.1	Early branching	0	0	0	6.2.1.3	Straight	7	4	19
3.2	Late branching				6.2.1.4	S' shape	1	4	
3.3	No branching				6.2.2	Size			
4	Nodes				6.2.2.1	Long	4	5	1
4.1	Axillary buds				6.2.2.2	Medium	17	16	20
4.1.1	Protruding	0	0	0	6.2.2.3	Short	4	7	7
4.1.2	Sunken	0	0	0	6.2.3	Angle			
4.1.3	Normal	25	27	28	6.2.3.1	Acute	22	24	27
4.2	Leaf scars				6.2.3.2	Horizontal	3	3	1
4.2.1	Pronounced margin	20	12	8	6.2.3.3	Optuse	0	0	0
4.2.2	Normal margin	5	15	20	6.3	Petiolule			
4.3	Nature of leaf scars				6.3.1	Orientation			
4.3.1	Sunken	5	3	0	6.3.1.1	Upward	14	17	16
4.3.2	Normal	20	24	28	6.3.1.2	Horizontal	2	3	7
5	Leaf storey				6.3.1.3	Downward	9	7	5
5.1	Shape				6.3.2	Size			
5.1.1	Conical	0	1	0	6.3.2.1	Long	1	8	2
5.1.2	Truncate	1	1	2	6.3.2.2	Medium	18	18	22
5.1.3	Bow shaped	1	1	1	6.3.2.3	Short	6	1	4
5.1.4	Hemispherical	23	24	25	6.3.3	Extra floral nectary			

Table 1 (contd.)

Sl.no	Characters	AC	RO	MT	Sl.no	Characters	AC	RO	MT
5.2	Separation				6.3.3.1	Prominent	14	10	21
5.2.1	Well separated	20	13	20	6.3.3.2	Less prominent	11	17	7
5.2.2	Not well separated	0	3	1					
5.2.3	Intermediate	5	11	7					
6.4	Leaflets				6.4.8	Appearance (Cross section view)			
6.4.1	Colour				6.4.8.1	Straight	11	12	24
6.4.1.1	Green	20	21	22	6.4.8.2	V' shaped	2	1	1
6.4.1.2	Dark green	5	6	6	6.4.8.3	Boat shaped	12	14	3
6.4.1.3	Yellowish green	0	0	0	6.4.8.4	Convex shaped			
6.4.2	Luster				6.4.9	Leaf apex			
6.4.2.1	Glossy	0	4	5	6.4.9.1	Aristate	1	0	1
6.4.2.2	Dull	25	23	23	6.4.9.2	Accuminate	2	2	5
6.4.3	Texture				6.4.9.3	Cuspidate	0	3	1
6.4.3.1	Smooth	0	0	0	6.4.9.4	Apiculate	22	22	22
6.4.3.2	Leathery	25	27	28	6.4.10	Lateral appearance			
6.4.4	Shape				6.4.10.1	Flat	16	15	21
6.4.4.1	Elliptic	24	21	26	6.4.10.2	Convex	1	0	0
6.4.4.2	Lanceolate	0	0	0	6.4.10.3	S' shape	8	12	7
6.4.4.3	Obovate	1	6	2	6.4.11	Arrangement			
6.4.5	Size				6.4.11.1	Margin touching	4	6	7
6.4.5.1	Large	11	11	2	6.4.11.2	Overlapping	0	4	1
6.4.5.2	Very large	1	0	0	6.4.11.3	Separated	21	17	20
6.4.5.3	Medium	13	16	26	6.4.12	Vein colour			
6.4.5.4	Small	0	0	0	6.4.12.1	Yellow	23	25	28
6.4.6	Thickness				6.4.12.2	Light green	2	2	0
6.4.6.1	Thick	2	4	0	6.4.13	Nature of vein			
6.4.6.2	Thin	23	23	28	6.4.13.1	Prominent	9	12	4
6.4.7	Margin				6.4.13.2	Not prominent	16	15	24
6.4.7.1	Smooth	14	8	15	6.4.14	Leaf blade dorsal side			
6.4.7.2	Wavy	11	19	13	6.4.14.1	Smooth	25	25	22
					6.4.14.2	Irregular	0	2	6

Table 2. Variability for morphological traits in a population of wild germplasm at the age of 18 months

Trait	Mean values for the genotypes from the three provenances			Range		General mean	Control
	RO	AC	MT	Minimum	Maximum		
No. of flushes	12	10	13	4	21	11.2	13.6
Leaves in 3 rd flush	12	10	10	6	18	11	9.6
Leaf area index	2.0	2.3	2.5	0.59	4.3	2.2	2.6
Total leaf wt. (g)	193.6	224.8	243.5	63.2	444.6	215.2	275.4
Aerial biomass (g)	1153.5	1049.0	1096.0	393.5	2415.1	1106.5	1479.3
Leaf area ratio	18	22.7	23.4	9.05	42.41	20.76	17.62
Specific leaf wt.(g)	0.01	0.01	0.01	0.008	0.012	0.01	0.011
Leaf shoot ratio	0.22	0.29	0.31	0.107	0.594	0.262	0.229

Table 3. Variability for growth and structural characters in a population of wild germplasm at the age of 2 years.

Characters	Provenance range			General Mean	Controls	
	AC	MT	RO		GT 1	RRII 105
Girth (cm)	13.08 - 18.25 (15.43)	13.58 - 16.58 (14.95)	12.42 - 16.67 (14.66)	15.1	17.3	16.2
Height (cm)	436.00 - 766.00 (598.78)	424.00 - 614.00 (525.21)	443.00 - 689.00 (576.90)	570.9	561.0	538.0
Juvenile yield (g)	0.06 - 0.95 (0.38)	0.25 - 2.95 (1.07)	0.04 - 1.39 (0.38)	0.6	6.3	6.5
Leaf size (cm ²)	82.3 - 227.0 (141.84)	80.9 - 120.8 (93.74)	72.2 - 232.0 (118.89)	120.7	77.0	59.6
Bark thickness (mm)	1.83-3.17 (2.29)	2.16-3.04 (2.62)	2.0-2.79 (2.32)	2.37	2.92	2.92
TLVR	2.0-4.8 (3.52)	2.67-7.0 (4.06)	2.5-4.7 (3.60)	3.62	4.66	4.00
DLV	13.5-23.3 (19.35)	15.7-23.8 (20.09)	10.2-22.3 (18.78)	19.15	18.33	19.17
Diameter (µm)	18.86 - 26.23 (22.74)	14.29 - 27.99 (23.74)	17.78 - 24.16 (19.46)	22.01	21.98	22.16
TSCFZ	0.71 - 1.34 (0.97)	0.88 - 1.24 (1.07)	0.63 - 1.38 (1.0)	1.01	1.06	1.22
LCSA (mm ²)	0.72 - 3.95 (2.05)	0.63 - 8.82 (2.9)	0.93 - 3.42 (1.61)	2.17	2.82	2.42

TLVR: Total No. of Latex Vessel Rows; DLV: Density of Latex Vessels per 2 mm circumference of the plant; TSCFZ: Thickness of Stone Cell Free Zone; LCSA: Laticifer Cross Sectional Area.

(Values in parenthesis indicate provenance wise mean)

Table 4. Direct and indirect effects of some morphological and anatomical traits on test tapping yield.

Characters	Leaf size	SLW	BT	Girth	NLVR	DLV	Diameter	ADLR	Correlated with yield
Leaf size (cm ²)	-0.11	0.014	-0.025	-0.058	-0.063	-0.009	0.002	0.005	-0.24**
SLW	0.025	-0.060	0.022	0.060	0.111	-0.004	-0.009	-0.023	0.12
BT(mm)	0.036	-0.017	0.080	0.163	0.130	-0.004	-0.008	-0.014	0.39**
Girth(cm)	0.025	-0.014	0.052	0.250	0.100	-0.004	0.012	-0.005	0.42**
NLVR	0.019	-0.018	0.028	0.068	0.370	0.010	0.004	-0.057	0.43**
DLV	0.010	0.002	0.003	-0.010	0.037	0.100	-0.019	-0.005	0.12
Diameter (µm)	0.002	-0.005	-0.006	-0.030	-0.015	0.019	-0.100	-0.007	-0.14
ADLVR (mm)	-0.004	0.011	-0.009	-0.010	-0.163	-0.004	-0.005	0.130	-0.05

SLW: Specific leaf weight; BT: Bark Thickness; NLVR: Number of Latex Vessel Rows; DLV: Density of Latex Vessels per mm circumference of the plant; ADLR: Average Distance between Latex Vessel Rows

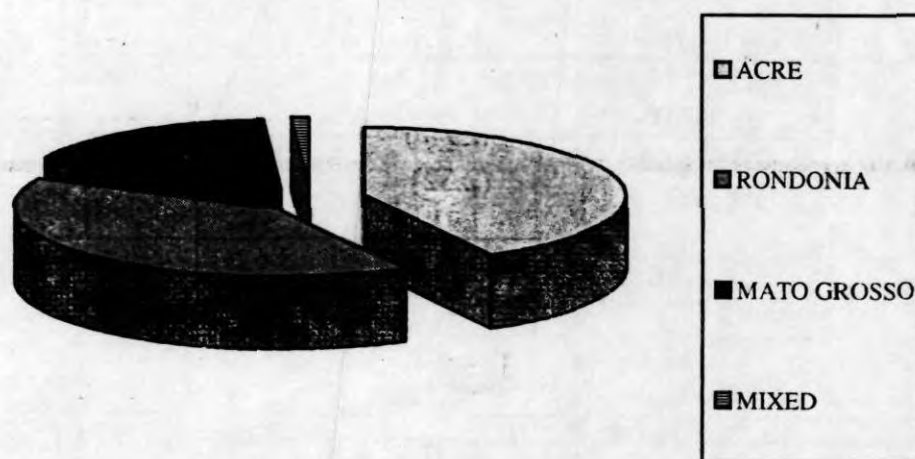


Fig. 1. Relative proportion of wild germplasm from different provenances.

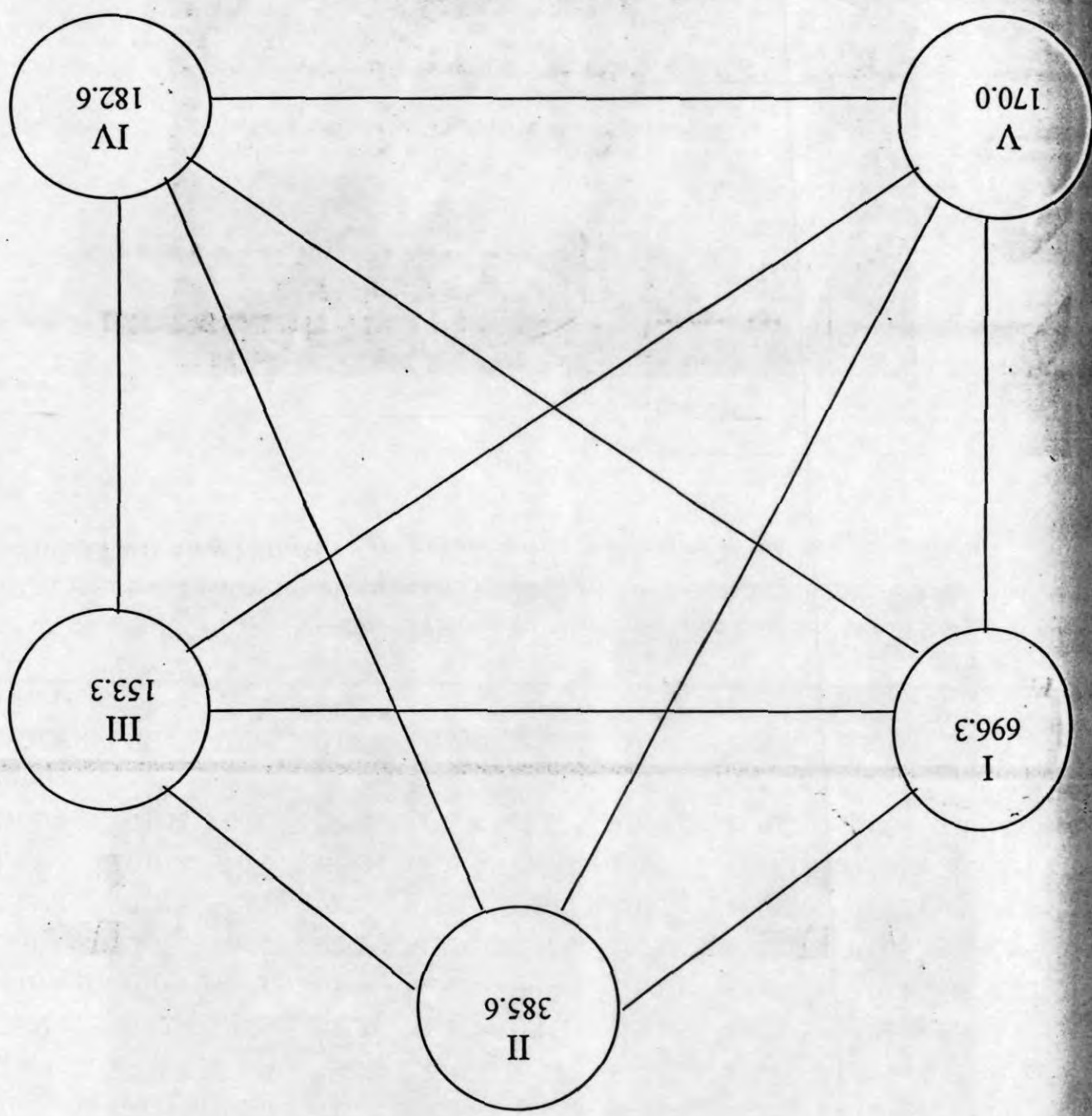


Fig. 2. Cluster diagram showing genetic diversity in the wild germplasm.



Figs. 3 a & b. Inflorescence of the variants identified in the wild germplasm.

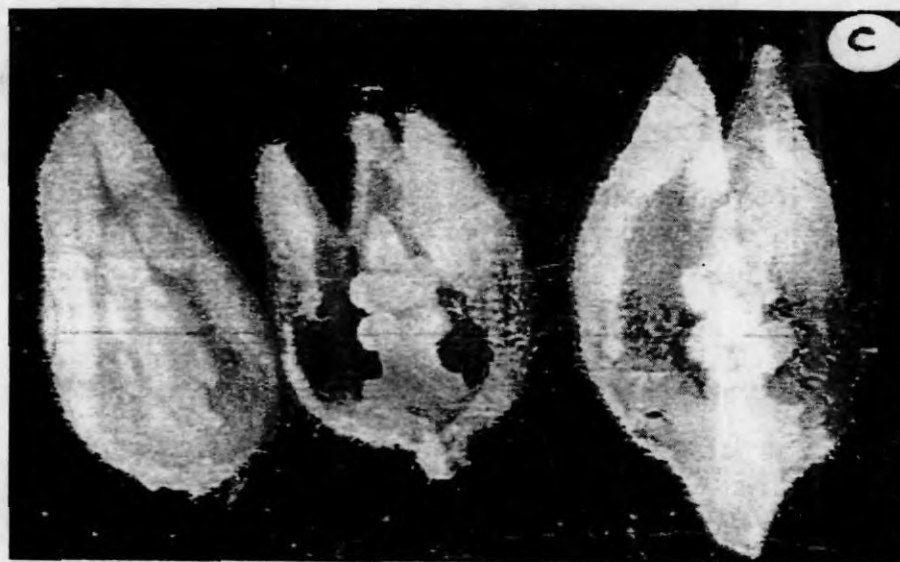


Fig. 3c. Dissected male flowers of the variants showing the staminal columns and the basal disks.

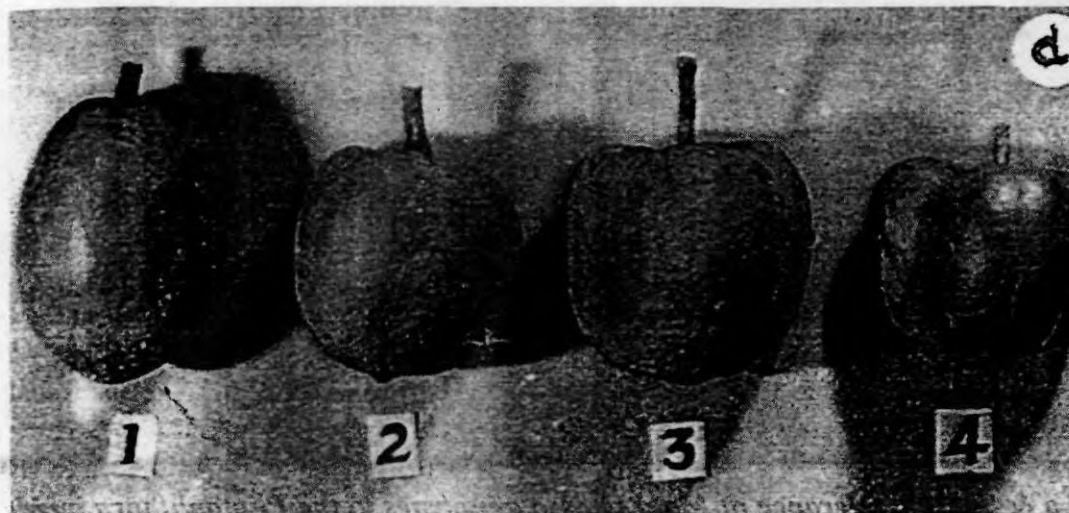
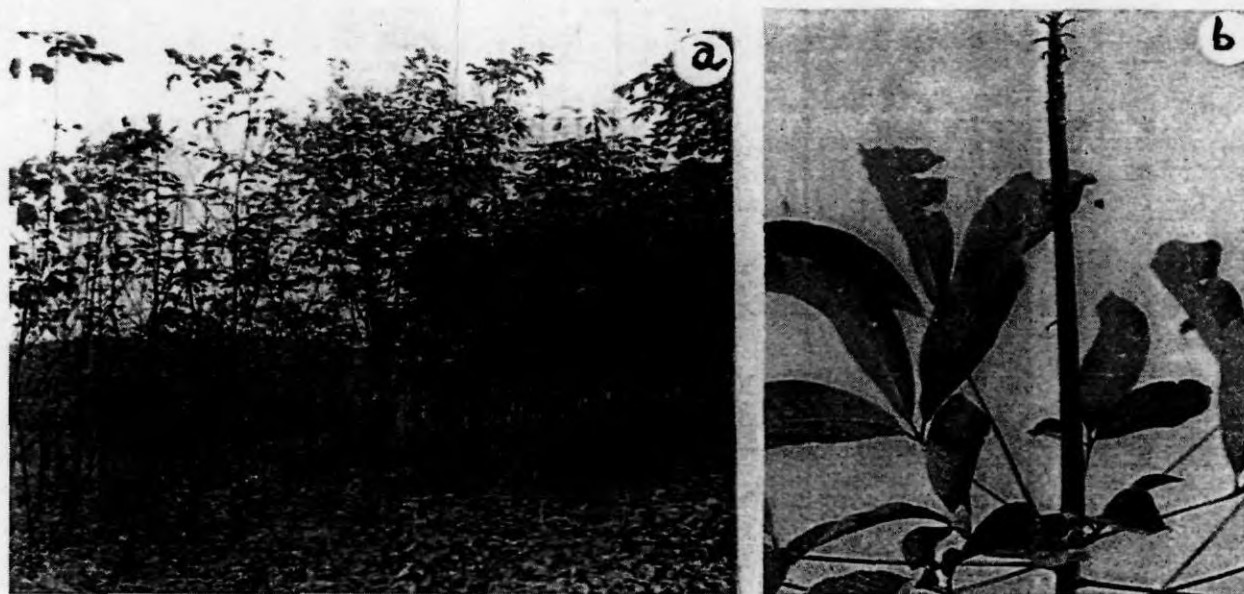


Fig. 3d. Fruits of *Hevea spruceana* (1), variants (2 & 3) and *Hevea brasiliensis* (4) showing the shape of the fruits.



Figs. 4 a & b. Differences in the leaf whorl characteristics of the wild *Hevea* germplasm.



Figs. 5 a & b. Field tolerance of Mato Grosso genotypes to the *Phytophthora* incidence.

Fig. 6. Variability for membrane thermostability of leaf tissues based on mean and standard deviation values in the wild germplasm

