

Review

The plant biotechnology flight: Is Africa on board?

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The development of plant biotechnologies has been very rapid in recent times, especially in the developed countries. The technologies have created a new branch of biotechnology known as molecular farming, where plants are engineered to produce pharmaceutical and technical proteins in large quantities. An evaluation of the status of plant biotechnology development in Africa revealed that majority of the countries that are involved in biotech activities are still at the level of tissue culture applications. This calls for urgent and sincere commitments on the part of the various stakeholders in Africa, especially the governments, to the development of biotechnology capacity.

Key words: African biotechnology, molecular farming, developing countries, African biotech capacity building.

INTRODUCTION

Since the first report of plant genetic transformation in the 80s, the technology has been deployed to produce the first-generation genetically modified (GM) plants, the herbicide-tolerant (Ht) and insect-resistant (*Bacillus thuringiensis* [Bt]) crops, that were engineered basically to increase farmers productivity. The first-generation GM crops have proven to be of tremendous benefits to the countries that have adopted them. For example, as high as 70 to 85% reduction in the application of herbicides and pesticides were reported for India and China (Bennett et al., 2006; Huang et al., 2003), hence impacting positively on the cost of these chemicals and overall production costs. The introduction of the Bt crops has led to reduction in insect damage and has reduced the labor costs by about half in South Africa (Morse et al., 2005) and by 66% in Australia (Fitt, 2003). It was estimated that Bt-cotton in the US led to 860,000 kg reduction in pesticide use and increased farmers' net income by \$100 million (Gianessi et al., 2002, cited in Konde, 2006). Additionally, these first generation GM crops have increased the yield at unprecedented level, for example, a striking 87% yield increase was recorded in the field trial of Bt cotton in India (Qaim, 2003) and additional 1.6 million Metric tons maize production was achieved with Bt-maize in the US (Gianessi et al., 2002, cited in Konde, 2006).

Plant biotechnology (plant biotech) has since moved on to engineering second-generation GM crops, which incorporate traits that lead to enhanced nutritional contents

of the farm products, for example, the engineering of the β -carotene biosynthetic pathway in rice for enhanced provitamin A content (Ye et al., 2000) and the engineering of tomatoes for increased folate production (Diaz de la Garza et al., 2007). The technology is actually on a flight at the moment, with the third-generation GM crops that are engineered as bio-factories for the production of different kinds of recombinant proteins for pharmaceutical and industrial applications. This plant-based production of biopharmaceuticals and technical proteins is known as molecular farming (Schillberg et al., 2005). Interestingly, there appears to be somewhat more favorable public perception about the plant molecular farming (PMF) crops than the first- and second generation GM crops (Costa-Font and Mossialos, 2005), possibly because of the need and potential benefits of the products and also probably because most of these crops are not intended for consumption but are only being used as production platforms. With the huge technological advances within short period, plant biotech is indeed poised to be the leading plant science of the century, but the question is, is Africa part of these developments? This review discusses the evolution and the development of PMF and overviews the various plant-derived recombinant pharmaceutical and non-pharmaceutical proteins that are at different stages of developments. It also highlights the status of development of PMF technologies in the developing countries, with emphasis on Africa and then discuss capacity building in African biotech development.

Table 1. Comparison of different production systems for recombinant proteins [data adapted from Biemelt and Sonnewald, (2005)].

System	Costs of Production	Time effort	Scale-up capacity	Product quality	Glycosylation	Contamination risk	Storage	Social acceptance level
Bacteria	Low	Low	High	Low	None	Endotoxins	Medium/ -20 °C	High
Yeast	Medium	Medium	High	Medium	Incorrect	Low	Medium/ -20 °C	High
Mammalian cell culture	High	High	Very Low	Very high	Correct	Viruses, oncogens	Difficult/ Liquid N ₂	Medium
Transgenic animals	High	High	Low	Very High	Correct	Viruses, oncogens	Difficult	Low
Plant cell cultures	Medium	Medium	Medium	High	Minor differences	Low	Medium/ -20 °C	High
Transgenic plants	Low	High	Very high	High	Minor differences	Low	Easy/Room temperature	Medium

THE EVOLUTION AND DEVELOPMENT OF PLANT MOLECULAR FARMING

Plants have been used right from the dawn of ages, as sources of natural medicaments for treating various ailments. In addition to being the major resource for drug exploration, plants are still being used hugely in complementary and alternative medicine in many developing countries and nowadays, in the developed countries (Fønnebø et al., 2007). Up till early 1970s, bioactive compounds of drugs were being extracted, purified and synthesized solely from plants. However, synthetic drugs, whose evolution started with the production of aspirin by the drug company Bayer in 1899, took the centre stage in pharmaceutical production, for the greater parts of the 20th century. Since the advent of genetic engineering technologies in the 1970s, living systems, such as bacteria, yeast and animal cells have been used as production systems for many valuable therapeutic and diagnostic proteins (Andersen and Krummen 2002; Harvey et al., 2002), thereby complementing chemical synthesis and extraction of bioactive compounds from living materials. However, due to the production constraints of these systems, which include poor quality and low yield, the development of plant-based expression systems for recombinant

proteins has been well-accepted as a promising cost-effective alternative platform for the production of safer and cheaper biopharmaceutical proteins. The comparative advantages of the plant-based system over the existing expression systems, which are summarized in Table 1, are the incentive for this.

Since the first recombinant plant-made pharmaceutical protein, in which the human growth hormone was expressed in tobacco and sunflower (Barta et al., 1986), there have been significant advances in the development of PMF technologies, which have largely demonstrated that plants could be turned into bio-factories for the large-scale production of recombinant proteins (Table 2).

Moreover, plants are now being engineered to mimic mammalian pattern of protein processing, that make these recombinant proteins fold properly and maintain their structural and functional integrity. As such, they are being made to produce even more complex functional mammalian proteins with therapeutic activity, such as human serum proteins and growth regulators, antibodies, vaccines, hormones, cytokines, enzymes and antibodies. With increasing demand for biopharmaceuticals, coupled with the high costs and inefficiency of the established production systems, there is now pressure to increase production capacity. Hence attention is being shifted to

transgenic plants as the new generation bio-reactors.

OVERVIEW OF PLANT-DERIVED RECOMBINANT PROTEINS

Several candidate recombinant proteins with potential use as vaccines have been expressed in plants, since the first plant-derived vaccine-relevant protein was reported 20 years ago (He et al., 2008; Hull et al., 2005; Liu et al., 2005; Maclean et al., 2007; Marquet-Blouin et al., 2003; McCormick et al., 2008; Mett et al., 2007; Moravec et al., 2007; Nochi et al., 2007; Qian et al., 2008; Rosales-Mendoza et al., 2009; Santi et al., 2006; Satyavathi et al., 2003; Sharma et al., 2008; Streatfield and Howard 2003; Tackett et al., 2000; Tiwari et al., 2009; Tregoning et al., 2005). Several plant-produced vaccine candidates are at different stages of clinical trials (Table 3). However, only one veterinary vaccine, the NDV vaccine for poultry, has been approved by the US Department of Agriculture (USDA) (www.the-poultrysite.com).

Also, several biologically active full antibodies have been produced in plants (Huang et al., 2001; Nicholson et al., 2005; Ramessar et al., 2008; Villani et al., 2008). Of all the different plant-derived monoclonal antibodies presently being tested in

Table 2. Major advances in the development of PMF [data adapted from Schillberg and Twyman (2007)].

Year	Major Advance	Reference
1986	Human growth hormone produced in tobacco and sunflower – the first plant-derived recombinant therapeutic protein	Barta et al., 1986
1989	Full-size IgG produced in tobacco – the first demonstration of the ability of plants to assemble heterologous complex biomolecules	Hiatt et al., 1989
1990	Human serum albumin produced in tobacco and potato – the first native human protein produced in plants	Sijmons et al., 1990
1992	Hepatitis B virus surface antigen produced in tobacco – the first plant-derived vaccine candidate	Mason et al., 1992
1992	α -amylase produced in tobacco – the first plant-derived industrial enzyme	Pen et al., 1992
1995	First secretory IgA produced in tobacco	Ma et al., 1995
1995	<i>E. coli</i> heat-labile enterotoxin (LT-B) expression in tobacco and potato – the first proof-of-concept of a plant edible vaccine	Haq et al., 1995
1996	Artificial elastin expression in tobacco – the first plant-derived protein polymer	Zhang et al., 1996
1997	First clinical trial using recombinant bacterial antigen delivered in a transgenic potato	Tacket et al., 1998
1997	Avidin produced in maize – the first commercialized plant-derived protein	Hood et al., 1997
1999	First glycan analysis of plant-produced recombinant glycoprotein	Cabanes-Macheteau et al., 1999
2000	Human growth hormone produced in tobacco chloroplasts	Staub et al., 2000
2000	Triple helix assembly and processing of human collagen produced in tobacco	Ruggiero et al., 2000
2001	First multi-component vaccine candidate expressed in potato – cholera toxin B and A2 subunits, rotavirus enterotoxin and enterotoxigenic <i>Escherichia coli</i> fimbrial antigen fusions for protection against several enteric diseases	Yu and Langridge, 2001
2001	Glycan modification of a foreign protein produced in a plant host using a human glycosyltransferase	Bakker et al., 2001
2003	Expression and assembly of a functional antibody in algae	Mayfield et al., 2003
2003	Bovine trypsin – the first marketed plant-derived protein, targeted towards a broad market	Woodard et al., 2003
2004	Glyco-engineered moss strains – the first plant system to be commercialized as bioreactor	Decker and Reski, 2004 (http://www.greenovation.com)
2005	First demonstration of most 'humanized' protein glycosylation patterns in plant production system	Huether et al., 2005
2006	Antibody against Hepatitis B – the first commercialized plant-derived antibody (marketed in Cuba)	Pujol et al., 2005
2006	HN proteins of Newcastle disease virus – world's first regulatory approval (USDA) for a plant-made vaccine for animals (poultry)	Vermin and Waltz, 2006
2008	Caro [®] – the first antibody vaccine for human application (prevention of tooth decay), to be approved by the EU	Kaiser, 2008
2009	Highest transient expression of full-sized IgG antibody in plants	Vézina et al., 2009
2009	Highest recombinant protein accumulation in plants so far – 70% total soluble protein for a proteinaceous antibiotic ^[40]	Oey et al., 2009

the clinical trials (Table 3), only one antibody for production of Hepatitis B Virus vaccine has been commercialized (Pujol et al., 2005). Several human proteins have been expressed in the plants (Arlen et al., 2007; Bai et al., 2007; Edelbaum et al., 1992; Elias-Lopez et al., 2008; Gutiérrez-Ortega et al., 2005; Musa et al., 2009; Nykiforuk et al., 2006;

Sadhu and Reddy, 2003; Weise et al., 2007), many of which are at various stages of clinical trials or commercialization. It is noteworthy that Merispase[®], a gastric lipase from the Meristem Therapeutics is already on the market (Sharma and Sharma, 2009, Table 3).

Plant-produced antimicrobial nutraceuticals, such

as human lactoferrin and lysozymes are now commercially available (Table 3). The Cobento Biotechnology's *Arabidopsis*-derived human intrinsic factor, which is to be used against vitamin B12 deficiency has just been approved by the EU (Key et al., 2008) and now commercially available (Sharma and Sharma, 2009).

Table 3. Plant-derived pharmaceuticals in clinical stages of development or on market.

Product	Disease	Plant	Clinical trial status	Company
Vaccines				
Hepatitis B antigen (HBsAg)	Hepatitis B	Lettuce	Phase I	Thomas Jefferson University, USA,
Fusion proteins, including epitopes from rabies	Rabies	Potato	Phase II	Arizona State University
Cancer vaccine	Non-Hodgkin's lymphoma	Spinach	Phase I completed	Thomas Jefferson University, USA
<i>Vibrio Cholerae</i>	Cholera	Tobacco	Phase II	Large Scale Biology, USA
Heat-labile toxin B subunit of <i>E. coli</i>	Diarrhea	Potato	Phase I	Arizona State University
Capsid protein Norwalk virus	Diarrhea	Maize	Phase I	ProdiGene ^a , USA
Antigen	Diarrhea	Potato	Phase I	Arizona State University
Antigen	Feline parvovirus (Dogs)	Potato	Phase I	Arizona State University
HN protein of Newcastle disease virus	Papilloma virus (Rabbit)	Tomato	Advanced	Large Scale Biology
Viral vaccine mixture	Newcastle disease (Poultry)	Tobacco	Early	Large Scale Biology
Poultry vaccine	Diseases of horses, dogs, and birds	Tobacco suspension cells	USDA Approved	Dow Agro Sciences
Gastroenteritis virus (TGEV) capsid protein	Coccidiosis infection	Tobacco suspension cells	Phase I	Dow Agro Sciences
	Piglet gastroenteritis	Canola	Phase II	Guardian Biosciences
		Maize	Phase I	ProdiGene ^a
Antibodies				
CaroRX	Dental caries	Tobacco	EU approved as medical advice	Planet Biotechnology, USA
DoxoRX	Side-effects of cancer therapy	Tobacco	Phase I completed	Planet Biotechnology
RhinoRX	Common cold	Tobacco	Phase I completed	Planet Biotechnology
Fv antibodies	Non-Hodgkin's lymphoma	Tobacco	Phase I	Large Scale Biology
IgG (ICAM1)	Common cold	Tobacco	Phase I	Planet Biotechnology
Antibody against Hepatitis B	Vaccine purification	Tobacco	On market	CIGB, Cuba
Therapeutic human proteins				
Gastric lipase, Merispase [®]	Cystic fibrosis	Maize	On market	Meristem Therapeutics
α -Galactosidase	Fabry disease	Tobacco	Phase I	Planet Biotechnology
Lactoferrin [™] (α -interferon)	Hepatitis B & C	Duckweed	Phase II	Biolex, USA
Interleukin	Crohn's disease	Tobacco	Field trails	Southern Crop Protection and Food Research Centre, Canada
Fibrinolytic drug (thrombolytic drug)	Blood clot	Duckweed	Phase I	Biolex
Human glucocerebrosidase	Gaucher disease	Carrot suspension cells	Marketing expected in 2010	Protalix Biotherapeutics, Israel
Insulin	Diabetes	Safflower	Commercialization expected for 2010	SemBioSys, Canada

Table 3. Contd

Apolipoprotein	Cardiovascular	Safflower	Phase I	SemBioSys
Nutraceuticals				
Human intrinsic Factor, Coban	Vitamin B12 deficiency	<i>Arabidopsis</i>	On market	Cobento Biotech AS
Human lactoferrin	Anti-infection, anti-inflammatory	Rice	Advanced, on market as fine chemical	Ventria, USA
Human lysozyme	Anti-infection, anti-inflammatory	Rice	Advanced, on market as fine chemical	Ventria
Immunosphere™	Food additive for shrimps	Safflower	Marketing expected in 2010	SemBioSys

Data adapted from Basaran and Rodríguez-Cerezo (2008), Kaiser (2008), Key et al. (2008), Lau and Sun (2009), Sharma and Sharma (2009) and Spök et al. (2008).

^aWinded up activity.

The plant-derived industrial proteins, most of which are enzymes, including avidin, trypsin, β -glucuronidase, peroxidase, laccase, cellulose, amongst others are now commercialized (Basaran and Rodríguez-Cerezo, 2008). The molecular farming of cell-wall deconstructing enzymes holds great promise for the biofuel industry, with respect to the production of cellulosic ethanol (Lee et al., 2008; Sticklen, 2008). Non-hydrolytic proteins with cell wall disrupting and loosening properties, such as the carbohydrate binding modules of cell wall deconstructing enzymes and the expansins have been demonstrated to alter cell wall structure (Obembe et al., 2007a; Obembe et al., 2007b), as such are good candidate PMF proteins for the production of cellulosic ethanol.

STATUS OF PLANT MOLECULAR FARMING DEVELOPMENT IN THE DEVELOPING COUNTRIES

It is exactly a decade after one of the foremost campaigners for plant biotech in Africa, Florence Wambugu gave a wakeup call to all stakeholders in Africa to rally and stimulate research and development in plant biotech for solving, especially, the food insecurity problem of the continent (Wambugu, 1999). An assessment of the status of plant biotech development for generating the first and the second generation GM crops shows that

Africa does not seem ready to catch up with the trends in other parts of the world, as most of the countries are still at the stage of tissue culture applications, while genetic engineering is limited to only three countries, South Africa, Kenya and Zimbabwe (Ayele et al., 2006; Cohen, 2005), with South Africa as the leader (Thomson, 2008). The groups of Edward Rybicki and Jennifer Thomson both of the University of Cape Town are the trail blazers for the entire continent. Recently, they reported the world's first maize streak virus (MSV) resistant transgenic maize (Shepherd et al., 2007). This GM maize is also the first all-African produced transgenic crop plant, as well as the first genetically engineered crop developed wholly by a developing country (Sinha, 2007).

With respect to the development of PMF technologies in the developing countries, an analysis of recent data compiled by Basaran and Rodríguez-Cerezo (2008) indeed revealed that the developing countries account for 37% of the world PMF activities while the developed countries account for 63%. Although, this seems encouraging, however, on further analysis, the margin between the two worlds widens remarkably with respect to the actual numbers of PMF centers. The analysis shows that 87% of the PMF centers are located in the developed countries, while the developing countries can only boast of 13%, which is just one-third of the numbers of centers located in the

US (Figure 1).

It should be noted that Africa, through the sole activities of Rybicki's group, accounts for less than 1% of the world PMF centers. The research activities of his laboratory in the development of plant-derived vaccines have secured a seat for Africa on the plant biotech flight! In his review article, published in the January 2009 issue of *Drug Discovery Today* and titled "Plant-produced vaccines: promise and reality", Rybicki illustrated the evolution of the PMF activities in his laboratory, which dates back to 1997 (Rybicki, 2009). It was such a delight to read the success story of PMF technologies in his laboratory, from the early years of little beginnings to the landmark advances in recent times, in the development of plant-produced tumor vaccine, papillomavirus vaccines (Maclean et al., 2007; Varsani et al., 2006), which could be made available at affordable prices, thereby placing them at the reach of the resource-poor patients. This feat is particularly inspiring, as we can only hope that these pioneering activities would eventually rob off on the rest of the continent, with time, especially when biotech capacity improves generally.

BUILDING CAPACITY FOR PLANT BIOTECHNOLOGIES IN AFRICA

Discussion on the problem of wide spread

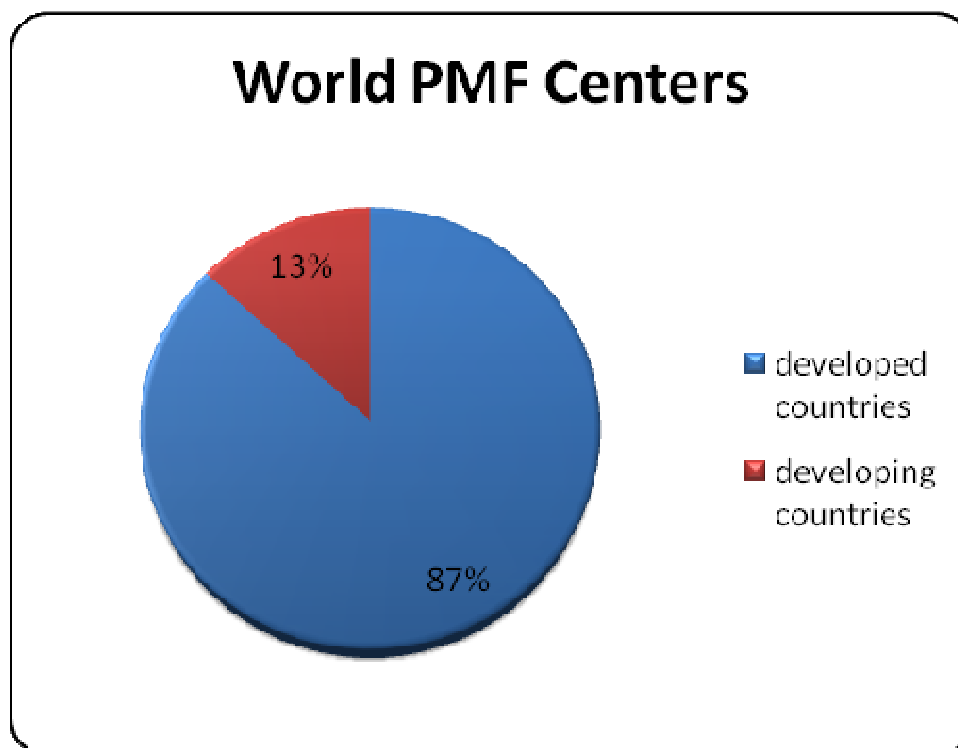


Figure 1. Distribution of plant molecular farming centers between the developed and the developing countries.

inadequacy in infrastructural capacity for biotech generally, in Africa cannot be over flogged and the solution to the prevailing dearth of plant biotech research and development activities, in particular, in most of the continent, is believed not to be beyond reach. Several models and recommendations have been proposed for taking the continent out of the woods, with respect to plant biotech development, in particular (Ayele et al., 2006; Delmer, 2005; Konde, 2006; Machuka, 2001; Ozor, 2008; Singh and Daar, 2008; Wambugu, 1999). It remains to be seen whether the various stakeholders in Africa really have the strong will, like other developing countries in Asia and Latin America, to drive this through and not to be disinterested further by recent external negative attitudes against GM crops, which is keeping the technology out of Africa (Paarlberg, 2008). The impacts of biotech on the economic growth of these emerging economies are glaring for all. As such, I believe that biotech can also work in Africa if it is working elsewhere. The strategies adopted by some of these other developing economies are worth publicizing, to serve as good templates for Africa's biotech development. In this regard, a paper on the biotech exploits and bio-economic growth of India was presented at the Knowledge Management Africa (KMA) Conference 2009, which was held in Dakar, Senegal from 4th through to 7th May, 2009 (Obembe and Dike, 2009). Some of the recommendations presented at the Conference, based on India's

strategic plans, are highlighted below.

- 1) Deliberate and aggressive awareness campaigns about the new technology, with respect to the potential benefits and to allay public fear over their safety.
- 2) Revisiting the educational policies to encourage and stimulate interest of young people in Science and Technology at the primary and secondary levels. Also, the redesigning of the curricula at the tertiary level to make biotech courses compulsory component. This agrees with other viewpoints that the development of man power for biotech base should be long term trainings and not through workshop and seminars.
- 3) Provision of basic infrastructures for low-techniques, such as tissue culture and nucleotide analyses for the Universities, as well as funding of research activities of researchers in these Universities.
- 4) Provision of motivation and incentives in order to retain the highly educated human resources and to make those trained overseas return home.
- 5) Investment in basic infrastructures-reliable power supply, portable water, roads, modern information and telecommunications facilities. ICT Infrastructures in particular, will enhance acquisition of knowledge and its application and also reduce transaction costs.
- 6) Setting up specialized biotech centers. This is in line with the NEPAD initiatives of establishing four specialized biotech centers of excellence across Africa. This initiative

will ensure capacity building in core and priority areas where expertise and resources already exist.

7) Establishment of collaborative ventures/Technology Park/incubators with private companies, to facilitate that biotech products get to the market. This sort of venture will eventually be self-funded and also ensure placements for trained workforce. Alternatively, the Government can provide loans to small and medium scale companies that might be interested in such ventures.

8) Attraction of foreign investments and fostering international partnership and linkages, all of which can only be established when there are functional basic facilities on ground.

9) Setting up/strengthening of existing biosafety, regulatory and Intellectual property bodies to formulate more efficient biotech policies and guidelines and also to set up testing and certification facilities.

CONCLUDING REMARKS

By and large, for any meaningful change to happen at the national levels for instance there must be substantial financial commitment on the parts of the various governments. The African countries cannot expect to get the same results as other developing countries when most of them are committing less than 0.01% of their Gross Domestic Products (GDPs) to Science and Technology on the whole, while countries like India, Korea, Brazil and Cuba are committing more than 1.0% of their GDPs to biotech research and development alone. The transformations that are being celebrated in the bio-economies of these countries today attest to the saying that "your harvest is a proportion of your sowing".

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