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Review

An overview of the potentials of natural rubber (*Hevea brasiliensis*) engineering for the production of valuable proteins

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Nigeria has in recent times exhibited great commitment to the use of biotechnology as a tool to enhance agricultural and general socioeconomic development. Plant biotechnology and genetic engineering have led to the production of various pharmaceutical proteins from plant sources. Plants are potential bio-farming factories because they provide an inexpensive and convenient system for the large scale production of valuable recombinant proteins. The objective of this paper is to highlight the prospects and potentials of transgenic rubber plant as a unique protein factory which will act as additional source of income to the rubber farmer especially in Nigeria. Rubber has the advantage of having continuous harvesting from same tree for a minimum of twenty years. The International Rubber Research Organizations have successfully developed transgenic rubber plants that produce foreign proteins of potential commercial value. Among such routines is an antibody and human serum albumin. Tapping rubber trees for valuable proteins will be more profitable compared to other options. The technologies have been developed and only needs to be adapted to our local conditions. It is hoped that this paper will be instructive to rubber farmers, policy makers, executors of policies or citizens wishing to join the Nigerian rubber farmers in their bid to increase their prosperity or alleviate their poverty.

Key words: Natural rubber, transgenics, protein factory.

INTRODUCTION

Natural rubber (NR) is a term applied to a wide variety of elastic substances produced from over 500 plant species. The commercial species, *Hevea brasiliensis* Muell Arg. is native of Brazil and was introduced into Nigeria in 1895 from Kew Botanical Gardens in England. It has 6 - 7 years gestation period and economic life of about 25 years. Natural rubber is raw material for over 40,000 products. Rubber grows well in deciduous rain-forest regions of lowland tropics with temperature ranges of between 21 - 35℃ and a well distributed rainfall of 2000 mm or more on a well drained soil (Aigbekain et al., 2000; Samarappuli, 2001). Nigeria's rubber belt includes Edo, Delta, Ondo, Ogun, Abia, Akwa Ibom, Cross River, Imo and Rivers State which lies between 15° N and 10° S. Recently in a bid to increase rubber production in Nigeria, efforts at plantation establishment is being made in non traditional rubber growing areas, for example, in Kaduna state. Rubber in Nigeria according to grouping of crops and importance based on national support and foreign exchange earnings belongs to the majority group. Though Nigeria is not the top most natural rubber producer, its production is still contributing substantially to the world market and still leads the other producers in Africa (Table 1). Rubber plantations provide gainful self employment and sustainable livelihoods and serves as a foreign exchange earner for the country besides ecological benefits including carbon sequestration. Abolagba et al. (2003) and Giroh et al. (2006), however, noted the poor wages to the rubber farmers and poor prizes of natural rubber, hence low productivity. There is, therefore, the need for alternative means of increasing the value of the crop and hence an enhanced income for the farmer.

Plant biotechnology and genetic engineering have led to the production of various pharmaceutical proteins from

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Table 1. Production of natural rubber in Africa 2000 - 2005.

Country	Production (Internation $ 1000)					
	2000	2001	2002	2003	2004	2005
Nigeria	57,929	57,929	65,975	76,166	76,166	76,166
Cote d'Ivoire	68,603	68,603	60,075	69,729	73,415	72,411
Liberia	57,393	57,393	58,465	59,002	62,756	62,756
Cameroon	28,357	28,357	27,892	24,616	24,616	24,616
Ghana	6,437	6,437	6,437	6,437	6,437	6,437
Gabon	5,900	5,900	5,900	5,900	6,260	6,260

Source: FAOSTAT (www.fao.org).

plant sources. Plants are potential biofarming factories because they provide an inexpensive and convenient system for the large scale production of valuable recombinant proteins (Maziah et al., 2006). The transfer of selected genes in a single generation by genetic transformation has been especially interesting for the rubber tree, since its improvement through conventional breeding is limited by long breeding cycles and high levels of heterozygosity. Besides improving crop productivity, however, genetic transformation technique has another promising application that is less publicised. Transgenesis has the potential to turn crop plants into living factories for the production of commercially valuable proteins such as peptide-based pharmaceuticals, industrial enzymes, proteins used in personal care products, etc. Proteins that are produced through the process of transgenesis are known as recombinant proteins. In the medical field, a number of pharmaceutical products have been made using GM organisms for many years. For example, insulin, growth hormones, heart disease medication, anticancer drugs and cystic fibrosis medication are all produced by genetically modified animal cells or bacteria in large bioreactors (e.g. Biogen at www. Biogen.com). This paper highlights the prospects and potentials of transgenic rubber plant as a unique protein factory which will act as additional source of income to the rubber farmer in Nigeria.

HARVESTING FOREIGN PROTEINS FROM TRANSGENIC MICROORGANISMS, ANIMALS AND PLANTS

The production of recombinant proteins by transgenic organisms is hardly new. The synthesis of commercially important proteins, particularly pharmaceuticals, by mirco-organisms (commonly bacteria and yeasts in bioreactors) involve processes well entrenched in industry. While the manufacture of these proteins in high-tech bioreactors is costly, drug manufacturers recoup investment through appropriate pricing of their products. An alternative to the bioreactor has emerged in recent years. DNA engineering in animals has enabled the expression of foreign proteins (commonly therapeutic proteins) in the milk of animals such as sheep, goats and cows. These animals become, essentially, living bioreactors that support the sustained yield of target proteins which include human hormones, enzymes, blood coagulating factors and immunological agents. An attraction of using transgenic animals to produce recombinant proteins in milk lies in the lower costs of maintaining animals as compared with brick and mortar factories. Another important consideration is that the animals can be milked continually, thus enabling continual production of the target protein.

Like transgenic animals, plants can also be genetically transformed to express protein-based pharmaceuticals and other valuable proteins. In fact, plants are even cheaper to maintain than animals. Grown in the field, plants require little more than sunlight, water and basic horticultural input and as protein manufacturing factories; they are solar-powered and ecologically friendly. Plants have yet other advantages over animals for protein production. Their multiplication through inbred seeds is relatively simple and efficient and they can also be propagated by vegetative means (e.g. by cuttings, bud-grafting, etc) without the use of seeds. In fact, vegetative propagation serves more than just a means to multiply plants. Since such plants are clonal copies of one another, genetically identical copies of the best cultivars can be easily reproduced in very large numbers.

THE RUBBER TREE AS A UNIQUE TRANSGENESIS MODEL

The many advantages of transgenic plants for 'biopharming' notwithstanding, their one significant weakness is the difficulty in recovering the recombinant protein. Unlike transgenic animals where there is continual protein production in the milk, harvesting of the recombinant protein involves destruction of the plant or a portion of it, whether the desired protein is to be found in the seeds, leaves or shoots. After every harvest, it takes time for new growth to take place before the next harvest is possible. As a result, protein recovery is more likely to be batch-wise, rather than a continual process. Taking into consideration the strengths of the transgenic animal (continual protein production in the milk) and the transgenic plant (low cost of maintenance, simple clonal propagation) for recombinant protein production, it would obviously be beneficial to have a production system that combines both advantages. The ideal plant for recombinant protein production would be one that is cheap to maintain and easy to multiply clonally, while allowing for continual harvesting of the protein. This is where the transgenic rubber tree has the distinct advantage when compared with other transgenic crop plants.

In the bark of the rubber tree is a complex network of laticifers, or latex vessels, each vessel merely one-third the thickness of a human hair. These laticifers contain natural rubber latex that is exuded when the bark is cut. Rubber tapping that is routinely practiced in estates and smallholdings is essentially the systematic and regulated cutting of the bark to harvest the latex. Since rubber tapping is a non-destructive method of latex extraction and harvesting, the tree can be tapped every alternate day throughout the year without pause. Among plants, the rubber tree is unique in its capacity to produce voluminous latex upon tapping and to replenish this supply rapidly in readiness for the next tapping. If *H. brasiliensis* were transformed with a gene encoding a foreign protein, the transgenic *Hevea* system would allow for continual production of the target protein, a feature lacking in any other transgenic plant system. In the transgenic *Hevea* system, therefore, modern techniques in biotechnology melt with the generations-old practice of rubber tapping to add new value to the rubber tree.

INSERTING FOREIGN GENES INTO *HEVEA BRASILIENSIS*

The basic methods employed for genetic transformation of the rubber tree follow procedures well-established for other plants (Sambrook et al., 1989). As with many plants, genetic transformation of the rubber tree involves inserting the selected gene into callus tissue (unorganised clusters of cells) and then regenerating the transformed callus tissue into the complete plantlet (Arokiaraj et al., 1994). *Hevea* callus tissue cultures are established from anther walls of the rubber tree male flowers. The first transgenic *Hevea* plant was produced through particle bombardment of callus tissue whereby DNA was coated on to microscopic gold particles that were then shot into callus tissue under high pressure. The transformed callus was subsequently regenerated into the complete plantlet. Since this initial success, genetic transformation has also been achieved through *Agrobacterium* mediation (Arokiaraj et al., 1996; Venkatachalam et al., 2007) and is today the preferred method for transforming *Hevea*. By this approach, foreign genes are transferred into a bacterium called *Agrobacterium* and this is then allowed to infect the callus tissue. The foreign gene is incorporated into the genetic make-up of the *Hevea* callus tissue during this process. As only a small proportion of the callus cells would be successfully transformed, a mechanism has to be available to sort out cells that are successfully transformed from those that are not. For this reason, the DNA assembly that is used in transformation contains a second gene that confers antibiotic-resistance to transformed callus cells. When the callus tissues are transferred to culture medium containing the antibiotic, untransformed cells perish, while the transformed cells - armed with the means to resist the antibiotic-continued to thrive. The surviving callus cells proliferate and some develop into embryo-like structures that go on to form plantlets.

MULTIPLYING SUCCESS

From a number of transgenic plants that have been produced, the ones that show the strongest protein expression are multiplied for further study. Neither new nor expensive technology is needed here. By the use of the horticultural practice of *Hevea* bud-grafting, unlimited clonal copies-each genetically identical-can be generated from a single selected transformant. The amenability to clonal propagation has been proven through successful multiplication by bud-grafting over four successive vegetative generations of plants bearing the *gus* gene (Arokiaraj et al., 1998). Besides demonstrating the efficiency of up-scaling transgenic *Hevea*, these results also confirm the stability of the genetic transformation in this plant. In the research carried out at RRIM, transgenic rubber plants have successfully synthesised in the latex a bacterial enzyme (beta-glucuronidase or GUS) and a mouse antibody fragment (Yeang et al., 2002). Significantly, these proteins are functional proteins in that their operational characteristics are retained. The recombinant GUS protein shows its characteristic enzymic properties when supplied with its designated substrate, while the antibody fragment is immuno-reactive to its matching antigenic protein. In another set of experiments, transgenic *Hevea* has produced a human protein - human serum albumin- in its latex (Arokiaraj et al., 2002).

TOWARDS COST-EFFICIENT PRODUCTION OF AFFORDABLE PROTEINS

Its obvious, commercial potential notwithstanding, that the production of recombinant proteins from transgenic *Hevea* is not about profit making alone. Cost-efficient production by transgenic plants can alter the economics of recombinant protein synthesis. For example, hitherto prohibitively expensive chemotherapy could be brought within reach of the man in the street. Commercial proteins from transgenic plants need not be confined to high-cost pharmaceuticals either. Moderate-value proteins such as industrial enzymes or proteins used in personal care products may also be harvested from engineered plants such as the rubber tree. In fact, the low cost of maintaining transgenic plants make them especially suited to high volume production of less expensive proteins that otherwise cannot be produced cost-effectively in conventional bioreactor systems.

ADVANTAGES OF THE TRANSGENIC RUBBER TREE AS A LIVING PROTEIN FACTORY

There are several advantages in using transgenic *Hevea* for the production of commercially valuable proteins. Among these are:

a) The concept is a novel approach to cost-efficient production of high value proteins in the latex of transgenic rubber trees, which essentially serve as production lines.
b) The approach is environment-friendly. The process is driven by the sun and is therefore energy-efficient and essentially pollution-free. Rubber (*H. brasilensis*) is primarily being cultivated for the extraction of latex, however by now, its importance has extended to timber production and sequestering of atmospheric carbon in the mitigation of climate change. Being a long-term sink of atmospheric carbon, importance of rubber in Clean Development Mechanism (CDM) to combat the climate change has already been emphasized and is being exploited by various Institutions globally.
c) Rubber trees require no special attention beyond routine horticultural maintenance. Their use is thus highly cost-efficient as compared with conventional bioreactor systems.
d) Production of the target protein is continual through a system of non-destructive harvesting (tapping) of the rubber tree.
e) Glycosylation of eukaryote proteins (binding of sugars to certain proteins to render them functional), which does not occur in bacterial systems of protein production, can take place in the transgenic rubber tree.
f) The latex that exudes from the rubber tree is free of animal viruses and other contagion vectors. These include pathogenic viruses such as those causing AIDS or hepatitis and prions that cause mad cow disease and its human variant.
g) Successful transformation of the rubber tree for a specific gene needs to be achieved only once. Rubber trees are amenable to vegetative propagation and an unlimited number of genetically identical plants (clones) can be generated by conventional horticultural methods.
h) The methodology does not involve the use of animals and hence the issue of animal rights does not arise.
i) From the biosafety viewpoint, the transgenic rubber tree raises far fewer objections as compared with other crops. *Hevea* is not native to Nigeria and propagation is normally by vegetative means. Hence, it is not expected to have adverse effect on the environment or on the crop. Unlike transgenic food products, recombinant proteins from *Hevea* are purified from the transgenic elements that are not presented to the consumer.

CONCLUSION

It is imperative that any effort at addressing the gaps in biotechnology in the Institute considers these constraints. A key constraint is laboratory infrastructure followed by funding. The acute constraints of frequent power cuts and low voltage also bedevils the Institute. Other constraints include availability of laboratory spare parts and trained repair technicians, ease of procuring laboratory chemicals, lack of current journals and poor private sector linkage. These constraints notwithstanding, application of biotechnology tools to rubber production in Nigeria promises great benefits and returns for the Nigerian farmers and the country's economy. Several molecular markers have been developed for the rubber tree. Genes for transformational works on rubber are available. Nigeria has in recent times exhibited great commitment to the use of biotechnology as a tool to enhance agricultural and general socioeconomic development. Biotechnology development policy and biosafety guidelines have been drawn and RRIN staff has been trained on Biosafety issues and represents the Institute at the national level. RRIN scientist participated in the training for regulators and applicants in field testing of bioengineered crop varieties. Improved infrastructure and funding will therefore see a great impact of biotechnology tools on rubber production. Some of the technologies have been developed elsewhere and only needs to be adapted to local conditions.

It can be seen that although the rubber tree was brought out from the wilds of the Amazon and has been 'domesticated' in Nigeria for more than a hundred years, it can still contribute in many new ways to protecting the environment and providing us with valuable new products. Application of these biotechnology tools to rubber production in Nigeria promises great benefits and returns for the Nigerian farmers and the country's economy.

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