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Possible areas for molecular intervention for crop improvement in *Hevea brasiliensis* - theoretical considerations

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ABSTRACT

The last decade has seen worldwide efforts to use molecular biology methods to transform crop plants by introduction of agronomically desirable genes. In this new molecular approach, it has become possible to bring about specific plant genome changes by addition of one or more genes. In conventional breeding, entire sets of chromosomes are combined and desired parental genotypes are not necessarily reconstituted. The new approach offers the potential to make relatively quick and specific changes in elite cultivars without disrupting their otherwise desirable genotypic constitution. While highly attractive in concept with tremendous scientific potential, the generation of a transgenic crop plant is confronted with innumerable technical hurdles. The paper discusses progress to date and the opportunites for further progress in the future.

Introduction

The history of breeding in *Hevea brasiliensis* presents a story of success. For a tree crop, which requires about a 30-year-cycle for the generation of clones, the achievement of a ten times increase in productivity through hybridization and selection within the short span of six decades of breeding efforts is certainly significant. Compared to this outstanding initial success, the subsequent yield gains achieved through hybridization during the last three decades have been slow and limited and in many cases less stable (Figure 1). Besides the general law of increasing complexities and constraints for yield enhancement when the gap between the yield levels already achieved and the theoretical potential maximum gets narrower, a major factor in *Hevea* breeding is the narrow genetic diversity available to the breeders. The possibility of exploiting high yielding traits from the 1981 IRRDB collection of Brazilian germplasm now appears far less promising than originally expected.

The last decade represents worldwide efforts to use molecular biology methods to transform crop plants by introduction of agronomically desirable genes. In this new molecular approach, it has become possible to bring about specific plant genome changes by addition of one or more genes. In conventional breeding, entire sets of chromosomes are combined and desired parental genotypes are not necessarily reconstituted. The new approach offers the potential to make relatively quick and specific changes in elite cultivars without disrupting their otherwise desirable genotypic constitution. While highly attractive in concept with tremendous scientific potential, the generation of a transgenic crop plant is confronted with innumerable technical hurdles. Although nearly 900 transgenic varieties have been tested worldwide, only one transgenic plant, tomatoes with the transformed characteristic of delayed ripening and better flavour, has come onto the market, in the US from May 1994. The

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technical problems involved in transformation and regeneration are much more severe and demanding in woody, perennial species. *Hevea* represents one of the most difficult systems. At the same time, rubber is a high value crop and a generated transgenic plant could be vegetatively multiplied with comparative ease, hence justifying investment in this new technology.

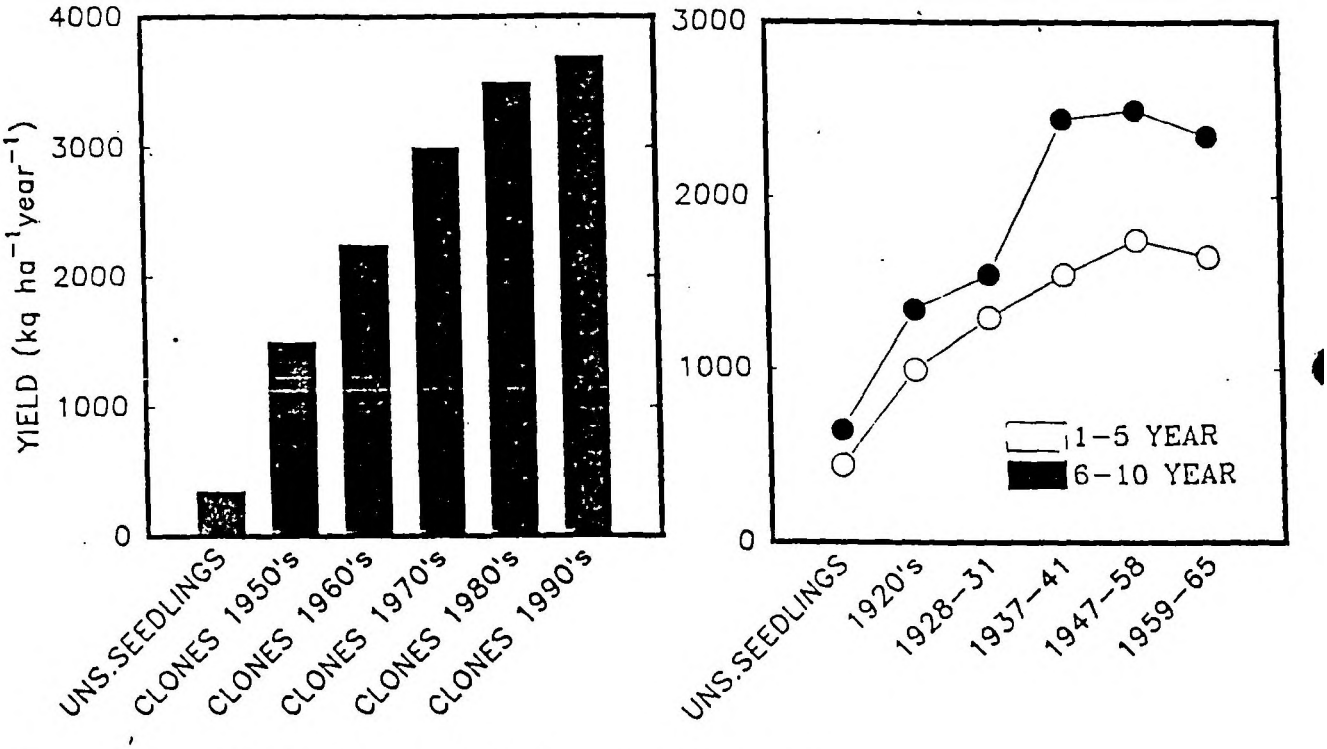

Figure 1 ***Yield increment through breeding/selection***

Factors influencing the decision to engineer genetic transformation.

Conceptual clarity is an essential pre-requisite for the adoption of genetic engineering to modify plant genotypes for any agronomic gain. Some of the factors that need careful consideration are listed below:

1. An acceptable cost for transformation and regeneration of the transgenic plant as compared with its expected economic return.
2. The possibility of achieving the target genotype by manipulation of only one or only a few genes.
3. A feasible strategy and protocol for the isolation of target genes.
4. An understanding of the requirements for the regulation of the target gene(s) expression and the availability of methods for achieving adequate control.
5. A system to introduce genes effectively into the target species.

Inability to meet any one of these requirements can proclude any positive economic impact of the programme.

Theoretical potential for transgenic rubber plants with specific agronomic traits.

Theoretically, the generation of a transgenic rubber plant incorporating agronomic gains such as drought tolerance, disease tolerance, tolerance to abiotic stress situations as well as tolerance to Tapping Panel Dryness (TPD) syndrome are attractive objectives.

An Economic Model

Assumption: A clone with a commercial productivity of 1.5 tonnes dry rubber per hectare per annum is genetically transformed incorporating drought resistant or disease resistant gene(s) and in the resultant transgenic plant the annual productivity is enhanced by 20 per cent. These transgenic plants are mass multiplied to plant in 100,000 hectares over a period of five years.

A 20% yield gain in a clone having a productivity of 1.5 tonnes/ha/year will be 300 kg/ha/annum. From 100,000 hectares, the incremental production will be 30,000 tonnes per annum. The total incremental yield from the transgenic plants during a 20-year-period of economic exploitation from 100,000 hectares would be 600,000 tonnes. Even at todays natural rubber price, (US$1130/tonne), the value of this incremental yield would be US$780 million.

This model is presented with the sole intention of infusing courage and confidence in potential investors.

The possibility of achieving certain specific agronomic gains by introducing one or a few genes into rubber.

A brief review of work done on molecular aspects of latex production in *Hevea* may be appropriate here.

Laticifer-specific gene expression has been reported by Kush *et al*[1]. They found that the known plant defence genes encoding, chitinases, PR proteins, prenyl alanyne ammonia lyase etc show a 10-15 times higher expression in laticifers than in leaves indicating the probable response of rubber trees to tapping and ethylene treatment.

Broekaert *et al*[2] reported wounding-induced accumulation or mRNA combining a hevein sequence in laticifers of the rubber tree.

Chye *et al*[3], using a hamster HMGR cDNA clone as a heterologus hybridization probe, could isolate and characterize 'cDNA and genome clone of HMGR from rubber. Southern analysis using 3' -end cDNA probes indicated the presence of at least two HMGR genes in *Hevea*. Northern blot analysis indicated that the HMGR1 transcript of 2.4kb is more highly expressed in laticifers than in the leaf.

Molecular cloning and nucleotide sequencing of a rubber elongation factor could be accomplished[4].

Molecular cloning, characterisation and expression of Mn-SOD have been accomplished from *Hevea*[5].

The role of hevein, a lectin-like protein, on the process of latex vessel plugging has been indicated by the studies of Gidrol *et al*[6].

Karthika and Kush[7] isolated a full length cDNA which encodes a 47 KDa protein with strong homology to farnesyl pyrophosphate synthetase (FPPS), using synthetic oligo-nucleotides corresponding to a partial aminoacid sequence of rubber transferase as probes to screen laticifer specific cDNA library.

A transformation system has been developed for *Hevea brasiliensis* using the particle gun method[8].

Variation in RAPD profiles between TPD-affected and normal plants selected from a seed-propagated rubber plantation has been reported[9]. Similar variations in RAPD pattern have been observed between *Oidium*-resistant and *Oidium*-susceptible genotypes of *Hevea* by Zheng Eueqin et al[10]. Intensive efforts are under way in different laboratories to perfect systems of transformation and regeneration.

Potential areas for molecular intervention.

Incorporation of drought tolerance

With the increase in the demand for natural rubber, many rubber producing countries, such as Thailand, China, India and Brazil, have made efforts to extend rubber cultivatiion to non-traditional areas with varying agroclimatic constraints. A major constraint in such areas is a prolonged period of drought.

In view of the scientific constraints and the long timeframe required for the generation of drought resistant genotypes through conventional breeding cycles, there is new worldwide interest to resort to molecular intervention to engineer plants with either drought responsive genes or with genes expected to alter osmotic regulation. In many crops, exciting progress has already been made in mapping drought resistant genes, in elucidating the theoretical mechanisms involved in identifying drought inducible genes and in characterizing the phenotypes of plants engineered for drought resistance[11]. There is considerable clonal variation in the percentage reduction in rubber yield in summer (7-30%). In general, high yielding clones show relatively greater percentage reduction in yield in the summer compared to low yielding clones (Figure 2). Drought tolerant rubber clones have been found to possess a more efficient osmo-regulation mechanism[12].

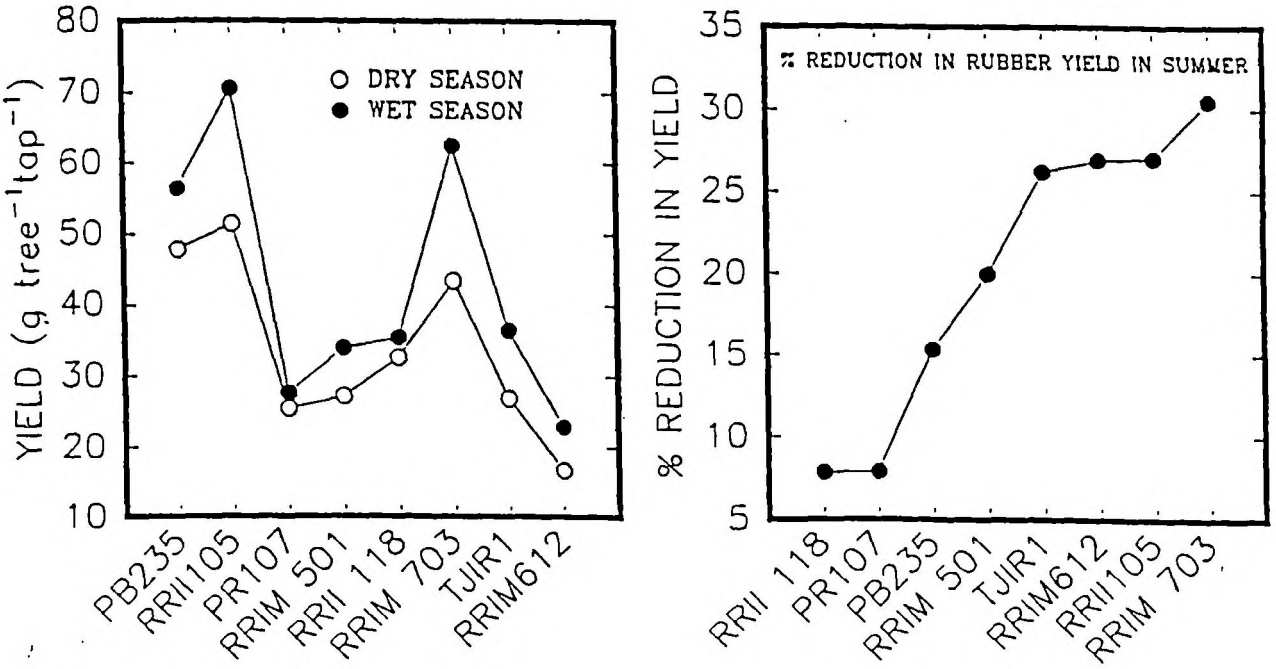

Figure 2 *Rubber yield of a few clones in wet and dry seasons*

Transgenic tobacco plants were engineered to over-express enzymes synthesising mannitol, fructan or betaine which can function as an osmoticum in a heterologous system. Another gene of interest is the one which encodes the synthesis of the sugar alcohol sorbitol from a common intermediate, glucose-6 phosphate, found in most plants. Sorbitol is a natural osmolyte and accumulates in many organisms that have to adapt to adverse environments.

Molecular approaches to mitigate the effect of TPD

a. Amplification of Mn-SOD

It has been widely documented that a high level of SOD in latex can counteract the action of free radicals, thus effectively providing protection to the system from developing the TPD syndrome. The ratio of free radicals to SOD levels may play an important role in determining susceptibility to TPD. Thus, a molecular intervention to amplify SOD generation in latex is an attractive concept. Mn-SOD from *Hevea* has already been cloned and characterized[5].

b. Regulation of in vivo ethylene production.

On the basis of theoretical conceptualisation, though not yet with actual experimental validation, climacteric rise in endogenous level of ethylene in response to exploitation stress is believed to be implicated in the development of the TPD syndrome. From a theoretical point of view endogenous ethylene production can be effectively regulated by introduction of an antisense gene for ACC synthase. Hamilton *et al*[13] have demonstrated that this approach can be made functional in plants. By molecular intervention one can regulate endogenous ethylene levels in the laticiferous tissue. It may have control over generation of free radicals and the subsequent events leading to TPD.

c. Promotion of endogenous production of cytokinin

Krishnakumar *et al*[14] comparing the trans-zeatin riboside (tZR) levels in the bark samples collected individually from TPD-affected and normal rubber plants of the clone RRII 105 obtained comaratively higher tZR content in normal plants than in TPD-affected plants. A protective role by cytokinin against the biochemical action of endogenous ethylene and free radicals can be theorectically assumed. Cloning and introduction of an ipt gene with the ultimate objective of amplification of endogenous cytokinin production is technically feasible. Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentanyl transferase gene has already been accomplished in tobacco plants[15].

d. Laticiferous system for molecular farming

Exciting possibilities have been predicted in using the laticiferous system for producing noval plant products by introduction of specific genes into this system[16]. The presence of all essential cytoplasmic components in latex as well as the ease with which the transformed latex can be extracted through tapping are to be considered as aspects of great economic advantage. A molecular strategy to produce novel plant products which can be derived from serum proteins, inositols and isoprenoid units by appropriate genetic engineering of *Hevea* is no longer to be considered a scientific fantasy but the problems associated with tissue specificity of expression are bound to be a major constraint to this approach.

e. Resistance to major diseases

In recent years, considerable efforts have been made to utilize molecular methods to combat diseases caused by fungi, bacteria and viruses[17]. The possibilities of incorporating resistance to *Phytophthora, Oidium* etc through molecular methods should be explored.

Other future prospects

The results of many RFLP, RAPD and AFLP analysis of *Hevea* that are being carried out in different laboratories in relation to TPD, disease resistance, stress tolerance etc can lead to specific molecular approaches to address these problems effectively.

I have no intention of making a comprehensive survey of the potential areas where molecular approaches can be gainfully used in rubber. The known innumerable technical problems involved in various stages of transformation, regeneration, regulation of temporal and spatial expression of the introduced genes etc are well documented and are not discussed in this paper. This paper has only the limited objective of provoking rubber scientists to harness this powerful tool to achieve their agronomic objectives within a shorter timespan and with more specificity. I believe that the prospects are bright provided we have a sound conceptual plan for the future.

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