GENETIC COMPLEXITY IN HEVEA BRASILIENSIS: SOME THEORETICAL CONSIDERATIONS

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INTRODUCTION

Hevea brasiliensis (Wild. ex A. de Juss) Muell. - Arg., the natural rubber tree, is one of the domesticated wild forest trees, and is one among the least genetically manipulated crops of the world. The basic reasons for the lesser genetic manipulation were the extensive outcrossing nature of the tree along breeding cycle and narrow genetic base when compared to other cultivated species. Apart from these, the major economic output from the tree-the natural rubber-is of physiological and complex biochemical origin.

HEVEA BRASILIENSIS AND ITS CONGENERS

Cytogenetic studies on *Hevea* spp. accomplished so far had shown that all of them carry 2n = 36 with a base number of x = 18 (Majumder, 1964). However this is unlike to any other euphorbiaceous plants which either have a basic number of 7, 8, 9, 10 or 11. This made the researchers doubt that *Hevea* spp. may be natural tetraploid or amphidiploids, but no probable progenitor for *Hevea, has been so far traced out. The present discussion is limited to* *H. brasiliensis* alone, being the only *Hevea* species of commercial importance.

REPRODUCTION AND GENETIC CONSTITUTION

H. brasilensis is a monoecious, entomophilous (pollinated by insects) and allogamous (cross pollinated) tree, and the extent of self pollination may be around 14-28%. It flowers once or twice a year but this behaviour is highly location specific (Simmonds, 1989).

Being an allogamous species genetic constitution of *Hevea brasiliensis* is both heterozygous and heterogenous in progenies but is heterozygous and homogeneous when multiplied vegetatively (budding). When allowed to breed freely they are believed to produce a panmictic (Mendelian) population due to random mating.

In effect no Mendelian genetic analysis is done in rubber (Simmonds, 1989). A little is known about oligo (major) and poly (minor) genic control of different traits. However available information tells about more recessive genes (like yellow seedlings) than any major genes.

Carryover of undesired recessive like lethal or semi or sublethel genes is a peculiarity of the cross pollinated plants over generations. These genes are carried on in heterozygous conditions masked by their dominant alleles. The exhibition of heterosis and in breeding depression and survival of the Zygotes after fertilisation, therefore depend of the quantum of such recessive alleles carried forward over generations (Borojevic, 1990). In rubber, however the extent of their presence in the genome is uncertain.

THE CYTOPLASMIC ROLE OF THE LATEX

As mentioned earlier the economic product of the rubber tree-the rubber-is the product of some complex genetic regulatory mechanisms. Based on the principles of gene regulation and expression in eukaryotic (with true nucleus) organisms this complexity can be figured out. Rubber particles are the product of a biochemical pathway takings up a diversion from the catabolic pathway of carbohydrates (sugars) immediately after the glycolysis where acetyl coenzyme A is converted to aceto acetyl coenzyme A, instead of citric acid which should have been formed usually when the catabolism enters the TCA (tricarboxylic acid) cycle. Moreover the diverted pathway itself is not a catabolic one but an anabolic where isoprene monomeres are being synthesised (figure).

The isoprene synthesis pathway is controlled by specialised enzymes like acetoacetyl Co A acyl tmasferase, 3-hydroxy 3-methyl glutaryl co A synthetase, 3-hydroxy 3-methyl glutaryl Co a reductase, Mevalonate Kinase, phosphomevalonate Kinase, 5-diphosphomevalonate decarboxylase, isopentenyl diphosphate isomerase, prenyl transferase, and rubber transferase starting from the conversion of acetyl Co A to acetoacetyl Co A (Kekwick, 1989). Biochemically all enzymes are polypeptides and polypeptides are direct gene products. Polypeptide synthesis in eukaryotic system is always under complex genetic regulation (Lewin, 1990). In rubber tree all the regulatory units controlling the expression of all these gene products must be distributed among all of its 36 chromosomes. Every genome has its chromatin equilibrium, the equilibrium between the euchromatin and heterochromatin fractions in chromosomes and every mRNA (messenger RNA) is the product of a specialised splicing action, governed by more specialised splicing genes. These split genes work precisely on intron-exon junction and open reading frames (OR) of the parent DNA strand. Every regulatory unit of a gene comprises of may polycistronic polynucleotide sequences which function either as structural genes or regulator genes or promotor genes etc., which my be found together or distributed in different chromosomes (Crick, 1979; Sharp, 1981; Gilbert *et al.*, 1986). Hence, synthesis of every molecule of the enzymes involved must be under the control of different regulatory sequences and this clearly divulges the intricateness of the genetic regulation in producing every single enzyme in the anabolic pathway of isoprene monomeres.

Recent biochemical investigations reveal that the isoprene monomeres are being synthesised in laticifer system itself, as the latex is found rich in all the enzymes required for the biosynthetic track. However the conversion of pyruvate to acetyl co A is found to be distributed in and outside the mitochondria, which are retained in the laticifers even under the tapping flow (d' Auzac and Jacob, 1969). The presence of DNA and related rRNA (ribosomal RNA), tRNA (transfer RNA) and mRNA in the latex (Tupy, 1985) confirms this concept and that the rubber synthesis is self-regulatory and laticifer-specific.

Reconfirmation of this concept was evidenc[illegible]ently by Kush *et al.*, (1990) through [illegible] in vitro translation studies of the translatable mRNA. They observed that laticifer mRNAs are 20 to 100 fold enriched with transcripts encoding enzymes involved in rubber synthesis. Thereby it can be presumed that laticifer - specific genetic regulation of isoprene synthesis will be under the control of nuclear and extranuclear genome of rubber. By and large, gene expression under such regulatory system follows a central dogma, that is there will be inter as well as intra communications between nucleus and extrachromosomal genetic organelles like chloroplasts and mitochondria, for that the attempts on artificial manipulation of the genes for bettering the efficiency of rubber production (higher yield) is hardly an easy task. Because, if mutation is induced this may impair many steps in the metabolic track ultimately resulting in accumulating more undesirable genes besides all the existing ones.

The cytoplasmic role of the latex is complete when one comes to know about the particles other than rubber found in it. Lutoids are membrane bound particles containing the B-serum, which are highly osmotic sensitive and play a major role in the plugging mechanism. The precise mechanism of plugging is now known to be both electrostatic and enzymatic.

Lutoids are considered to be polydispersed lysosomal vacuome containing osmosensitive protein bodies like hevein and ergothionein and lysosomatic muramidinases and chitinases (Tata *et al.*, 1983), esterases and a range of acid hydrolases. They also carry peroxidases and several lutoid peroxidases. The lutoids are thus lysosomes of the latex cytoplasm and even the membrane of the lutoid is proved to be a vacuolysosomal tonoplast (d' Auzac and Jacob, 1989).

COMPLEXITY IN RUBBER BREEDING

Nevertheless as in any crop plant, the economic trait and its contributing traits in rubber tree are also believed to be polygenetically controlled and selection on phenotypic value is the only successful breeding strategy so far put forth. But the prediction of breeding values of characters like combining abilities (both general and specific) and also the nature of genetic variances by which those characters are being fixed in a population, was done by quite a few workers and the information available is too inadequate to bring out general conclusions.

Though a lot of inadequacies have been confronted everytime by the rubber breeder, he has been always trying to combine and recombine or even transgressing the desirable polygenes which may altogether produce either heterotic effects, transgressive segregation, rare recombinations or even combinations of all. And they are too optimistic to meet with any of such chances, because like all out breeders *H brasiliensis* too shows heterosis and inbreeding depression.

Severe selection pressure excerted by the breeders on *Hevea* populations, infact has been producing distortion of genetic variability since its domestication, for that the selected plants are always being propagated clonally and the unselected are always being discarded, severely cutting down the base of genetic variation. Hence the breeders have to keep in mind that they have to conserve the genetic variability all the while for their wide range of selection.

Due to the selection pressure quantification of genetic regulation fo characters using quantitative genetic tools becomes almost meaningless because it confronts with all the limitations in the Hardy-Weinberg's equilibrium of random mating populations. There will be considerable genetic drift or genetic flux and/or negligible natural heritable variations. Hence formulation of allelic testing designs always go beyond the Hardy-Weinberg's expectations, though the plants may be perfectly random mating.

Theoretically speaking, selections operating on genes which are partially or fully dominant with respect to the fitness tend towards the complete elimination of one or the other allel and finally the gene frequency. Hence the genetic equilibrium is being rather adjusted in fovour of the allel which has more selection efficiency (Falconer, 1989). However no clearcut information of overdominance of heterozygotes in rubber tree for any character, and hence the concept has no immediate relevance to the breeder.

Though not immediate a distant adverse effect can be envisaged in rubber breeding if the same selection pressure is allowed to exist sans giving priority to maintain the genetic variability.

In future this may create intermitting of genetically less flexible relatives, though they might have evolved in two distant centres as genetic variability is narrowing down, and will produce more unstable progenies which may exhibit 'genetic homeostasis' (Lerner, 1954). It is the effect when artificial selection is carried out and suspended before much of the variation has been lost by fixation, natural selection will tend to bring back the gene frequencies to an equilibrium resulting in the reversion of the means of the selected characters to its original magnitude. This effect however may not be a threat to the rubber breeding immediately as the selection products are propagated asexually. At present the concept of early fixation of characters is a reverie as far as rubber genetics is concerned.

CONCLUSION

With all the bottlnecks and intricateness in genetic constitution of *H. brasiliensis* in the fray breeders still have to go a long way to unfurl its mysteries. The discussion above is neither exhaustive nor implies that only rubber has such complex genetic regulation, and infact all the organisms have it in one way or the other. But considering the economic output of the tree and the yielding span more attention must be given in formulating future improvement programmes than relying upon the informations accumulated from other crop species. It is very easy to promulgate on utilisation of biotechnological tools in genetic manipulation of rubber, but rubber is said to be a recalcitrant species. On hand a little information is available so far how much resistance rubber genome may offer or how much lissom it is to the biotechnologist who attempts such manipulations at cellular level. Such responses will be very drastic as they are directly expressed in very few cells and tissues when compared to a whole organism.

Taking into consideration the various avenues where a rubber breeder and geneticist can work, one can be optimistic in knowing more about the genetic system in rubber. However a breeder should always keep in mind all these intricacies before framing future programmes.

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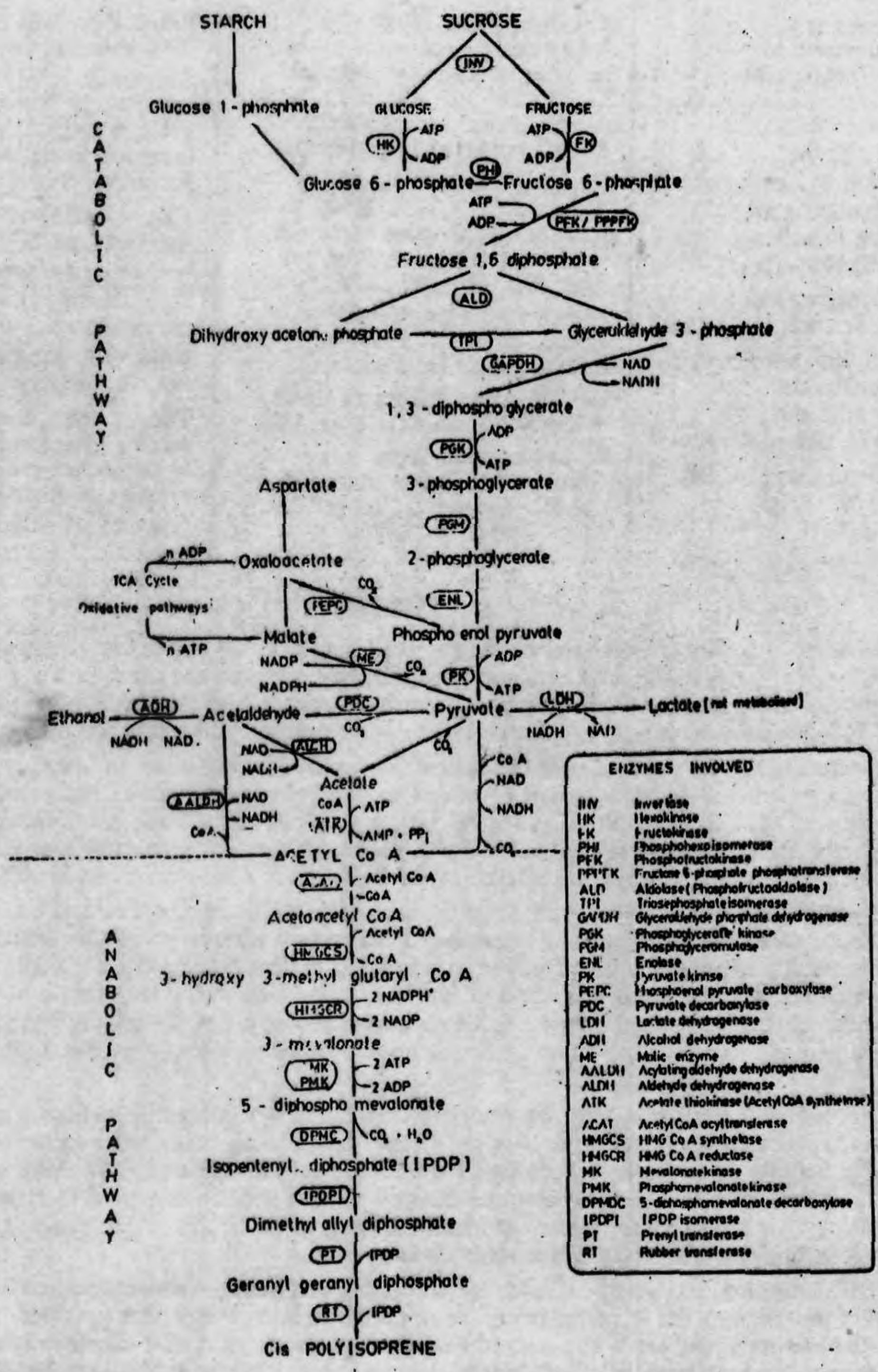

Fig 1: Pathways leading to the biosynthesis of rubber in Hevea brasiliensis

Hevea brasiliensis as a laticiferous cytoplasm. In Physiology of Rubber Tree Latex', J.d' Auzac, J.L. Jacob and J. Chrestin (eds). CRC Press, Florida, USA. p.59-88.

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Rubber Pact Talks

The International Natural Rubber Organisation (INRO) which met in a two day session late last month failed to resolve the differences between the producers and consumers that comprise the organisation. In the crucial area of market stabilisation programme, both the parties were poles apart but unlike the acrimony that attended the meeting of INRO, the latest meeting was characterised by some degree of cordiality among the participants.

Though no concrete proposals emerged over the contentious issue of how to shore up natural rubber prices, the meeting agreed to establish an adhoc group of rubber market experts as soon as practicable to provide an independent view of the rubber market situation during the past two years. Analysts however believe that in practical terms, this move does not amount to much. This because, essentially, the new group is expected to no more than supplement the work already undertaken by the buffer stock committee of the INRO.

In terms of the agreement, producers and consumers are to nominate three members each to the new group which would evaluate the state of affairs pertaining to natural rubber and submit its report to the next meeting of INRO in May. In this ensuing meeting, producers are likely to be vocal in demanding early renegotiations of the rubber agreement, which according to them, has not lived up to its role of balancing the interest of both the groups. Their current disenchantment springs from the fact that, natural rubber prices has sought lower levels during the past two years and the efforts of the buffer stock manager to shore up prices has been both half-hearted and ineffective.

By the time of the INRO meeting in May, the delegates from the largest consuming nations namely the US, and the European Communities are likely to finalise their views on the time table for renegotiations of the rubber pact. Observers believe that without concessions from natural rubber consumers, the May meeting may not achieve much, except the restatement by both the factions of their position in regard to a new natural rubber agreement. Already, the producers are sore at the inordinate delay in the reform of the rubber agreement and may take recourse to radical measures to influence rubber prices.

Prior to the INRO meeting, producing countries had a meeting in Thailand to hammer a common strategy with the chief aim of preventing a fall in rubber prices below the lower intervention price provided for in the existing rubber pact. Unfortunately, this move met with stiff resistance from the consumer members at the regular INRO meet. They argued that market realities justified the current lower price bands for natural rubber. The irony is that, after the last INRO meeting in October, this body indicated that it would enter the market in a big way in the new year. But, although prices continued to dip, the purchase operations proved to be modest so that there was no fundamental change in the market situation.