SUMMARY AND CONCLUSIONS

Five spontaneous male sterile clones are identified from the existing exotic and indigenous populations of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. out of which two (Ch 2 and RRII 35) are reported for the first time in this crop.

Polyploidy was induced in a clone (RRII 105) and a tetraploid having a chromosome complement of $2n = 4x = 72$ was evolved. An artificial triploid ($2n = 3x = 54$) was synthesised by crossing diploid with the tetraploid. A radiation induced mutant and mutagen (Ethyl methane sulphonate) induced mutant showing male sterility were evolved.

Comparative studies on the morphology, cytology and genetics of these male sterile clones were carried out. A fertile clone, RRII 105, was investigated for comparison. There is distinct morphological variations in foliar and floral characteristics. Significant differences are noted among the clones with regard to growth attributes. The spontaneous male sterile clones GT 1, Ch 2, RRII 35, spontaneous triploid and tetraploid had comparatively more vigour
In terms of diameter and height, while radiation induced mutant was comparatively less vigorous.

Deviating from the typical trifoliolate condition of normal *Hevea* clone, variation in the number of leaflets (2 to 5) at the nursery stage is noted for induced triploid and tetraploid. Even though triploids are reported to be vigorous in the present investigation induced triploid is not vigorous. This may be due to the effect of back crossing.

The correlation of growth attributes of these clones at 30 months growth has shown significant correlation (\(P < 0.01\)) between height and girth.

The dwarf stature exhibited by induced triploid and radiation induced mutant can be incorporated in the breeding programme of *Hevea*.

The spontaneous triploid and RRII 17 have no fruitset, hence the incidence of leaf diseases are reported to be comparatively less. They can be incorporated for the crown modification.

Significant variations in flower size is also noted among these clones. The flowers are bigger in size for tetraploid, tri-
ploids and Ch 2 and comparatively smaller in radiation induced mutant and GT 1.

Mitotic studies have confirmed that the chromosome complement of triploid and tetraploid is \(2n = 3x = 54\) and \(2n = 4x = 72\) respectively, whereas the diploid shows \(2n = 2x = 36\).

Meiosis is normal in the spontaneous male sterile clones GT 1, Ch 2, RRII 35 and EMS induced mutant up to the formation of tetrads after which complete degeneration of cytoplasm and nuclei resulted in total male sterility.

Abnormalities in the tapetum are noted in GT 1 and Ch 2. Proliferation and persistence of tapetum is noted in GT 1 and Ch 2 whereas, complete degeneration of tapetum is noted in the normal fertile clone.

In the spontaneous sterile clone, RRII 17 wide spectrum of meiotic abnormalities are noted. Desynapsis is also observed. Predominant formation of univalents (8 to 32) is seen during metaphase I. Anaphase I is also highly irregular. Due to unequal segregation microspores of varying number (3 to 9) and size are observed.
In the radiation induced mutant, cytomelexls is observed in 30% of the meiocytes. Cytoplasmic connections are observed in all stages of PMCs ranging from early prophase to microspore stage. The male sterility in this plant is due to genetic imbalance caused by irradiation which result in wide range of meiotic abnormalities coupled with cytokinetic aberrations. Pollen conglomerates and megapollen are also observed.

Meiotic studies of triploid exhibited univalents, bivalents and trivalents. Anaphase I showed unequal segregation formation of laggards. The spontaneous triploid showed autotriploid nature since it showed 18III at metaphase I. The tetraploid showed large number of bivalents, followed by less formation of univalents, trivalents and quadrivalents. The distribution of nuclei during tetrad stage showed wide variations (2 to 6). Tetraploid showed 80% stainable pollen, induced triploid showed 5.5% whereas the spontaneous triploid did not have any stainable pollen.

Remarkable variation in the production of pollen grains was noted. Pollen production was practically absent in GT 1, RRII 35, mutagen induced mutant and spontaneous triploid. RRII 105 clone showed 150-200 pollen per anther (Table 3).

Variations in the number and size of germpores as well as
shape of pollen grains are reported in tetraploid and triploid compared to that of the diploid. A general correlation between polyploidy, genome complement and pollen characteristics was observed in the different cytotypes of *H. brasiliensis*.

The size of microsporocytes and microspores at different developmental stages of flowers of control RRII 105, Ch 2, triploid and tetraploid had shown considerable variations. The maximum size was noted at tetrad stage and minimum for the microspores within tetrad.

Scanning electron microscopic observations have given a better understanding of exine ornamentation in polyploid and sterile pollen grains. The sterile pollen grains showed normal exine formation, but exhibited a more or less aberrant pattern. Exine ornamentation is comparatively large for polyploids. In the sterile pollen differences in the exine pattern and size of columella are noted. However, the exine ornamentation is not perfect as in the case of fertile clone.

Estimation of 4 C DNA content in the various cytotypes has shown that cytotypes with higher ploidy level have corresponding higher DNA level.
Manifestation of cytoplasmic male sterility in GT 1 was noted for the first time in Hevea. However, further detailed genetic studies are essential to draw a conclusion.

Evaluation of the seedling progenies of male sterile clones GT 1, Ch 2 and RRII 35 along with that of fertile clone Mil 3/2 showed that the progenies of male sterile clones showed significant difference in vigour and juvenile yield. They also showed high $h^2$, GCA GA indicating that these characters can be used for early evaluation. Among the three male sterile clones, GT 1 is showing superiority in growth attributes and juvenile yield.

Multiple correlation studies have shown that about 54% of total variation of yield is due to girth. The male sterile clones having good fruitset can be used for designing seed orchards. These materials can enrich the genetic reservoir of Hevea.
NEW FINDINGS

REPORTED FOR THE FIRST TIME FROM THE PRESENT STUDY

1. Identification of spontaneous male sterility in clones, viz. Ch 2 and RRII 35.

2. Successful induction of male sterility
   (a) Radiation induced mutant with dwarf stature
   (b) Mutagen (EMS) induced mutant.

3. Identification of spontaneous triploid (RRII 15).

4. Synthesis of artificial triploid by crossing diploid with tetraploid.

5. Desynapsis in RRII 17.

6. Cytomixis in radiation induced mutant.

7. Wide spectrum of cytokinetic aberrations in the radiation induced mutant.
8. SEM studies on pollen grains of tetraploid, triploid and sterile clones.


10. 4 C DNA estimation of polyploids.

11. Evaluation of progenies of male sterile clones.

12. Manifestation of cytoplasmic male sterility in the clone GT 1. The male sterile clones having good fruitset can be utilised for hybrid seed production. Among the male sterile clones GT 1 is a good combiner.
FURTHER SCOPE OF INVESTIGATIONS FROM THE RESULT OF PRESENT STUDY

1. Detailed studies on genetic mechanism underlying the male sterile clones incorporating $F_1$ and $F_2$ generation.

2. Breeding behaviour of tetraploid and triploids.

3. Incorporation of clones showing desirable secondary attributes in the breeding programme of Hevea.

4. Male sterile clones having good fruitset can be incorporated in designing seed gardens for the production of hybrid seeds.

5. Field evaluation of the selected genotypes from the progenies of male sterile clones with the ultimate aim of selecting clones with high yield and good secondary attributes.

6. Induction of polyploidisation in the male sterile clones to study the effect of genome multiplication.

7. Biochemical characterisation of male sterile and newly evolved materials.