Plant Biotechnology on a flight: Is Africa on board?

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Abstract: The rate of development of plant biotechnologies has been huge 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 commercial quantities. An evaluation of the status of plant biotechnology development in Africa revealed that the very few countries, with the exception of South Africa, that are involved in biotechnology activities are still at the level of tissue culture applications. This calls for sincere commitments on the part of various stakeholders in Africa, especially the governments, to the development of biotechnology capacity.

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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, including the developing ones. 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 even by a higher percentage (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 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 favorable public perception about the plant molecular farming (PMF) crops, possibly because of the potential benefits of the products, and also probably because most of these crops are not intended for consumption but are only being used as vehicles for high-value molecules. With the huge technological advances within short period, plant biotechnology 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. It then gives an overview of the various plant-derived recombinant pharmaceutical and non-pharmaceutical proteins, which are at different stages of research, clinical and commercial developments. Lastly, I shall assess the status of development of PMF technologies in the developing countries, with particular emphasis on Africa and then discuss issues bothering on capacity building in African biotechnology development generally.

The evolution and development of PMF

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 *et al,* 2002; Harvey *et al,* 2002; Heyer, 2005; Jones *et al,* 2003; Kuroiwa, 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, low yield, and non-flexible scalability, 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 are summarized in Table 1 below, are the incentive for this.

Table 1. Comparison of different production systems for expression of recombinant pharmaceutical proteins (Data adapted from Biemelt and Sonnewald, 2005).

System	Production costs	Time effort	Scale-up capacity	Product quality	Glycosyla-Tion	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

Since the first recombinant plant-made pharmaceutical protein, 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. Major advances in the development of PMF are shown in table 2. Moreover, plants are now being engineered to mimic mammalian pattern of protein processing (including N-glycosylation), 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 (Liénard *et al*, 2007). With increasing demand for bio-pharmaceuticals, coupled with the high costs and inefficiency of the established production systems (Knablein, 2005), there is now pressure to increase production capacity. Hence attention is being shifted to transgenic plants as the new generation bio-reactors.

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 fiirst plant-derived recombinant therapeutic protein and the first proof-of-concept of plants as bioreactors	Barta *et al*, 1986
1989	Full-size IgG produced in tobacco – the first plant-derived recombinant antibody and 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	E. coli 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	Highest recombinant protein accumulation achieved in plants so far – 46.1% total soluble protein for *Bacillus thuringiensis* Cry2Aa2 protein	De Cosa *et al*, 2001
2001	First multi-component vaccine candidate expressed in potato – cholera toxin B and A2 subunits, rotavirus enterotoxin and enterotoxigenic *Escherichia coli* 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	HN proteins of Newcastle disease virus – the world's first regulatory approval (USDA) for a plant-made vaccine for animals (poultry)	Dow AgroSciences, 2006
2006	Antibody against Hepatitis B – the first commercialized plant-derived antibody (marketed in Cuba)	Pujol *et al*, 2005
2006	Rapid high-yield (transient) expression of full-size IgG antibodies in	Giritch *et al*, 2006

	plants	
2008	Caro^{RX} – 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

Overview of plant-derived recombinant proteins

Plant-derived vaccines

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 (Tiwari *et al*, 2009). Recent examples include the hepatitis B surface antigen (He *et al*, 2008; Qian *et al*, 2008), the heat labile enterotoxin B subunit (LTB) of *Escherichia coli* (Moravec *et al*, 2007; Rosales-Mendoza *et al*. 2009), and the cholera toxin B subunit (CTB) of *Vibrio cholera* (Nochi *et al*, 2007; Sharma *et al*, 2008). Others include the L1 protein of human papillomavirus type 11 and 16 (Liu *et al*. 2005; Maclean *et al*. 2007), the Norwalk virus capsid protein (Tacket *et al*, 2000), and the Hemagglutinin protein from measles virus (Marquet-Blouin *et al*. 2003). It should be noted that the US Defense Department has been sponsoring research and development of various bio-defense vaccines against lethal bioterror agents including anthrax and plague (Hull *et al*. 2005; Mett *et al*. 2007; Santi *et al*. 2006).

Several plant-produced antigens have been reported to induce immune responses, conferring protection against challenge in mouse model systems (Satyavathi *et al*. 2003; Streatfield and Howard 2003). Oral administration of several plant-produced antigens has also been reported to induce protective immune response in mice and humans (McCormick *et al*. 2008; Tregoning *et al*. 2005). However, much lower dose of vaccine administered orally is required by injection delivery (Streatfield and Howard 2003). Several plant-produced vaccine candidates, which are at different stages of clinical trials, are summarized in Table 3 below. To date only one veterinary vaccine, the NDV vaccine for poultry, developed by Dow AgroSciences, has been approved by the US Department of Agriculture (USDA) (www.thepoultrysite.com).

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 Potato	Phase I Phase II	Thomas Jefferson University, USA Arizona State University
Fusion proteins, including epitopes from rabies	Rabies	Spinach	Phase I completed	Thomas Jefferson University, USA
Cancer vaccine	Non-Hodgkin's lymphoma	Tobacco	Phase II	Large Scale Biology, USA[a]
Vibrio Cholerae	Cholera	Potato	Phase I	Arizona State University
Heat-labile toxin B subunit of *Escherichia coli*	Diarrhea	Maize Potato	Phase I Phase I	ProdiGene[a], USA Arizona State University
Capsid protein Norwalk virus	Diarrhea	Potato, Tomato	Phase I	Arizona State University
Antigen	Feline parvovirus (Dogs)	Tobacco	Advanced	Large Scale Biology, USA[a]
Antigen	Papilloma virus (Rabbit)	Tobacco	Early	Large Scale Biology, USA[a]
HN protein of Newcastle disease virus	Newcastle disease (Poultry)	Tobacco suspension cells	USDA Approved	Dow Agro Sciences, USA
Viral vaccine	Diseases of horses,	Tobacco suspension	Phase I	Dow Agro Sciences,

mixture	dogs, and birds	cells		USA
Poultry vaccine	Coccidiosis infection	Canola	Phase II	Guardian Biosciences, Canada
Gastroenteritis virus (TGEV) capsid protein	Piglet gastroenteritis	Maize	Phase I	ProdiGene[a], USA
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, USA
RhinoRX	Common cold	Tobacco	Phase I completed	Planet Biotechnology, USA
Fv antibodies	Non-Hodgkin's lymphoma	Tobacco	Phase I	Large Scale Biology, USA[a]
IgG (ICAM1)	Common cold	Tobacco	Phase I	Planet Biotechnology, USA
Antibody against Hepatitis B	Vaccine purification	Tobacco	On market	CIGB, Cuba
Therapeutic human proteins				
Gastric lipase	Cystic fibrosis	Maize	Phase II trials, commercialization expected for 2010	Meristem Therapeutics France
α-Galactosidase	Fabry disease	Tobacco	Phase I	Planet Biotechnology, USA
Lactoferon™ (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, USA
Human glucocerebrosidase (prGCD)	Gaucher disease	Carrot suspension cells	Marketing expected for 2010	Protalix Biotherapeutics, Israel
Insulin	Diabetes	Safflower	Commercialization expected for 2010	SemBioSys, Canada
Apolipoprotein	Cardiovascular	Safflower	Phase I	SemBioSys, Canada
Nutraceuticals				
Human intrinsic factor	Vitamin B12 deficiency	*Arabidopsis*	EU Approved	Cobento Biotech AS (EU)
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, USA
Immunosphere™	Food additive for shrimps	Safflower	Marketing expected for 2010	SemBioSys, Canada

Data adapted from Basaran and Rodríguez-Cerezo (2008), Spök *et al.* (2008), Kaiser (2008), Key *et al.* 2008 and Lau and Sun (2009).
[a]The firm has filed for bankruptcy and since winded up activity

Plant-derived antibodies

Two main approaches are being employed to produce biologically active full antibodies in plants. These are cross-pollination of individually transformed plants expressing light or heavy chains (Huang *et al.* 2001) and co-transformation of the heavy and light chain genes (Nicholson *et al.* 2005; Villani *et al.* 2008). One of such plant-derived antibodies, a surface antigen of *Streptococcus mutans* has been developed into a clinical product, CaroRX^{TM}, and has recently been approved by the EU to be used as medical advice, for the prevention of tooth decay (Kaiser, 2008). Moreover, a maize seed-produced HIV-specific monoclonal antibody was found to exhibit high antigen-binding activity (Ramessar *et al.* 2008), as such has proven to be a good candidate for clinical development as HIV microbicide for topical vaginal application, to prevent HIV transmission . There are five different plant-derived monoclonal antibodies presently being tested in the clinical trials, as listed in Table 3. To date, only one plant-made antibody for production of Hepatitis B Virus vaccine has been commercialized (Pujol *et al.* 2005).

Therapeutic and nutraceutical proteins

Several human proteins have been expressed in the plants, including the epidermal growth factor (Bai *et al,* 2007), α-, β- and γ-interferons, which are used in treating hepatitis B and C (Arlen *et al.* 2007; Edelbaum *et al,* 1992; Sadhu and Reddy, 2003), erythropoietin, which promote red blood cell production (Musa *et al,* 2009; Weise *et al,* 2007), interleukin, which is used in treating Crohn's disease (Gutiérrez-Ortega *et al,* 2005; Elias-Lopez *et al,* 2008), insulin, which is used for treating diabetes (Nykiforuk *et al*, 2006) and several others. Contained in Table 3 is the list of some of these therapeutics, which are at various stages of clinical trials or at the verge of being commercialized.
Plant-produced antimicrobial nutraceutics, such as human lactoferrin and lysozymes are now commercially available, but only as fine chemicals (Table 3). The Cobento Biotechnology's human intrinsic factor, produced in transgenic *Arabidopsis* plants, and which is to be used against vitamin B12 deficiency has just been approved by the EU (Key *et al.* 2008). Other nutraceutical products at various stages of development are listed in Table 3.

Plant-derived industrial proteins

The plant-derived industrial proteins, most of which are enzymes, including avidin, trypsin, β-glucuronidase, peroxidase, laccase, cellulase and so on (Basaran and Rodríguez-Cerezo, 2008) are now commercialized. The molecular farming of cellulases as well as other cell-wall deconstructing enzymes such as hemicellulases and ligninases 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, which have been demonstrated to alter cell wall structure (Obembe *et al,* 2007a; Obembe *et al,* 2007b), are potential candidate PMF proteins for the production of cellulosic ethanol.

Status of PMF development in the developing countries

It is now exactly a decade since one of the foremost campaigners for plant biotechnology in Africa, Florence Wambugu gave a wakeup call to all stakeholders in Africa to rally and stimulate research and development in Plant Biotechnology for solving, especially, the food insecurity problem of the continent (Wambugu, 1999). An assessment of the status of plant biotechnology 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 counties 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 clear leader (Thomson, 2008). The group of Prof EP Rybicki at the University of Cape Town is the trail blazer for that country and indeed for the entire continent. Recently, they reported the world's first maize streak virus (MSV) resistant transgenic maize (Shepherd *et al*, 2007). This MSV-resistant 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 world PMF centers are actually located in the developed countries, while the developing countries can only boast of 13%, which is just about one-third of the number of centers located in the US (Figure 1).

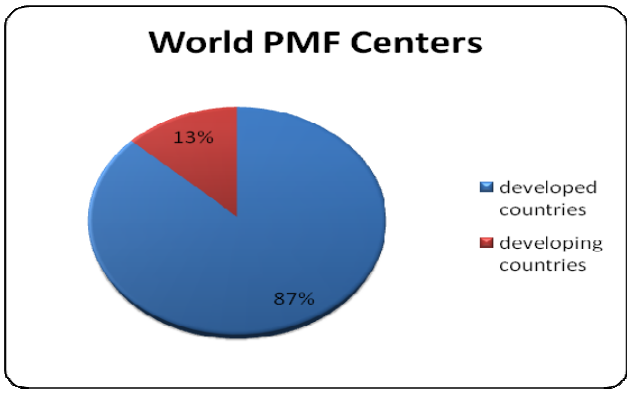

Figure 1. Pie Chart showing the distribution of PMF centers between the developed and the developing countries.

It should be noted that Africa, through the sole activities of Prof. 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 biotechnology flight! In his review article, published in the January 2009 issue of Drug Discovery Today, and titled "Plant-produced vaccines: promise and reality", Prof. 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 tumour 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 poor patients. This feat is particularly inspiring, as we all hope that these pioneering activities would eventually rob off on the rest of the continent, with time, especially when biotechnology capacity improves generally.

Building Capacity for Plant biotechnologies in Africa

Discussion on the problem of wide spread inadequacy in infrastructural capacity for biotechnology generally, in Africa cannot be over flogged and the solution to the prevailing dearth of plant biotechnology 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 biotechnology 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 biotechnology on the economic growth of these emerging economies are glaring for all. As such, I believe that biotechnology 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 biotechnology development. In this regard, a paper on the biotechnology 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.

i. Deliberate and aggressive awareness campaigns about the new technology, with respect to the potential benefits and to allay public fear over their safety.
ii. Revisiting the educational policies to encourage and stimulate interest of young people in Science and Technology at the primary and secondary level. Also, the redesigning of the curricula at the tertiary level to make Biotechnology courses compulsory component. This agrees other viewpoints that the development of man power base for biotechnology should be long

term local and international trainings and not through workshop and seminars.

iii. 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.

iv. Provision of motivation and incentives in order to retain the highly educated human resources and to make those trained overseas return home.

v. 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.

vi. Setting up specialized biotechnology 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.

vii. 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.

viii. 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.

ix. Setting up / Strengthening of existing biosafety, regulatory and Intellectual property bodies to formulate more efficient biotechnology policies and guidelines, and also to set up testing and certification facilities.

By and large, for any meaningful change to happen at the national level for instance there must be substantial financial commitment on the parts of the various governments, as there is no segment of the field that is cheap to run. 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 GDP to Science and Technology on the whole, while other countries like India, Korea, Brazil and Cuba are committing more than 1.0% of their GDPs to biotechnology activities 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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