O. Agronomico, Campinas, SP, 41(1), 1989

BIOLOGY, CYTOGENETICS AND PLOIDY OF SPECIES OF THE GENUS HEVEA

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I. Introduction

The genus Hevea belongs to the Euphorbiaceae family which includes other important genera of tropical crops, such as Ricinus (Castor), Manihot (Tapioca) and Aleurites (Oiticica). Hevea brasiliensis (Wild, ex. A. Juss.) Muell. Arg. is the most important species of this genus. In Asia it is cultivated as the main source of natural rubber, more than 80% of the production being concentrated in Malaysia, Indonesia and Thailand. The total area under rubber cultivation in the world is estimated at 7 million hectares. Traditionally Havea is cultivated in the equatorial region, situated between 10°N and 10°S of the equator. Unique among natural products, natural rubber combines elasticity, plasticity, resistance to friction and impermeability to liquids and gases. Although synthetic rubber presents better qualities for some uses, it is not as good for the tyre industry which consumes 75% of the world production of natural rubber.

A native of the Amazon region, its natural occurrence is limited to the Amazon region of Brazil, where there are ten species of this genus, and to bordering countries such as Bolivia, Peru, Colombia, Ecuador, Guayanas, Surinam and Venezuela (WYCHERLEY, 1977).

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As typical genus of Hileia Amazonica, with great amplitude of ecological ambience, it presents great morphological variability (SEIBERT, 1947) ranging from high forests to bushy forests (BRASIL, 1971).

Hevea is not an ideal plant for genetic research. Its number of chromosomes, 2n = 36, was determined about 50 years age by RAMAER (1935). Among the unfavourable factors we may mention the similarity of forms of chromosomes of metaphase and their small size (MAJUMDAR, 1964). However, being an allogamous plant, it is ideal for interspecific hybridization and for improvement works, even, with its low percentage of fructification, around 3%. Although the plant can grow in poor soils, it responds very well to suitable manures.

The objective of this paper is to summarize and integrate the available information on different aspects of cytogenetics and ploidization of Hevea. Even with scarce literature, we hope that the information will be of utility in future research on the topics mentioned above, as well as in future programmes of genetic improvement of the genus.

2. Botany and Taxonomy

According to PIRES (1973), the genus Hevea is a natural taxon, that is, a group, a definite taxonomical entity, very well defined and easy to recognize.

Botanically this Brazilian Rubber Tree, locally called "Seringa" is a dicotyledonous monoecious plant, that is, it has male and female flowers in a same plant. The flowers are unisexual, small, yellowish, and arranged into racemes. The leaves are long-petiolate and divided into three lobes. The fruit is a big capsule which generally contains three seeds.

All the species are woody and arboreous; in general they range from average trees to very tall forests, with the exception of H. Camporum and H. camargoana, which are small trees of the size of field shrubs (GONCALVES et al., 1983).

The genus as a whole shows evident signs of periodicity such as: senescence (wintering); intermittent growth, variation in the distancing of leaves along the branches and formation of moderatily marked scaly rosettes, which possibly should be related with the formation of growth rings in the wood (SEIBERT, 1947).

+ The first discription of a species belonging to the genus Hevea dates back to 174g. According to BOUYCHOU(1969), it was the French engineer Francois Fresnau who first described the properties of the Brazilian rubber tree (Seringa) and it was the botanist Fusee Aublet who classified it in the genus Hevea, and within it, in the species H. guianensis.

The most recent taxonomic studies are those conducted by BALDWIN JUNOR (1947), SEIBERT (1947), PIRES (1973) and, more recently, SCHULTES (1977). BALDWIN JUNIOR (1947), based on an accurate study of Heveae native to the Amazon region, and combined with cytological observations, came to the conclusion that the genus Hevea possessed nine species. In Peru SEIBERT (1947) recognized eight species. The studies of SCHULTES (1977) recognized nine species and four varieties and PIRES(1973) recognized eleven species, including in the group the most recent species, H. camargoana, found in the island of Marajo (PIRES, 198

The present classification of the species of the genus Hevea is based on the studies conducted by Bailon and Mueller Argoviensis, cited by ALBUQUERQUE (1978), and by HIBBER PAX and DUCKE, cited by BRASIL, (1971) and SCHULTS(1977). Eleven species are recognized in Brazil (GONCALVES et al., 1983), while in Asia only nine are recognized (WYCHERLEY, 1977). Of the eleven species known in Brazil. H. Guianensis Aubl., H. benthamiania Muell. Arg., H. brasiliensis ((Willd. ex. A. Juss.) Muell. Arg., H. pauciflora (Spr. ex. Bth.)Muell. Arg., H. nitida Mart. ex. Muell. Arg., H. microphylla Ule, H. spruceana (Bth.) Muell. Arg., H. paludosu Ule Jahrb., H. nigidifolia (Spr. ex. Bth.) Muell. Arg., H. camporum Ducke and H. camargoana Pires, only H. brasiliensis, H. benthamiana and H. guianesis produce commercially acceptable latex (GOMES, 1981).

Hybridization and introgression between species occur frequently. Experimental crossings show absences of genetical barriers of reproduction (GONCALVES and CARDOSO, 1987). WYCHERLEY (1979) advances the hypothesis that the speciation of HEVEA occu in relation to the autonomy of flight of pollinating insects and synchronism in the flowering of these species.

3. Reproductive Biology

3.1. Physiological aspects of senescence (wintering) and flowering

The Brazilian rubber tree (Seringa) is a plant of deciduou habit, more pronounced in regions where dry periods are constant In Amazon regions, where the dry periods are less rigid, falling of leaves and flowering are irregular. According to WITMORE (1975), in deciduous forest, the availability of water is perhap the most important ecological factor which affects wintering and flowering, although both are influenced by the geographical situation, conditions of climate and nature of the vegetal material.

According to PINHEIRO (1981), in the conditions of Belem, PA, with a climate without a marked dry season, depending upon th clone there is a disuniformity of flowering, although there may be a greater concentration in the months of July and August. In regions of climate with marked dry season wintering occurs in this period, as is the case of Tracuateua in Para, which has a dry season which goes from August to November and wintering occurring from August to October. Another example is the case of Acailandia, in Maranhao, which has a dry season of four months and flowering occurs in this period.

In the State of Sao Paulo, it is found that the regions studied in the Sao Paulo plateau, like those of Sao Jose do Rio Preto, Campinas, Ribeirao Preto and Marilia, apt for the cultivation of rubber for presenting definite dry climate. In these regions, normally, wintering occurs in the period of June-August It is observed that in Campinas the falling of seeds occurs in the month of March to April, that is, one month after the fall of seeds in the region of Sao Jose do Rio Prelo.

In rubber plantations in general, wintering starts when the tree changes its habit of growth and this occurs, generally, after the third or fourth year after the planting; although variations occur in relation to clone, density of planting, genetic material and other factors (BOUYCHOU, 1969), there exist grafted trees which flower in one to two years, as well as there exist others which flower after seven years.

Trees of H. brasiliensis flower once in a year in the Amazon region, normally during April-July. In Malaysia they flower twice a year, normally between March and April and between August and September, with more pronounced flowering during the first season (PARANJOTHY, 1980). In Vietnam and Cambodia, according to BOUYCHOU(1969), wintering occurs from February to March.

The formation of annual rings of growth, observed in the transverse direction of the stem, in association with wintering, has been quite marked, similar to the plants of temperate climate (RAO, 1972). Meanwhile, according to La Rue[6], cited by SEIBERT 1947), the formation of the annual rings of growth is related with regions of definite seasons of rain and dryness. Seibert observed in the Iberia region of Peru 211 annual rings of growth in a tree of 84 cm diameter after the first year of decomposition, leading us to believe that these rings were related with the alternation of these seasons.

3.2. Inflorescence and flower

A little after the annual wintering, monoecious inflorescences appear in the extremities of the branches. They consist of a main axis, with about twelve pubescent axes, on which the flowers are distributed in the form of a raceme.

The small flowers are of two types, that is, male and female, situated in the extremities of the main axis and secondary axes. The proportion of male flowers to female is generally 60:1, that is, one female flower for 60 male flowers (BOUYCHOU, 1966), proportionally varying between the different clones (GEORGE, 1967).

The flower do not show petals, but there is a perianth with five lobules. The male flowers are smaller, more pointed than the female flowers and its involucre is directly inserted on the peduncle. The female flowers are distinguished by the presence of a swollen disc at its base, from where originates the floral involucre.

In the conditions of the Sao Paulo Plateau; the inflorescence matures in a period of 1 to 2 weeks. The male flowers, in general, open earlier than the female flowers and anthesis (full bloom) generally occurs in the second half of the morning, and is completed by noon, because of which controlled pollination should be carried out in the morning time.

A floral bud produces, on an average, about ten racemes, each one, on an average, with six female flowers. Flowering takes place at the beginning of the vegetative cycle of the rubber tree, starting a little before or after the launching of the new leaves and generally is spread over the whole period of refoliation. (BRASIL, 1971).

Like all the plants native to tropical zones, rubber tree, according to BOUYCHOU (1969), is a plant of short days for its flowering. Its photoperiod does not seem to have been studied yet, and we do not have till now any data on the limits of daily necessity.

The male flowers possess ten sessile stamens, directly inserted on a small control conical column called androphore. The stamens are arranged into two altemados verticilos of five sessile stamens each (GOZCALVES, 1979). The stamens have elongated shape, oriented towards the same direction as the androphore and a distinct groove on the external surface (Fig.1).

(6) LA RUE, C.D. The Hevea rubber tree in the Amazon valley. Washington, US Department of Agriculture, 1926. 70P. (Department Bulletin 1922).

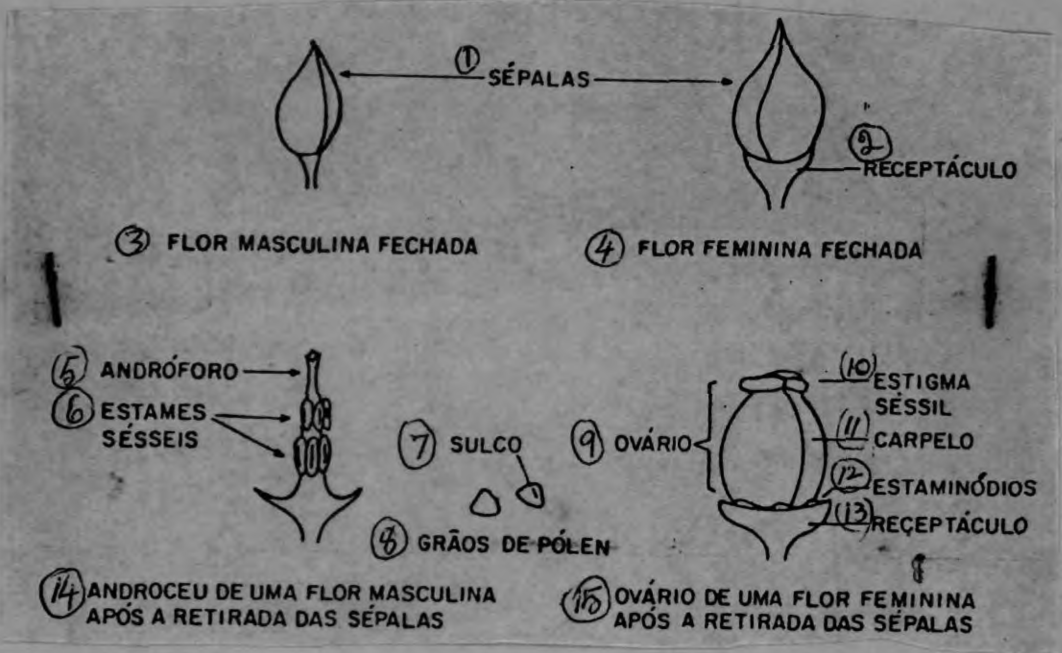

Fig.1. Diagramme of male and female flowers of the genus Hevea (source: BOUYCHOU, 1969).

(1) Sepals (2) Receptacle (3) Closed male flower (4) Closed female flower (5) Androphore (6) Sessile stamens (7) Groove (8) Pollen grains. (9) Ovary (10) Sessile stigma (11) Carpel. (12) Staminodiums (13) Receptacle (14) Androeceum of a male flower after removal of sepals (15) Ovary of a female flower after removal of sepals.

The female flower is isolated at the end of the main axis and in the secondary ramifications of the racemes, or still less frequently, in the tertiaries. It is easily distinguished by noting that the base of the calyx is much more voluminous than in themale flower, and we can observe also a green coloration in the base, which is not found in the male flower. It consists of a triangular ovary formed by three welded carpels, on which is mounted a sessile stigma, which in the process of pollination receives pollen grains (GONCALVES, 1979). Between the ovary and the perianth there exist ten staminodia arranged on the receptacle (Fig.1).

Both in the female flower and in the male there are rudiments of other organs. According to FERRAND (1944), this is due to the fact that the flowers are constituted on a plan of herma-phrodite flowers and that in the course of their development one of the organs is retained in the evolution.

3.3. Male sterility

The phenomenon of male sterility described by many authors in different taxons of angiosperms (LASER and LERSTEN, 1972, GRUN, 1976) is a physiological anomaly of little importance for the researcher who wishes to utilize the sexual way for producing a heterozygotic structure on a big scale (DUVICK, 1959).

According to DUVICK (1959), whatever may be its origin, mal sterility permits to profoundly modify the system of reproduction of a plant. Utilizing it, the researcher has the possibility of producing allogamy in two autogamous plants, with the objective of obtaining the effect of heterosis.

In the case of Hevea, the occurrence of male sterility was reported for the first time by RAMER (1935), who described it as irregularity in the myosis of the mother cell of the pollen. Through the cytological study of mother cells of the pollen grain this author observed clones of H. brasiliensis which showed complete or partial male sterility, and concluded that irregular myosis was responsible for the sterility.

Meanwhile, it was in the period of 1962 to 1964 that researches were carried out in Malaysia (MAJUMDAR, 1967) to discover macho sterility in clones of H. brasiliensis. The objectiv was to establish seed farms for economically producing hybrid seeds on large scale. According to this author in that period, out of the two thousand clones examined three clones with complete male sterility were found.

In practical works of improvement conducted in Indonesia (DIJKMAN, 1951) and in Brazil by IAC (CAMPINAS, 1989) various grades of male sterility was observed in different clones, ranging from poor production to complete absence of pollens, because of which clones like GT1 can be used as female parentals in crossings. Sterile male clones are not different from normal clones in their morphological characters (MAJUMDAR, 1967), although the male flowers take more time to attain maturity.

3.4. Longevity of pollen

The pollen of Hevea shows the characteristic of being sticky (FERWERDA, 1969) and, if stored without special precautions, quickly loses viability. DIJKMAN (1938), by storing androphore (column of anthers) in 67 to 80% relative humidity and at $\pm 6^{o}C$ temperature, achieved viability of pollen for seventeen days. Storage studies conducted by MAJUMDAR (1966) showed that a good degree of viability, around 20%, could be obtained for a week if the pollen is stored in a temperature between 5 and $0^{o}C$ and 75-81% relative humidity. Pollen stored in this way makes a good fertilization possible.

3.5. Fertilization and fructification

After the fertilization of the ovules the fruit, generally with three seeds, takes about five months to attain complete development. During the period of development a large quantity of small fruits are aborted, especially during the first two months. According to WYCHERLEY (1979), fructification succeeds in less than 1% of the female flowers in natural conditions, and about 4% in the case of artificially pollinated flowers. In the Sao Paulo Plateau the greater or lesser percentage of fructification, in natural or artificial conditions, is related to the low relative humidity of the air in the period of pollination.

4. Centre of Genetic Diversity

The Centre of genetic diversity of the genus Hevea is Rio Negro, in the confluence with the Amazon river (WYCHERLEY,1977). In this region seven species are naturally found: H. benthamiana, H. guianensis, H. microphylla, H. nitida, H. pauciflora, H. rigidifolia and H. spruceana. Of course, there exist in this region putative hybrids, which represent the majority of the combinations of existing species, and consequently, overlappings of the geographic area of one or more species. These overlappings induce the occurrence of hybridization and introgression in such dimension that it led BALDWIN JUNIOR (1947) to conclude that some lines of specific points of the genus happen to lose their identities.

The distributionof H. comporum is away from Rio Negro and H. brasiliensis has its area outside the primary centre and is therefore in the secondary centre of diversity. According to WYCHERLEY (1977) this centre covers a vast area in the surroundings of the municipality of Borba, in lower Rio Madeira, where five species naturally occur.

Geomorphologically, the centre of diversity is situated in the proximities of the northern margin of the Amazon basin where the Guyanas Plateau begins. The soils of this centre proper are constituted by latosols of low land and fluvial glaze, since the region which surrounds the centre includes a variety of plateau soils (WYCHERLEY, 1977).

As regards climate, the centre of diversity is within the equitorial region with constant super humidity (ORTOLANI, 1986).

An important aspect which should be emphasized is that the species found there seem to have evolved under this constant humid climate, showing the existence of big variation with regard to resistance to leaf disease, caused by the fungus Microcylus ulei. Besides, the species which spread beyond the region which surrounds the centre have adopted themselves to conditions of long dry periods each year, partially contributing to the behaviour of wintering and, consequently to the flowering of the trees.

5. Cytogenetics of the Genus Hevea

The use of cytogenetics in the improvement of economic characters of Hevea can, in principle, favour the understanding of the model of inheritance of the plant. An aspect of great importance in this direction is that it provides the researchers the choice of plant material to be worked on and, consequently, a good planning of the programme of improvement. Excepting HEUSSER (1919) and BANGHAM (1931); various researchers, such as: RAMAER (1935); PERRY (1943); PADDOCK (1943); MENDES (1946); BALDWIN JUNIOR (1947); ROSS(1959); BOUHARMONT (1960); CONAGIN (1971a, b) and ONG (1975), concluded that the majority of the species of the genus Hevea possess $2n = 36$ chromosomes (Chart 1).

Chart 1. Number of chromosomes in species and interspecific hybrids of Hevea

Species	2n	References.
H. brasiliensis	16	HEUSSER (1919)
	34	BANGHAM(1931)
	36	ONG (1975, 1980); CONAGIN (1971a, b); RAMAER (1935); PERRY (1943); PADDOCK (1943); BOUHARMONT (1960); ROSS (195[illegible] MENDES (1946); DIJKMAN (1951).

H. guianensis	34	BANGHAM (1931)
	36	MAJUMDAR (1964); RAMAER (1935); BOUHARMONT (1960); ONG (1980).
H. spruceana	34	BANGHAM (1931)
	36	MAJUMDAR (1964); RAMAER (1935); PERRY (1943); PADDOCK (1943); BOUHARMONT (1960); ONG (1980); MENDES(1946).
H. pauciflora	18	BALDWIN JUNIOR (1947)
	36	MAJUMDAR (1964); ONG (1980).
H. benthamiana	36	ONG (1980); BOUHARMONT (1960).
H. Nitida	36	ONG (1980).
H. Spruceana x H. brasiliensis	36	RAMAER (1935).
H. Colina x H. brasiliensis	36 36	RAMAER (1935).
H. rigidifolia(1)	?	-
H. microphylla	?	-
H. paludosa	?	-
H. camporum	?	-
H. camargoana	?	-

(1) Cytologic or cytogentic studies do not exist.

5.1. Myosis

In Hevea, myosis studies are few and not very systematic. HEUSSE (1919) was the first author to conduct research on reproductive organs of H. brasiliensis with regard to myosis. In trying to make improvement through controlled pollination, the study of the organs of reproduction, according to this author, is very important for the success of the work. However, in the discussion of myosis, HEUSSER did not enter into details, but mentions only the most perceptible study and, with a brief discussion, its connection with the phenomenon of heredity.

Years later, BANGHAM (1931) published a paper on the number of chromosomes of some species of Hevea, in which the number of n = 8 chromosomes of H. brasiliensis, estimated by HEUSSER (1919) was corrected . For all the species researched, BANGHAM (1931) found 2n = 34 chromosomes. However, his work hardly contains information on the myotic division.

It was RAMAER (1935) who first studied the cytology of Hevea in detail. He observed that the haploid number of all the species and forms researched, whose countings were done in diacenesis, metaphase I, anaphase I and metaphase II, in a total of hundreds of cells in myotic phases, ~~did not total of hundreds of cells in myotic phases,~~ did not leave margin for any doubt.

The microsporogenesis of five species of Hevea was studied by BOUHARMONT (1960), who observed that in thebeginning of prophase the nucleus of the sporocytes were voluminous, possessing one, two or even three nuclei. The nucleus, hardly stainable, was full of numerous thin filaments and this gave it a granular and finely reticulated look. The results of the two researches showed concordance with regard to the number of chromosomes observed by RAMAER (1935), PERRY (1943), PADDOCK (1943), MENDES (1946), BALDNIN JUNIOR(1947) and ROSS (1959).

In Malaysia, MAJUMDAR (1964), with the intuition of finding male sterile clones for use in the improvement programme of the Rubber Research Institute of Malaysia (PRIM), studied microsporogenesis in four species of Hevea, finding n = 18 chromosomes in all of them. This author observed that in the pachytene stage the chromosomes were in the form of fine threads, whi in diacinesis they appeared short and thick. The presence of 18 chromosomes was also observed in the poles of anaphase I and met phase II. The chromosomes appeared more or less irregular in shape. Anaphase I appeared irregular, except in some cases where one or two retardatories or chromation bridges were seen. The author also observed in anaphase II the normal seggregationof 18 chromosomes for each pole.

In Brazil cytological researches in microporogenesis, carried out in the Agronomical Institute (IAC) by CONAGIN (1971a, b) on diploid and polyploid clones, showed that in diploid clones (2n = 36) the microgenesis appeared normal and it was possible to observe in diacinesis 18 bivalents which separated in anaphase I regularly, without showing retardatories. According to this author, the phases of the second division also appeared normal, giving 100% normal tetrads. Already in the polyploid clones (2n = 72) several irregularities were observed in the chromosomic separations, giving rise to abnormal tetrads, with microcytes and empty pollen grains.

Recently, ONG (1980), utilizing floral primordials of several species of Hevea, conducted the most complete study of myotic chromosomes, finding in all of them the usual configuration of 18 bivalents in metaphase I. This author observed that among the 18 chromosomes three were ring-shaped, while the remaining had the shape of a stick, and among the eight bivalents, two were long, four of average length and the remaining short. It is interesting to point out that occasionally this author observed also one or two quadrivalents, although the frequency of occurrence of this configuration was low and the number of chiasmas per cell was about 28 in all the species examined.

5.2. Analysis of the caryotype

Analysis of the caryotype includes the study of the number, size and morphology of chromosomes. Total length of the ratio of arms of the chromosomes are useful in systematic and phytogenetic researches. According to JAUHAR (1981), Levistskii and Avdulov were the pioneers in the use of cytological data as a help in the study of taxonomic and phyllogenetic studies between plant species and genera. Although the basic number, size and morphology of the chromosomes can in fact be utilized in taxonomic classification (CONSTANCE, 1957), these parameters could be subsidiary to morphological characters in any taxonomic study. Modern cytological techniques, liking banding of chromosomes with Giemsa (VOSA, 1973), and models of heterochromatin coloured with fluorochromes, like Chinacrine mustard (VOSA, 1970), can furnish information of phyllogenetic value.

As regards caryomorphology, the chromosomes are generally measured in metaphase after pre-treatments (ONG, 1980; RAMER, 1935); PADDOCK, 1943). The main disadvantage inherent in these studies is that the magnitude of error in the measurements of contracted chromosomes is high. Small significant differences between chromosomes of a species, of intra-specific category or of different species, cannot be resolved accurately. However, caryomorphological studies can be carried out more precisely in chromosomes in the pachytene stage in rate of low chromosome number.

The genus Hevea is a homogeneous collection of species with number of chromosomes $2n = 36$, and $x = 9$. ONG (1975), through the analysis of the caryotype of seven species of the genus, suggests that the species diverge between themselves with respect to caryomorphology. Judging by the criterion of asymmetry of chromosomes, the species H. brasiliensis seemed to be more evolved, while H. Guianensis was found to be more primitive. According to ONG (1980), out of all the seven species studied, H. brasiliensis was found to be with least number of metacentric chromosomes (10) while the biggest number was found in H. guianensis (16). According to Chart 2, the order of evolution of the other species is as follows: H. benthamiana (11), H. rigidifolia and H. spruceana (12), H. nitida (14) and H. pauciflora (15). The wide differences in asymmetry of the caryotype between the seven species of Hevea support the affirmation that they are in fact distinct species.

Chart 2. Degree of asymmetry between seven species of Hevea

Species	H. brasilionsis	H. benthamiana	H. rigidifolia	H. spreceana	H. nitida	H. pauciflora	H. guianensis
Ratio of length of the longest and shortest pair of chromosome	1.94	1.81	1.88	2.00	1.89	1.88	1.94
Number of metacentric	10	11	12	12	14	15	16
Number of submetacentric	7	11	3	2	2	2	1
Number of acrocentric	1	1	2	2	1	1	1
Presence of satellite	Yes	Yes	-	-	Yes	Yes	-

Source: ONG(1980)

The conclusions of the study of morphology of the caryotype, firstly conducted by RAMAER (1935) and later confirmed by BOUHARMONT (1960) and ONG (1975, 1980) are that the genus Hevea is a segmental allopolyploid with basic number of chromosome of X = 9 instead of n = 18. The explanation for this fact is the existence of big similarities between the different pairs of chromosomes in the caryotypes, suggesting duplication of pairs of chromosomes. According to ONG (1980), these duplications of pairs of chromosomes would be expected if the genus was of allopolyploid origin. Besides, the low valency of multivalents, observed in the researches conducted by ONG (1975), suggests that the genus Hevea is a segmental allopolyploid.

The fact that a large number of species of existing plants, including Hevea among others of our cultivated plants, are allopolyploids, clearly shows the importance of polyploidy in evolution. According to BURNHAM (1964), at least a third of all the species of angiosperms are polyploids, but in some groups, the percentage is higher.

Allopolyploids probably arose in nature through the duplication of chromosomes after interspecific crossings. Duplication occurs naturally in experimental hybrids between species. The frequency of duplicated sectors is low, but it can be increased by various agents. The result is as if the zygotic number of the two species was combined in a single individual. These are known as amphipolyploids or amphidiploids if both the parental species are diploids(BURNHAM, 1964).

6.+ Haploidy

It was Bergner, in1921, who discovered thefirst haploid in higher plants (BLAKESLEE et al., 1922), and three years later

Blakeslee and Belling (CHASE, 1969) made the first suggestion for the use of these plants in genetic improvement, and there exist at present a series of theoretical possibilities of its use suggested by NITZCH & WENZEL (1977). According to SEARS (1939), in the field of cytogenetics, haploid plants are important as basic materials in the production of monosomic series, which have important applications in cytogenetics and in the improvement of plants. In improvement, due to the non existence of dominance in haploids, they can be utilized with great success, considering that the phenotype of these plants faithfully reflect the genotype of the same (ABEL, 1955). JAUHAR (1981) emphasises that haploids can be utilized in various areas of research, such as studies of induced mutagenesis, effects of dosage and interaction of gene and analysis of genic bonding, contributing to accelerate a programme of genetic improvement.

6.1. Haploids (Spontaneous dihaploids)

The first researcher to carry out cytological studies on Hevea (H. brasiliensis) was HEUSSER (1919), in Sumatra, who observed that the plant possessed 2n = 16 chromosomes. Only much later, in studies conducted by various authors, it was observed that the species of Hevea till then studied possessed 2n = 36 and n = 18.

However, the studies of HEUSSER (1919) remained for a long time as an isolated example, explained by the use of cytologic technique inadequate at that time, until the casual discovery of a haploid specimen of H. pauciflora, in 1947, by Baldwin Junior. According to DIJKMAN (1951), after counting the chromosome, the tree of 2n = 18 chromosomes was abandoned and it lost its identi weeks. Two years later, in Malaysia, attempts were made by PRIM, in 1975, for improving the development of the embryoids obtained from the somatic corns **derived** from the anther, through modification of the culture medium, although this attempt was not successful (ANNUAL REPORT, 1975). In the same period, PARANJOTHI and GHANDIMATHI (1975) reported that corns of various explants were obtained and some of these were maintained through repeated subcultures. Differentiation of embryoids was obtained in culture of corns derived from somatic tissues of anther, and could take root, although they did not develop into plantlets, in the same way, with explant culture of young stem of H. brasiliensis, WILSON and STREET (1975) and WILSON et al. (1976) obtained structures similar to embryoids which also failed to develop into plantlets.

The first work on plantlets of Hevea from anther cultured in vitro was reported in 1977 (CHEN et al. 1977). Two years later, CHEN et al. (1979) studied the relation between corn obtained from anther and formation of embryoids from pollen of Hevea . They observed diploid cells (2n = 36) in 80% of cells in metaphase of myotic division after 20-30 days in culture medium, while 50 days after the inoculation, only 10% were deploids and 69% were haploids. The results showed that after 50 days of culture the corn derived from the walls of anthers was degenerating itself, while the haploid tissue of the microspore was dividing naturally.

Subsequent studies of reduction of ploidy of culture media and development of microspores into embryoids in the process of culture of anther were conducted systematically (CHEN et al. 1981 CHEN et al. 1981a). According to CHEN(1983), hundreds of plant-

Later, Baldwin Junior returned to the old place of the tree and collected seeds of the place with the object of making new verifications of chromosomes in the seedlings possibly originated from it. These seedlings, however, showed 2n = 36 chromosomes. I was not possible to collect new samples in the place and in this way the discovery remained at the margin. However, the fact that the tree in question had a number of characters similar to those found in H. brasiliensis and that it was verified later as being probably H. pauciflora, made Baldwin Junior conclude that Heusser's discovery of 2n = 16 could be related to an individual of H. pauciflora confused as H. brasiliensis.

The chromosome number 2n = 18, according to DIJKMAN (1951) is considered by Baldwin Junior as an expression of a diploid condition, and groups possessing 2n = 36 chromosomes would represent an approximation of a tetraploid condition.

7. Induced haploids

Production of pure lineages through many generations of self-fecundations of allogamous perennial species, such as Hevea, is almost impossible due to its long generation cycle. However, homozygotic diploid plants can be obtained by duplication of the number of chromosomes of a haploid plant obtained from pollen through culture of anther. By meansof this procedure, pure lineages of different genotypes could be obtained by providing the base for production of hybrids of Hevea exhibiting heterosis.

The first attempts to obtain anther culture and pollen grains culture in Hevea were made by SATCHU THANANTHAVALE & IRUGULBANDARA (1972) and SATCHUTHANANTHAVALE (1973) in Sri Lanka. They observed formation of corns after four weeks in culture medium maintaining good growth through six subcultures in a period of si lets from pollen havealready been obtained and some have already been cloned. This author emphasises that with the definition of the technique of anther culture, normally, 100 to 150 embryoids are formed in every hundred anthers inoculated. The frequency of viable plantlets from pollen has been increased to 3% (number of plantlets from pollen per 100 anthers inoculated) (CHEN et al.,1981b) besides, GUO et al. (1982) recently reported that two plaintlets were obtained from ovules (female gamete) of H. brasiliensis, needing, however, the study of degree of ploidy.

8. Triploidy

Triploids, in a general way, occur rarely and spontaneously in nature. The first citation of a triploid tree ($2n = 3x = 54$) was made by BALDWIN JUNIOR (1947) in a clone of H. guianensis. Much later, NAZEER and SARASWATHY AMMA (1987), in India, reported another plant of H. brasiliensis which was a triploid of spontaneous occurrence.

In nature, triploid plants can originate from crossing between triploid plants or by fusion of female ovule with two male nuclei BURNHAM(1964). However, the most true hypothesis for spontaneous triploids is the result of fertilization of reduced and non-reduced gametes, also observed in other species by various researchers (NEWTON and DARLINGTON, 1929; NAZEER 1980, 1981).

Induced triploids were obtained in Brazil by BOAVENTURA(7) and in India, by SARASWATHY AMMA et al. (1980), involving colchi tetraploid clones and diploid clones side by side in the field. In both the cases, considering that the vigour of the plant is a phenomenon of heterosis, the above-mentioned triploids show luxuriant vigour, greenish dark coloration and thickness and more pronounced leaf veins than the diploid.

9. Poliploidy

According to WRIGHT (1976), polyploids can be subdivided into antopolyploids if the whole set of chromosomes are derived from the same parental species, or allopolyploid if the whole thing originated from different parental species. In a typical autotetraploid there exist groups of homologous chromosomes, while in a typical allotetraploid chromosomes are quite different from the species which show two groups of chromosomes.

Many of our cultivated plants, such as wheat, oats, cotton, tobacco, potato, peanut, alfalfa and Napier grass show success in polipolody. Their parental progenitors are already, in many cases, extinct or, if they still exist, they do not compete with the same (HARLAN, 1967).

Although the initial expectations of success of polyploidy as a mode of improvement of plants have not turned out to be a reality it has contributed in some way for the improvement of ornamental plants and fodder plants. According to JAUHAR (1981), the areas of utility include the following: (1) Breaking of sterility barriers, facilitating the transfer of genes; (2) Application of special crossing techniques similar to the method of haploidy of crossing of diploids; (3) Widening of the genetic variability.

Considering that the constitution of the genome of a vegetal organism is in equilibrium and the induced duplication of chromosomes (autopoliploidy) disturbs this equilibrium and produces sterility, at chromosome level, the main problem is the irregular

(7) BOAVENTURA, Y.M.S. Personal communication1989. (Cytology Section IAC, Campinas.

myotic behaviour which results in unbalanced and sterile gametes. In the case of the genus Hevea spp. the existence of studies in this direction in autopoliploid clones has been practically nil.

9.1. Induction of polyploidy

The interest of polyploidy in plants was initially given incentive in 1927 when colchicine, a drug extracted from Colchicum autumnale, showed that it inhibited the formation of cellular spindles and acted in the division of centromers in plant cells. Since then researchers have made various attempts to obtain new, superior crops by utilizing this technique, considering the fact that polyploids possess bigger cells in relation to diploids and can produce agronomically superior plants and fruits.

The induction of polyploid clones in Hevea was first reported by Ford in Sri Lanka (Rubber Research Board of Ceylon, 1942). without success. Researches in this direction were conducted by MENDES and MENDES (1963) in Brazil, SHEPHERD (1969) in Malaysia and MARKOSE et al. (1974) and MARKOSE (1975) in India. The first results of these researches created great interest due to the increase of vigour of the plants resulting from this process.

In Brazil, MENDES and MENDES (1963) were the first researchers to observe that by utilizing concentration of 0.2 to 0.4% of colchicine solution it would be possible to induce duplication of 36 to 78 chromosomes in cells of new plants, and that the induced plants had bigger stomata in lesser number and showed differences in leaf morphology and in the stem, when compared with normal plants of H. brasiliensis. In production mini test, plants with age less than one year showed an increase of around 320% in production (MENDES, 1971; PINHERO et al., 1980). However, the plants showed mixoploidy; trying to minimise the problem MORAES

(1982) has been carrying out attempts for the refining of the method.

Later, SHEPHERD (1969), in Prang Besar, in Malaysia, tried to introduce polyploidization in seeds and buds of H. brasiliensis. He observed in the induced materials thicker leaves of dark green coloration, bigger stomata in smaller number per foliar area. It was observed, also, that the bark of the trees were thicker than those of normal diploids. Lastly, it was observed that the putative polyploids had a number of undesirable characters, which restricted their use in commercial plantation.

Recently, GONÇALVES et al. (1982), through induction and investigation of hybrid polypolid clones of H. benthamiana x H. brasiliensis, observed a high degree of mixoploidy in the polyploids obtained. Morphologically, the polyploid clones differed from diploid clones in lesser height, lesser ratio of length and width of stomatum, bigger petiole and bigger stomatum. Chromasome of the leaves of the five different clones and polyploids including diploid, were counted with the object of determining the degree of ploidy. Metaphases of the cells were studied. The cells of the leaves in all the branches revealed a considerable variation in the number of chromosomes (Chart 3), with the majority of the cells possessing 36 to 72 chromosomes. CHEN et al. (1982) also observed a considerable variation in the number of chromosomes in the metaphase of cells of the leaves of haploid plantlets. A possible explanation for the case of mixoploidy is that in the induction of polyploidy, the meristomatic tissue is composed of two layers, one external and the other internal, with the cells in division. According to WRIGHT (1976), generally it is difficult to obtain such a concentration which causes the diffusion of the solution of colchicine into the interior of the meristematic cells, in all the cells or in both the layers. In Hevea, in particular, according to MARKOSE (1975), the absorption and diffusion of the colchicine solution in the interior layer is improbable, and consequently only the outer cells are affected.

Even considering the little emphasis given to polyploidization of Hevea by research centres and the limited progress of polyploids in Hevea, SANTOS et al. (1987) in Bahia, ONG et al. (1984) in Malaysia and ZHOZHONGYU et al. (1982) in China, reported preliminary results of the experiment in production of Brazilian (IAC), Malayan(RRIM) and Chinese (HK) polyploid clones clearly demonstrating the possibility of obtaining new rubber plant clones capable of being more productive than the mother plants which after being polyploidized give use to the said polyploids.

Chart 3. Number of chromosomes in the metaphase of young leaf cells treated with colchicine in five polyploid clones of Hevea

Clones	Measurement of height of plants	No. of leaves	No. of metaphases	Chromosome count	
				35-54	55-72
IAN 717	1.20	15	42	40	2
PS01	0.81	8	45	6	39
PS02	0.92	10	23	5	18
PS03	0.75	12	33	5	28
PS04	0.86	6	22	4	18
PS05	0.93	2	20	7	13

Source: GONÇALVES (1982).

9.2 Hevea: a natural allopolyploid

The first information that Hevea is a natural allopolyploid was made by PERRY (1943) who determined the number of chromosomes of 109 species and varieties distributed among a total of 22 genera of Euphorbiaceae family. His study showed that the basic numbers of chromosomes (n) for the family were 6, 7, 8, 9, 10 and 11. On the basis of these results this author concluded that any member of the family which possessed number of chromosomes greater than these basic numbers would probably be an allopolyploid. In this way, the genus Hevea with n = 18 has as basic number x = 9 chromosomes.

Later, BALDWIN JUNIOR (1947) concluded that Havea was an allopolyploid probably originating from crossing between two primitive species of basic number x = 9. Although the genus Havea is quite distinct in the family Euphorbiaceae, it is accepted that it is very close to other genera of the same family confined to the Amazon region. Among these stands out the genus Micrandra, with 13 known species, out of which M. minor is being tapped as a rubber-producing plant (WYCHERLEY, 1977). The distribution of this genus is greater than that of Hevea.

However, systematic verifications on the possibility of this genus being allopolyploid were carried out by BOUHARMONT (196 who through cytological study of H. brasiliensis pointed out a certain degree of affinity existing between the pairs of bivalent chromosomes. Confirmations of the studies made by Bouharmont were obtained some time later by ONG (1980) through the formation of multivalents, especially quadrivalents, which occurred with greater frequency in the species studied. Based on this evidence, that is, on the presence of quadrivalence, the existence of a great similarity was observed between pairs of bivalents and same number of satellites and from this Ong concluded that H. brasiliensis is an allopolyploid. This idea is once again strengthened by the fact that many researchers, since a long time, opine that the basic number of x = 9 is considered quite high for the genus which belongs to the family Euphoribiace (RAMAER, 1935; PERRY, 1943).

10. Conclusions

1. The speciation of Hevea , in accordance with ecological adaptation, consists in the short range of the flights of pollinating insects (mosquitoes and small flies) and the lack of synchrony of flowering between some of the species and rarely even within the same species.

2. There exists an amplitude of fertility in the different genotypes of Hevea. Few clones are really sterile, whether self-pollinated or cross-pollinated. Some are only autosterile. Putative male sterility is probably complete sterility.

3. Hybridizations and introgression occur frequently. Experimental crossings show absence of genetic barrier. The area of geographic distribution of each species overlaps with the others and putative hybrids occur naturally, representing the major part of the combinations of the more common species of Hevea.

4. The analysis of the caryotype of seven species of the genus Hevea showed divergences between themselves with regard to the morphology of the chromosomes.

5. Judging by the criterion of asymmetry, the species H. brasiliensis seems to be the most evolved and H. guianensis the most primitive. Out of all the seven species studied by ONG(1980) H. brasiliensis was found to possess the least number of metacent-

centric chromosomes (10), while H. guianensis, the highest numbe3

6. Detailed analysis of the caryotype supports the contention that the genus Hevea is an allopolyploid with basic number of chromosomes 9 instead of 18, in relation to the duplication of pairs of chromosomes, observed in species of polyploid origin.

7. For many authors the myotic behaviour was normal in the clones of H. brasiliensis studied. Few were the anomalies observed, and these irregularities showed little effect on the fertility of the clones.

8. Cytological investigations revealed a high incidence of mixoploidy in the foliar tissue of artificially induced polyploid clones.

Acknowledgement

The authors are grateful to their colleague Yone M.S. Boaventura, Scientific Researcher of the cytology Section of the Agronomical Institute, for the critical comments and suggestions given during the course of conducting this research.

Bibliographic References

ABEL, B. Eine bethode zur erhaltung von homozygoten chlorophyll-mutaten. Naturwiss, 42: 372-373, 1955.

ALBUQUERQUE, J.M. Segment of Botany. In: Specialization course in Heveiculture, 3, Belem, 1978 Belem, FCAP. p.1-8.

ANNUAL REPORT. Kaula Lumpur, Rubber Research Institete of Malaysia, 1975. 300p.

BALDWIN JUNIOR, T. Hevea, a first interpretation - Journal of Heridity, London, 38: 54-64, 1947.

BAMGHAM, W.N. Chromosomes of some Hevea species. Journal of Arnold Arboretum, 12:287, 1931.

BLAKESLE, A.S., BELLING, J.; FARNHAM, M.E. & BERGNER, A.D. A haploid mutant in the Jumson weed, Datura stramonium Science, Washington, 55: 646-647, 1922.

BOUHARMONT, J. Taxonomic and caryologic researches on some species of the genus Hevea. Belgian Congo, INEAC, 1960, 64p. (Scientific Series, 85).

BOUYCHOU, J.G. Biology of Hevea. General Review of Rubbers and Plastics, Paris, 40: 933-1001, 1969.

BRASIL. Superintendence of Rubber. National Rubber Plan. The genus Hevea: Description of species and geographic distribution. Annexure IV. Rio de Janeiro, SUDHEVEA, 1971, 57p.

BURNHAM, C.R. Discussion in Cytogenetics. Minneapolis, Mimneso Burgess Publishing, 1964. 375 pp.

CAMPINAS. Agronomical Institute. Rubber Programme: Annual Report 1988. Campinas, 1989 n.p. (Typed).

CEN, M; CHEN, Z, QUIAN, C; WANG, C; HE, Y & XIAO, Y. Investigation of ploidy in the process of anther culture of Hevea Brasiliensis Muell. Arg. Sinica Genetics Proceedings, Beijing, 8:169-174, 1981.

CHASE, S.S. Monoploids and monoploid derivatives of Maize (Zea mays L.) . Botanical Review, New York, 35: 117-169, 1969.

CHEN, C; CHEN, F; CHIEN, C; WANG, C: CHAN G,S; HSU, H, OU, H: HO, Y & LU, T. Introduction of pollen plants of Hevea brasiliensis Mull. Arg. In: Symposium on Anther Culture, 1. Beijin 1977. Proceedings Beijing, 1977. p.3- 8.

CHEN, Z. Rubber (Hevea). In: EVANS, D.A.; SHARP, W.R.; AMMIRATO P.V. & YAMADA, Y. Handbook of Plant cell Culture. New York Macmillan, 1983.

CHEN, Z; QUIAN, C.; QUIN, M; XU, X. & XIAO, Y. Recent advances in anther culture of Hevea brasiliensis (Muell. Arg.).

Theoretical and Applied Genetics, 62: 103-108, 1982.

CHEN, Z; QIAN, C; XU, X, QIN, M; XIAO, Y; LIN, M.D.Z.; WU, S & HUAN, N. Recent advances in anther culture of rubber tree and sugarcane. Annual Report. Beijing, Institute of Geneti of Sinica Academy, p.103, 1981 b.

CHEN, Z; QIAN, C; QIN, M; WANG, C; SUO, C; XIAO, Y & HSU, H. Recent advances in anther culture of rubber tree. Annual report. Beijing Institute of Genetics Academia Sinica, p.88-90, 1979.

CHEN, Z; QIAN, C, QIN, M; WANG, C; SUO, C; XIAO, Y & XU, X. Relationship between somatic cells and microspores in the process of anther culture of Hevea brasiliensis Mull. Arg. Act.Bot. Bor-Occ. Sin., 1:31-37, 1981a.

CONAGIN, C.H.T.M. Microsporogensis in normal and tetraploid clon of Hevea braziliensis Muell-Arg. Bragantia, Campaninas, 30((2):: 11-18, 1971a.

CONAGIN, C.H.T.M. Cytological study in clones. Polimeros, Rio de Janeiro, 1(2): 19-20, 1971b.

CONSTANCE, L. Plant taxonomy in an age of experimentl American Journal of Botany, Baltimore, 44: 88-92, 1957.

DWKMAN, M.J. Hevea, thirty years of research in far East. Florid University of Miami, 1951-329p;;

DWKMAN, M.N. Preliminary data on storage of Hevea pollen. Archives v.d. Rubbercultuure: in Nederlands ch. India 22: 239-259, 1938.

DUVICK, D.N. The use cytoplasmatic male - sterility in hibrid seed production. Economic Botany, Baltimore, 13:167-195,195

Ferrand, M. Phytotechny of Hevea brasiliensis: Botanical improve ment, Cultivation and exploitation. Paris, Geneblouy. Jules Ducolat, 1944, 435p.

FERWERDA, F.P. Rubber. In: FERWERDA, F.P. & WIT, F. Outline of perennial crop breeding in the tropic. Wageningen, H. Veenman & Zonen N.V., 1969, 427p.

GEORGE, P.J. Observations on the floral biology and fruit set in Hevea brasiliensis Muell. Arg. Rubber Board Bulletin, Kottayam, India, 9:18-27, 1967.

GOMES, J.I. Anatonical study of the secondary xylem of species of Hevea in Brasilian Amazon Region Curitiba, UFPR, 1981 205 P. Thesis (for postgraduate).

CONCALVES, P. de S. Floral Biology of Seringa. In: Intensive Course of Heveiculture for agricultural technicians, 2, Manuas, 1979.

CONCALVES, P. de S & CARDOSO, M. Origin and domoesticationof seringa. O Agronomico, Campinas, 39(3): 277-287, 1987.

CONCALVES, P. de S; PAIVA, J.R. de & SOUZA, R.A. de. Retrospective and actuality of genetic improvement of rubber. (Hevea Sp.). In Brazil and in Asian countries. Manaus, EMBRAPA/ CNPSD, 1983. 69 p (document series, 2).

CONCALVES, P. de S, VALOIS, A.C.C. & PAIVA, J.R. de. "Induction and investigation of polyploidy in IAN 717 rubber tree clone A. preliminary study . Pesquisa Agropecuana Brasileira, Brasilia, 18(7): 789-796, 1982.

GRUN, G. JAI, X. & CHEN, L. Inductionof plantlet from ovule in vitro of Hevea brasiliensis. Hereditas, Lund, 4(1): 27-28, 1982.

HARLAN, J.R. Plant introduction and biosystematics. In: FREY, K.J. ed. Plant breeding. Symposium held at Lowa State University Press, 1967. 430 p.

HEUSSER, C. The reproductive organs of Hevea brusiliensis. Archives Rubberculture. Batavia, 3: 455, 1919.

JAUHAR, P.P. Cytogenetics of Pearl Millet. In: BRADY, N.G., ed. Advances in Agronomy, New York, 34: 407-485, 1981.

LASER, K.D. & LERSTEN, N.R. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms . The Botanical Review. New York, 35: 425-454, 1972.

MAJUMDAR, S.K. Chromosome studies of some species of Hevea. Journal Rubber Research Institute of Malaya, Kuala Lumpar, 18: 269-275, 1964.

MAJUMDAR, S.K. Male sterile. Clones in Hevea brasiliensis. Canadian Journal of Botany, Otawa . 45: 145-146, 1967.

MAJUMDAR, S.K. Pollen longevity studies in Hevea brasiliensis. Transaction Kentucky Academy of Science, 27:16-18, 1966.

MARKOSE, V.C. Colchyploidy in Hevea brasiliensis (Muell. Arg.) Rubber Board Bulletin, Kottayan, 12(1): 3-5, 1975.

MARKOSE, V.C., SARASWATHYAMMA, C.K., SULOCHANAMMA, S. & BHASKARAN NAIR, V.K. Mutation and polyploidy breeding in Hevea brasiliensis Muell, Arg. In: SCIENTIFIC SYMPOSIUM, Cochin, India, 1974. International Rubber Research and Development Board, p. 151-159.

MENDES, L.O.T. Preliminary investigations on duplication of the number of chromosomes in rubber by the action of colchicine. Belem, Agronomic Institute of Norte, 1946. 62P. (Technical Bulletin, 7).

MENDES, L.O.T. Polyploidization of rubber: A new test for determination of the capacity of production of young rubber plant Polimeros, Rio de Janeiro, 1:22-30, 1971.

MENDES, L.O.T. & MENDES, A.J.T. Artificial polyploidy in rubber Bragantia, Campinas, 22(3): 383-392, 1963.

MORAES, V.H.F. Organogenesis in apical meristemia of the stem of rubber (Hevea spp.). Brazilian Agricultural Research, Brasilia, 17(6): 893-894, 1982,

NAZEER, M.A. Cytology of a triploid. Sansevieria. Current Scienc Bangalore, 48: 34-35, 1980.

NAZEER, M.A. Triploidy in Heteroptens Kumth. Emend. Gnseb. Current Science, Bangalore, 48: 34-35, 1980.

NAZEER, M.A. & SARASWATHY AMMA, C.K. Spontaneous triploidy in Hevea brasiliensis (Wild ex. Adr. de Juss) Muell. Arg. Journal of Plantation Crops, Kasara god, 15(1): 1-71, 1987.

NEWTON, W.C.F. & DARLING TON, C.D. Meiosis in polyploids. Part 3 Triploid and pentaploid tulips. Journal of Genetics . London, 21: 1-15, 1929.

NITZCH, W. & WENZEL, G. Haploids in plant breeding. Berlin, Verlag Paul Parey, 1977. 101 p. (Advances in Plant Breeding, 8).

ONG, S.H. Chromosome morphology at the pachytene stage in Hevea brasiliensis. A preliminary report. In: INTERNATIONAL RUBBER CONFERENCE, Kaula Lumpur, 1975. Proceedings. p.1-19.

ONG S.H. Cytology of Hevea, In: FRIM. Short Course in Hevea breeding Kuala Lumpur, FRIM, 1980, p.1-21.

ONG, S.H.; IBRAHIM, N., GHANI, M.N.A. & TAN, H. Results of FRIM work on induced mutation and polyploidy breeding. In; COLLOQUE HEVEA 84. EXPLOITATION PHYSIOLOGIE AMELIORATION, IRRDB-1RCA, Montepellier, 1984, p. 383, 399,

ORTOLANI, A.A. Agriclimatology and rubber cultivation In: Symposium on rubber cultivation inthe State of Sao Paulo. Piracicaba, 1986. Campinas, CARGILL Foundation, 1986. Chapter 2, p. 11-32.

PADDOCK, E.F. On the number of chromosomes inHevea. Chronica Botanica, Walthan, Mass, 7: 412, 1943.

PARANJETHY,K. Physiolbgical aspects of wintering flower induction and fruit-set in Hevea. In: PRIM Hevea breeding course Kuala Lumpur, PRIM, 1980, p.1-17.

PARANJOTHY, K. & GHANDIMATHI, H. Tissue and organ culture in rubber tree. In: INTERNATIONAL RUBBER CONFERENCES, Kaula Lumpur, 1975, Proceedings. Kaula Lumpur, 1975, v.2, p.59-8

PERRY, B.A. Chromosome number and phylogenetic relationships in the Euphorbiaceae. American Journal of Botany, Baltimore, Md., 30: 527, 1943.

PINHEIRO, F.S.V. Genetic improvement of rubber. In: Brazilian Enterprise of Agricultural Research/CNPSD/FCAP. Specialization course in Heveiculture. Belem, 1981, 64p.

PINHEIRO, F.S.V.; PINHEIRO, E. & CONCEICAO, H.E.O. Evaluation of polyploid clones of rubber (Hevea spp.) of the IAC SERIES. In: National Seminar of Rubber, 3, Mandus, 1980. Anais. Rio de Janeiro, SUDHEVEA, 1980, p.349-364.

PIRES, J.M. Notes of herbarium I(Hevea camargoana n.sp.). Belem, Emilio Goeloli Parense Museum, 1981. 11b (Botanica, 52).

PIRES, J.M. Review of the genus Hevea, discription of species and geographic distribution. Annual Repott 1972, Belem, Institute of Agricultural Research of Norte. Botany project, sub-project: review of the genus Hevea "SUDHEVEA/ DNPEA/IPEAN. p.6-66,1973.

RAMAER, H. Cytology in Hevea. Genetica, The Hague, 17:193-236,,1935.

RAO, A.N. Periodic changes in the cambial activity of Hevea Brasiliensis. Journal of the Indian Botanical Society. 51:13-25, 1972.

ROSS, J.M. Chromosomes of H. brasiliensis. Planter's Belletin, Kaula Lumpur, No.41: 47, 1959.

RUBBER RESEARCH BOARD OF CEYLON, Ceylon, Sri Lanka Report of the Botanicalland Micological Department. In: --- Report of the work of the Rubber Research Board in 1941. S.l. Rubber Research of Ceylon, 1942. 10p.

SANTOS P.M., GOMES, A.R.G., MARQUES, J.R.B & VIRGENS FILHO, A. de C. Study of diploid and polyploid clones of rubber in South of Bahia. Itabuna, CEPLAC/CEPEC, 1987-13p. (Technical Bulletin, 154).

SARASWATHY AMMA, E.K.; MARKOSE, V.C.; LICY, J: ANNAMMA X, PANIKKER, A.O.N. & NAIR, V.K.B. Triploidy in Hevea brasiliensis Muell. Arg. In: NATURAL RUBBER CONFERENCE, Kottayam Kerala, India, 1960. Rubber Research Institute of India,198(

SATCHUTHANANTHAVALE, R. Hevea tissue culture. Quarterly Journal Rubber Research Institute of Ceylon, Sri Lanka, 50: 91-97, 1973.

SATCHUTHANANTHAVALE, R. & IRUGULBANDARA, Z.E. Propagation of callus from Hevea anthers. Quarterly Journal Rubber Research Institute of Ceylon, Sri Lanka, 49:65-68, 1972.

SCHULTES, R.E. Wild Hevea: an untapped source of germ plasm. Journal Rubber Research, Sri Lanka, 54(1):227-257,1977.

SEARS, E.R. Cytogenetic studies with polyploid species of wheat 1. Chromosomal aberrations in the progeny of a haploid of Triticum vulgare. Genetics Baltimore, Md., 24:509-523, 1939.

SEIBERT, R.J. A study of Hevea in the Republic of Peru. Annals Missouri of the Botanical Garden, Saint Louis,, 34:262-352, 1947.

SHEPHERD, R. Inductionof polyploid in Hevea brasiliensis. preliminary abservations on trials conducted at Prang Besear Rubber Research Station. Plantus Bulletin of the Rubber Research Institute of Malaysia, 1, Kula Lumpur, 104: 248-256, 1969.

VOSA, C.G. Heterochromatin recognition with fluorochromes Chromo soma. Berlin. 30:366-372, 1970.

VOSA, C.G. Heterochromation recognition and analysis of chromosome variation in Scilla Sibirica. Chromosoma, Berlin, 43: 269-278, 1973.

WILSON, H.M., EISA, M & IRWIN, S.W.B. The effects of agitated liquid medium on in vitro cultures of Hevea brasiliensis. Physiologia Plantarum, Copenhagen, 36: 349-402, 1976.

WILSON, H.M. AND STREET, H.E. The growth anatomy and suspension cultures of Hevea brasiliensis. Annals of Botany London, 39: 617-682, 1975.

WITMORE, T.C. Tropical rain forests of the far East. London, Oxford university Press. 1975, 355 p.

WRIGHT, J.W. Introduction of forest genetics London, Academic Press, 1976, 463p.

WYCHERLEY, P.R. The genesus Hevea. In: WORKSHOP ON INTERNATIONAL COLLABORATION HEVEA BREEDING AND THE COLLECTION AND ESTABLISHMENT OF MATERIALS FROM NEOTROPIC, Kuala Lumpur 1977. 12p.

WYCHERLEY, P.R. Rubber In: SIMMONDS, N.W., ed. Evolution of Crop plants. Edinburgh. Scotland, Longman, 1979, pp.77-86.

ZHOUZHONGYU, Y.K., GUO, O & HUANG, X. Studies on themethod of for predicting rubber yield at the nursery stage and theoretical basis. Chinese Journal of Tropical Crops, 3(2): 20-25 1982.