Can rubber tree be genetically modified?

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INTRODUCTION

Genetically modified (GM) plants contain gene or genes, which has been artificially inserted into the plant. The inserted sequence may come from another related plant or from completely different species.

A plant breeder tries to assemble a combination of genes in a crop plant which will make it as useful and productive as possible. Desirable genes may provide features such as higher yield or improved quality, pest or disease resistance or tolerance to heat, cold or drought. Combining the best gene in one plant is a long and difficult process. Traditional plant breeding has been limited to artificially crossing plants within the same species or with closely related species to bring different genes together. But transgenic technology provides plant breeders to bring useful genes from a wide range of living sources, not just from within the crop species or from closely related plants. This technology provides a mean for identifying and isolating genes, controlling specific characteristics in one kind of organism and for moving copies of those genes into another quite different organism, which will then also have those characteristics.

The production of recombinant products by transgenic organism is a very common practice now. The synthesis of commercially important proteins and pharmaceuticals by micro-organisms through bioreactors has emerged in recent years. Genetically engineered farm animals such as goats, sheep and cows are used as living bioreactors produce sustained yield of target proteins from their milk cheaply and continuously. Like transgenic animals, plants can also be genetically modified to produce protein based products even more cheaper than farm animals. Plants require only sunlight, water and basic horticultural inputs and thus they can be used as a solar-powered, ecologically friendly protein manufacturing factories. Plants have other advantages over animals in that their multiplication through inbred seeds is relatively simple and efficient and they can also be propagated by vegetative means as a clonal material in very large quantities

In conventional plant breeding, there is a possibility to inherit some undesirable characters or some times to lose good traits, displayed by their parents. But in genetic transformation, the genes controlling specific characters are inserted into plant, leaving its original characters as it is. The techniques of tissue culture are totally engaged in the process of genetic transformation. Therefore, it is necessary to produce efficient plant regeneration through somatic embryogenesis which is essential in crop improvement through transgenic approaches. Large number of experiments have been carried out in this respect to enhance the frequency of somatic embryogenesis and plant regeneration from the embryo, by modifying the culture conditions, altering nutritional and hormonal requirement as well as changing the explant material.

Possibilities with rubber tree

A plant produces recombinant protein in plant parts such as seeds, leaves or shoots. Therefore after every harvest it takes time for new growth before next harvesting. As a result recombinant products are more likely to be batch-wise rather than a continual process. Taking into consideration the strength of transgenic animal (continued milk production) and the transgenic plant (simple clonal propagation and low cost) for recombinant product, it could be beneficial to have a production system that combines both advantages. The transgenic rubber plant can be an ideal candidate for this when compared with other transgenic crop plants. Transgenic rubber plants can be maintained cheaply and easy to multiply clonally, while allowing continued harvesting of latex.

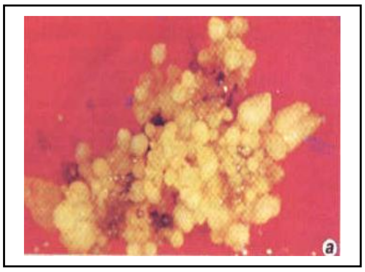
Can rubber tree to be genetically modified?

The shortest answer for this question is YES it can be genetically modified. Now, it is possible to precisely identify, isolate, copy and insert a single gene that exhibit a desirable characteristic in *Hevea.* The genetic information, incorporated into the expression vector necessary is coding sequence and promoter sequence. The coding sequence code for specified protein and the promoter sequence direct the expression of the protein at localized area or specified tissue type. The scientists engaged in rubber breeding and molecular activities in the world have been able to develop genetic transformation of rubber tree based on the somatic embryogenesis. By incorporating new genes into rubber trees and then tapping and milking, the scientist expect to get proteins, antibiotics, vaccines and hormones along with the latex. These products can be made cheaply, efficiently and pollution free. The advantages of the rubber trees are that these can be tapped often and throughout the year and the trees can last about thirty years in the field (Arokiraj, 2000 & Arokiraj *et al.,* 2002).

In addition, the scientists are engaged in producing genetically modified rubber plants by introducing genes controlling specific agronomic traits, such as the genes for disease resistance, tolerance to drought and other environmental stresses, enhanced rubber biosynthesis, higher timber yield and tolerance to tapping panel dryness *etc*. to high yielding rubber clones (Nair, 2005).

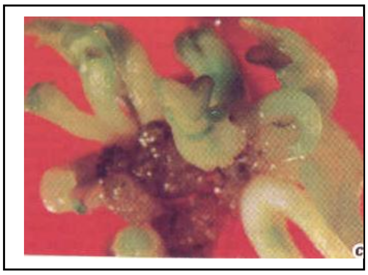
Progress with rubber tree

The basic methods employed for genetic transformation of the rubber tree follow procedures well established for other plants. As with other plants, genetic transformation of the rubber tree involve inserting the selected gene in to callus tissue and then regenerating the transformed callus tissue into complete plant. Recently, there has been an increasing interest in the induction of somatic embryogenesis of rubber especially for the genetic transformation of desirable gene. Somatic embryogenesis has now been reported from internal integument tissues as well as from immature anthers (Fig. 1). But reliable somatic embryo formation is limited to only for a few genotypes of *Hevea* (Kumari Jayasree *et al*., 1990., Seneviratne *et al*., 1996). Many techniques have been employed for the transfer of DNA into plant cell. Among them, the easiest, simplest and most successful gene transfer procedure is mediated by a bacteria called *Agrobacterium tumerfaciens* on friable callus lines and this step have been successfully optimized by suppressing calcium from the calli pre-culture medium as well as from the inoculation medium. In this process only a small proportion of callus cells would be successfully transformed and these transformed tissues are screened by through inoculation medium. The foreign gene inserted into *Hevea* over expressed the recombinant protein in the serum fraction of latex which can be easily recovered by tapping (Jayashree *et al*., 2003, Arokiaraj *et al.*, 1996, Montero *et al*., 2003).



Yellow colored Globular embryos

White Colored Heart & Globular Shaped embryos



Green cotyledonary embryos

Bipolar differentiation of somatic embryos into root & shoot poles

Fig. 1. Somatic embryogenesis from immature anthers (Source – Current Science. 76)

The rubber callus are inherently sensitive for the biotic and abiotic stresses followed by necrosis of the tissues and persistent bacterial contaminations following transferring. This can be overcome by optimization of growth regulator supply in the post transferring culture medium and, this allows callus growth recovery with reduced browning and sustain reporter gene beta-glucuronidase (GUS) activity (Kumari Jayasree *et al.*, 2001).

Today, scientists are able to produce human proteins from the genetically modified rubber trees (Anon, 2001). The gene introduced into rubber tree produced human serum albumin, a vital nutrient given to patients on drip feeds in intensive care. The transgenic rubber trees have become mini factories to produce protein based pharmaceuticals such as insulin for diabetics, blood clotting factors for hemophiliacs, clot dissolving plasminogens activities for cardiac treatment, tumor necrotic factors to combat cancer, eryhroprotein to treat anaemea, viral coat protein for vaccines *etc.* (Anon, 2001).

A group of scientists who are engaged in genetic transformation in rubber plants have been able to get patent for (United State Patent No. 5580768) a method of producing a genetically transformed fluid-producing plant which comprises;

i) inserting into the plant tissue a gene or gene fragment controlling the expression of a target product, and
ii) regenerating a plant from said tissue, the genetically transformed plant being capable of expressing the target product in the fluid that it produces.

And also it included, a fluid-producing plant which contain new genes in their DNA materials, such that the target product is expressed in the fluids which they produce. It is further described, a method of producing a protein or other target product which comprises:

i) harvesting the fluid from a genetically transformed fluid-producing tree or plant, or a clone thereof, and
ii) recovering the target protein or other product from said fluid. Most preferably, the plants are rubber (*Hevea*) plants and the genes are foreign genes that code for pharmaceutically valuable protein products which can be harvested in the latex produced by the plants (Anon 2008).

Researchers have already identified the genes that would provide drought tolerance, tapping panel dryness tolerance, elevated temperature and light tolerance. They are now able to produce genetically modified rubber plants that has better drought resistance and increased environmental stress tolerance (Vipin V Nair, 2005). These genes were introduced along with the gene coding for Hb superoxide dismutase (SOD) and the reporter gene which produce bacterial enzyme, beta-glucronidase (GUS). The SOD transformed tissues over expressed the gene when subjected to stress conditions (Sobha *et al.,* & Venkatachalam *et al.*, 2006).

The cloning of specific promoters from rubber tree has been undertaken with a view to optimizing and controlling transgene expression in genetically engineered rubber trees such as;

a) Ethylene inducible promoter – when ethylene treatments are practiced for stimulation the latex production, this promoter specifically help to over express the transgene.
b) Latex cell specific promoter – it limits the expression of transgene in latex producing tissues (Pujade - Renaud *et al.*, 2001)

Advantages of the GM rubber tree

- The procedure adopted in this process is ecological friendly. It is driven by solar power and therefore energy efficient and pollution free.
- Rubber tree requires only basic horticultural needs and therefore, it is highly cost effective when compared to other conventional bioreactor systems using micro organisms which need special care, making manufacturing overhead costs high.
- *Hevea* produces voluminous latex upon tapping continually as a non destructive mode as compared with other crop plants.
- The usual method of propagation of *Hevea* is bud grafting that helps to produce unlimited clonal copies of single selected transgenic plant. Therefore, successful transformation of the rubber tree for a specific gene need to be achieved only once.
- A significant advantage in using plants over other bioreactor systems is that glycosylation of the foreign protein (binding of sugars to certain proteins to render them functional) would occur in the plant system.
- The violation of animal right problems does not arise with transgenic rubber tree and no limitations from the national bio-safety framework.
- The latex that is taken from rubber tree is free from animal viruses and other contagion vectors such as those causing AIDS, Hepatitis or Mad cow disease *etc*.
- This can be used to produce proteins of moderate value, such as those in shampoos and toothpastes or enzymes for detergents and the valuable proteins like high cost pharmaceuticals (Anon, 2001).

Although the gene has been successfully inserted into the rubber plant, it does not guarantee that the protein encoded for particular gene will be successfully synthesized. Even when these genes are present, they can remain dormant. The other thing is that some proteins become modified and hence they do not produce exact linier sequences of amino acids of the native protein. Other than that, somatic embryogenesis in *Hevea* is highly genotype dependant and strictly specific to the genotype and medium interaction, non synchronized and very few embryos are converted into plantlets. Therefore, the production of transgenic rubber plant is rather difficult, laborious and time consuming process. But scientists involved in rubber breeding activities have been able to overcome those barriers and now they are able to extract proteins of value. With all these success, ultimately, rubber is likely to be a by-product of protein production in the future.

REFERENCES

Anon (2001). Tree of Life-Gene altered rubber plants are putting human proteins on tap. *New Scientist*. 1255.

Anon (2008). www.freepatentsonline.com/5580768.html

Arokiaraj, P, Jones, H, Hafsah Jaafar, Coomber, S and Charlwood, B V (1996). *Agrobacterium*-mediated transformation of *Hevea* anther calli and their regeneration into plantlets. *Journal of Natural Rubber Research* **11(2)**, 77-86.

Arokiaraj, P (2000). Genetic transformation of *Hevea brasiliensis* (rubber tree) and its application towards crop improvement and production of recombinant protein of commercial value. *Molecular Biology of Woody Plant*. (Eds. Subhsh, C., Minocha, S. and Mohan Jain). Vol. 2, 305-327.

Arokiaraj, P, Rueker, F, Oberayr, E, Shamsul Bahri, A R, Hafsah Jaafar, Carter, D C and Yeang, H Y (2002). Expression of human serum albumin in transgenic *Hevea brasiliensis*. *Journal of Rubber Research* **5** (3), 157-166.

Jayashree, R, Rekha, K, Venkatachalam, P, Uratsu, S L, Dandekar, A M, Kumari Jayasree, P, Kala, R G, Priya1, P, Sushma Kumari, S, Sobha, S, Ashokan M P, Sethuraj, M R and Thulaseedharan, A (2003). Genetic transformation and regeneration of rubber tree (*Hevea brasiliensis* Muell. Arg) transgenic plants with a constitutive version of an anti-oxidative stress superoxide dismutase gene. *Plant Cell Report* **22** (3), 201-209.

Kumari Jayasree, P, Asokan, M P, Sobha, S, Sankari Ammal, L, Rekha, K, Kala, R G, Jayasree, R and Thulaseedharan, A (1990). Somatic embryogenesis and plant regeneration from immature anthers of *Hevea brasiliensis* (Muell. Arg). *Current Science* **76**, 1242-1245.

Kumari Jayasree, P, Thomas, V, Saraswathyamma, C K and Thulaseedharan, A (2001). Optimization of parameters affecting somatic embryogenesis in *Hevea brasiliensis*. *Indian Journal of Natural Rubber Research* **14**, 106 -111.

Montero, P, Rattana, W, Pujade Ranaud, V., Michaux-Ferriera, N, Monkolsook, Y, Kanthapura, R and Adunsadthapong, S (2003). Production of *Hevea brasiliensis* transgenic embryogenic callus lines by *Agrobacterium tumefaciens*: roles of calcium. *Plant Cell Reporter* 11, 1095-1102.

Pujade-Renaud, V, Montero, P, Sanier, C, Phuangkosol, N, Kongsawadworakul, P and Chrestin, H (2001). Specific promoters for genetic engineering of rubber tree. *Proceeding of IRRDB Symposium* 2001, Montpellier, France.

Seneviratne, P, Withanage, S P, Nugawela, A, Wijesekera, G A S and de Zoysa, G M (1996). Somatic embryogenesis for *Hevea*; the techniques of anther culture. *Journal of the Rubber Research Institute of Sri Lanka* **78,** 79–88.

Sobha, S, Sushamakumari, S, Thanseem, I, Kumari Jayasree, P, Rekha, K, Jayashree, R, Kala, R G, Asokan, M P, Sethuraj, M R, Dandekar, A M and Thulaseedharan, A (2003). Genetic transformation of *Hevea brasiliensis* with the gene coding for superoxide dismutase with FMV 34S promoter. Research communications, *Current Science*, 85 (12), 1767 -1773.

Venkatachalam, P, Jayashree, R, Rekha, K, Sushmakumari, S, Sobha, S, Kumari Jayasree, P, Kala, R G and Thulaseedharan, A (2006). Rubber tree (*Hevea brasiliensis* Muell. Arg). *Methods Mol. Biol*. 344, 153 -164.

Vipin V Nair (2005). Research body develops GM rubber verity tolerant to drought, environment stress. A news paper article on Hindu Business Line 2005.05.26.

Yeang, H Y, Arokiaraj, P, Jaafar, H, Arif, S A M, Rajamanikam, S, Chan, J L, Sharib, J, Leelavathy, R, Hamzah, S and Van Der Logt, C P E (2002). Expression of a functional recombinant antibody fragment in the latex of transgenic *Hevea brasiliensis*. *Journal of Rubber Research* **5**(4), 215-225.