Key innovations in plant biotechnology and their applications in agriculture, industrial processes, and healthcare

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Abstract

The Sustainable Developmental Goals aim to secure immediate human needs, such as adequate food supply and healthcare and provision of clean, affordable, and accessible energy. These achievements have to be imbedded in a sustainability concept. Bioeconomy is at the core of this concept in which agricultural (plant) biotechnology plays a major role in delivering biomass for food, feed, and industry. Modern plant biotechnology comprises the genetic modification technology and various molecular biological tools which enhances the plant breeding potential. It results in increased food supplies, increased farm income worldwide, and reduced environmental damage. Here we review the innovations in plant biotechnology that are available on the market or at the late developmental stages and their application to agriculture, agroforestry, industrial processes, and pharmaceutical industry. Special emphasis is given to approaches adapted to meet heterogeneous local needs and help support more inclusive growth in low and middle-income countries.

Introduction

In the 21st century, humanity is faced by a myriad of socioeconomic and resource challenges to supply diverse emerging and recurrent global needs to feed, clothe, and fuel a population growing in size, age, and wealth. Pressure on resource competition and scarcity as well as the identification, evaluation, and quantification of the impact of the human pressure on the planet have catalysed a global concern on the sustainability of the continuous development of human societies. The Holocene – the warm period of the past 10-12 millennia – is the only state of the planet that we know for sure to support contemporary human societies and is now being destabilized. Indeed, since the later part of the 18th century, the effects of humans on the global environment have grown so dramatically that a new geological era, the Anthropocene, has been proposed (Crutzen, 2002). There is an urgent need of a paradigm shift to maintain the Earth System (ES) in a safely operating space for humanity. Sustainable developmental goals have to be implemented to guarantee immediate human needs, such as food supply, healthcare, and energy, alongside measures for a stable ES functioning. Nine critical processes/features have been proposed to regulate the ES functioning: climate change, biosphere integrity, land system change, freshwater use, biochemical flows, ocean acidification, atmospheric aerosol loading, stratospheric ozone depletion, and novel entities. Scientifically based planetary boundary levels of human perturbation have been established for these ES processes/features, beyond which the ES functioning may be substantially altered (Steffen *et al.*, 2015).

Embedded in this emerging ES thinking, the new bioeconomy proposes a global transition toward sustainability through a bio-based industry that integrates the use of renewable aquatic, and terrestrial resources and biological processes to create energy, materials and products with an environmentally friendly footprint. Besides bioindustry, bioeconomy also encompasses research, climate, environment, and development policies.

The deployment of bioeconomy relies on technological developments, among which biotechnology plays a key role. Biotechnology-based industry is an emerging reality that generates economic opportunities for agriculture, healthcare, chemical, and manufacturing sectors, with far-reaching potential impacts on socio-economic developments and environment. According to the Biotechnology Global Industry Guide (www.researchandmarkets.com/reports/41522/biotechnology_global_industry_guide), the total revenues of the global biotechnology industry were US$ 323.1 billion in 2014, representing a compound annual growth rate of 7.2% between 2010 and 2014. The biotech industry is revolutionary beyond industrial growth because it offers opportunities for society to walk a different path toward multiple sustainable goals. In the energy and chemical sectors, biotech innovation reduces dependence on petroleum and fossil fuels and, consequently, cleans the environment and fights global climate change. In the healthcare sector, the biotech industry has developed and commercialized drugs, vaccines, and diagnostics with significant impact on length and quality of life. In the agricultural field, biotech innovations simultaneously increase food supplies, reduce environmental damage, conserve natural resources of land, water, and nutrients, and increase farm income in economies worldwide.

The future of the biotech industry, more specifically, the industrial and agricultural sector, holds considerably in biomass production. Although biomass has since long been used as feedstock, e.g. wood-based materials, pulp and paper production, biomass-derived fibers, the transition toward the modern bioeconomy requires the sustainable raw material production and efficient biomass use, implying a set of principles that should be strived for: (i) increased yields for food, feed, and industrial feedstock with as minimal as possible increases in land, water, fossil fuels, and minerals for fertilizer production; (ii) flowing use of biomass as food, feed, material, and, finally, energy; and (iii) cyclic reaction in which products should be designed for disassembly and reuse, consumables should be returned harmlessly to the biosphere, durables should maximise their reuse or upgrade, and renewable energy should be used to energize the process (Mathijs *et al.*, 2015).

Agriculture is central for global development promotion within the biophysical limits of a stable ES. The conventional tools of intensive agricultural growth, i.e., mechanization, plant breeding, agrochemicals, and irrigation, diminish returns and threaten the ES resilience. Four ES features transgress the proposed planetary boundary levels: climate change, biosphere integrity, biogeochemical flows, and land system changes (Steffen *et al.*, 2015). As agriculture is the anthropogenic perturbation with the most prominent impact, it is challenged to produce sustainable yields. Of the novel technologies of several kinds needed to achieve sustainably high-yield agriculture, one of the most important implementation is modern plant biotechnology, i.e. genetically modified (GM) technology and various molecular biological tools, that enhances the plant breeding potential and reduces the negative impact both within fields and surrounding lands.

Plant GM technology originated back in the 1980s, when the first GM plant, resistant to the antibiotic kanamycin, had been developed (Van Montagu, 2011 and references therein; Angenon *et al.*, 2013). In the 1970s, Jeff Schell, Marc Van Montagu, and colleagues at the Ghent University (Belgium), who studied the tumor-inducing principle of *Agrobacterium tumefaciens*, discovered that a large plasmid was responsible for the formation of crown galls on infected plants and that part of its DNA was transferred to plant cells (Zaenen *et al.*, 1974; Van Larebeke *et al.*, 1975; Depicker *et al.*, 1978). After it had become clear that *Agrobacterium* could be used as a vector to transfer foreign DNA to plant cells, fertile transgenic tobacco (*Nicotiana tabacum*) plants were generated that expressed and transmitted the chimeric antibiotic resistance genes to their progeny. A first company on plant genetic engineering, Plant Genetic Systems (Ghent, Belgium), was founded (Van Lijsebettens *et al.*, 2013 and references therein) and the GM technology was soon employed worldwide both in fundamental science to study gene function and in agriculture to produce transgenic crops with useful agronomic traits. The commercialization of GM crops started in 1996. Since then, the acreage of GM crops cultivated worldly has increased steadily to up to 100-fold the area planted. The average agronomic and economic benefits of GM crops are large and significant (Klümper and Qaim, 2014) as is evidenced both in developed and developing countries. The agricultural sector is probably the segment of biotech industry that provides more benefits to the middle and low-income economies. In this introductory chapter we give an overview of the innovations in plant biotechnology that have been approved for commercialization or are at the late stages of development and their application to agriculture, agroforestry, industrial processes, and pharmaceutical industry.

Global GM crop plants

Genetic engineering has the potential to address the critical constrains of sustainable agriculture and the need for sufficient quantity of healthy food, feed, and biomass feedstock for the industry as well, but GM crops have delivered only a limited range of agronomic traits for the agriculture production. Of the possible GM crop options that have ever been commercialized in the world, only nine GM crops are grown commercially worldwide, among which soybean (*Glycine max*), maize (*Zea mays*), cotton (*Gossypium hirsutum*), and canola (*Brassica napus*) account for 99% of the worldwide GM crop acreage. In 2014, the largest share (50%) was for GM soybeans, followed by maize (30%), cotton (14%), and canola (5%) (James, 2014). Other crops that account for 1% of global GM planting are alfalfa (*Medicago sativa*), sugar beet (*Beta vulgaris*), papaya (*Carica papaya*), squash (*Cucurbita pepo*), and eggplant (*Solanum melongena*). Only three traits, herbicide tolerance (HT), insect resistance (IR), and hybrid vigor have been generated and introduced in almost all GM crops grown commercially over the past 20 years. In 2014, 57% of the world's land surface of GM crops was HT, 15% IR, and 28% both HT and IR, called stacked traits, whereas other traits, such as virus resistance and drought tolerance, collectively account for less than 1%. The drought-tolerant biotech corn varieties are cultivated since 2013 only in the USA (James, 2014).

In Africa, where the GM technology is most needed to foster agricultural transformation, the output is deceiving. Only three African countries cultivate GM crops: South Africa with 2.7 million ha of maize, soybean, and cotton; Sudan with 0.1 million ha of cotton; and Burkina Faso with 0.5 million ha of cotton (James, 2014).

Despite this quite unsatisfying output in terms of crops and traits, farmer's acceptance as well as global income, production, and environmental impacts of these biotech crops are impressive. Farmers who have been granted the opportunity, quickly adopted GM crops. By 2014, millions of farmers in 28 countries worldwide have chosen to plant GM crops over 181.5 million ha and grow almost half of the global plantings of soybean, maize, cotton, and canola. The GM traits have provided logistical advantages, risk reductions, and economic benefits.

Brookes and Barfoot (2015a) analyzed the changes in farm income thanks to the impact of GM technologies on yields, key production costs, notably seed cost and crop protection expenditure, but also impact on energy and labor costs where data were available, and the prospect of planting a second crop in one season. At the global level, GM technology has had a significant positive impact on farm income. The net economic benefits of the four major GM crops (soybeans, maize, canola, and cotton) at the farm level amount to US$ 133.4 billion for 18 years of commercialization between 1996 and 2013. Approximately 70% of these gains have derived from yield and production gains and 30% from cost savings, such as less ploughing, fewer pesticide sprays, and less labor. In 2013, the direct global farm income benefit was US$ 20.5 billion, which is equivalent to a 5.5% addition to the global production value of the four main crops. As expected, US farmers have been the largest beneficiaries of increased incomes, because they adopted the GM technology early on and more than 80% of the four crops are GM since several years. More relevant is that farmers in developing and emerging economies got approximately 50% of the economic gains. The additional income benefits for soybean and maize farmers in South America (Argentina, Bolivia, Brazil, Colombia, Paraguay, and Uruguay) and cotton farmers in Asia (China and India) were US$ 31.1 billion and US$ 32.9 billion respectively. Table 1 summarizes the economic impact of GM crops since their first commercialization year to 2013.

GM technology has also contributed to reduce the agriculture's environmental footprint by facilitating environmentally friendly farming practices (Brookes and Barfoot 2015b). The GM IR traits replaced insecticides used to control pest. Since

Table 1. Farm level economic benefits of GM crops

Biotech crop	Total cumulative farmer's income benefit 1996-2013 (US$ billions)	Biotech trait	Type of benefit	Country
Soybean	14.8	HT soybeans (1st generation)	Lower production costs	Brazil, USA, Canada, Uruguay, South Africa
			Lower production costs + second crop gains	Argentina, Paraguay
			Lower production costs + yield gains	Mexico, Bolivia, Romenia
		HT soybean (2nd generation with higher yield potential)	Lower production costs + yield gains	USA, Canada
		HT/IR soybean	Cost savings as 1st generation HT soybean + insecticide savings + yield gains	Brazil, Argentina, Paraguay, Uruguay
Maize	7.36	HT maize	Lower production costs	USA, Canada, South Africa, Colombia
			Lower production costs + yield gains	Argentina, Brazil, Philippines
	37.2	IR maize (resistance to corn boring pests)	Yields gains	USA, South Africa, Honduras, Argentina, Philippines, Spain, Uruguay, Colombia, Canada, Brazil, Paraguay
		IR maize (resistance to rootworm pests)	Yield gains	USA, Canada
Cotton	1.49	HT cotton	Lower production costs	USA, South Africa, Australia, Argentina, Uruguay, Paraguay
			Lower production costs + yield gains	Brazil, Mexico, Colombia
	40.78	IR cotton	Yield gains	USA, China, South Africa, Mexico, Argentina, India, Colombia, Burkina Faso, Pakistan, Burma
Canola	4.3	HT canola (tolerant to glyphosate)	Mostly yield gains where replacing triazine-tolerant canola	Australia
		HT (tolerant to glufosinate)/ hybrid vigor canola	Mostly yield gains	USA, Canada
Sugarbeet	0.14	HT sugarbeet	Mostly yield gains	USA, Canada

Adapted from Brookes and Barfoot (2015a).

1996, the active insecticide ingredient use in cotton and maize was reduced by 239 million and 71.7 million kg, respectively, with the highest benefits for cotton, because its culture requires an intensive treatment regime with insecticides. The adoption of GM IR cotton in China and India resulted in a cumulative decrease in insecticides of over 192 million kg for the period 1996-2013. IR soybeans were first grown commercially in 2013, mostly in Brazil, and the savings in active insecticide amounts in that year was above 0.4 million kg, corresponding to 1% of the total soybean insecticide use.

The environmental gains associated with the use of GM HT traits are related to the application of more environmentally friendly products and to simplified changes in farming systems. The adoption of conservation tillage has led to additional soil carbon sequestration and a reduction in tractor fuel use that amounted to 7,012 million liters between 1996 and 2013 (Carpenter, 2011). Less fuel, associated with fewer insecticide and herbicide sprays and less or no ploughing, corresponded to 28,005 million kg of CO_2 eliminated from the atmosphere or, in terms of car equivalents, to 12.4 million cars off the road for a year (Brookes and Barfoot 2015b).

The higher productivity of the currently commercialized GM crops alleviates the pressure to convert additional land for agriculture. To achieve the same tonnage of food, feed, and fiber obtained during the 1996-2013 period, 132 additional million ha would have been needed with conventional crops only (James, 2014).

GM crops approved for commercialization in the world

In contrast to the limited number of GM crops on the market, an important number of crops, events, and traits have received approval for commercialization. As of 11th October 2015, a total of 40 countries granted regulatory approvals to 29 GM plants and 383 GM events, covering 36 GM traits for use as food, feed and/or for cultivation (www.isaaa.org/gmapprovaldatabase). The fast-growing number of approved GM trait-containing varieties and hybrids shows that GM technology does not narrow the genetic diversity of the crop plant. In addition to the commercial HT and IR GM traits used to construct the vast majority of GM crops on the market, GM traits have been also approved for abiotic stress tolerance, altered growth/yield, disease resistance, modified product quality, and pollination control systems. Table 2 summarizes the GM traits approved per GM plant. Remarkably, 13 different GM traits aim to change product quality in 13 different crops.

A number of noteworthy biotech crops/traits have been recently approved. In November 2014, the US Department of Agriculture (USDA) endorsed commercial planting of two crops employing an RNA interference (RNAi) approach: a transgenic alfalfa with reduced lignin for improving fiber digestibility via RNAi of caffeoyl coenzyme 3-O-methyltransferase gene involved on the synthesis of guaiacyl lignin subunit and a potato (*Solanum tuberosum*) with reduced levels of several enzymes, among which one that produces the potentially carcinogenic metabolite acrylamide. This Innate™ potato (J.R. Simplot, Boise, Idaho) also suffers less wastage from bruising (Waltz, 2015). The Enlist™ Duo for maize and soybean (Dow AgroSciences, Indianapolis, IN, USA) that contains two stacked genes to confer tolerance to the herbicides glyphosate and 2,4-D-choline was approved in Canada in April 2014 and in the USA in September 2014 (James, 2014). Approval of the Arctic Apples, genetically engineered to resist browning associated with cuts and bruises by reduction of the browning-causing enzyme levels was granted by the USDA in February 2015 and by the Food and Drug Administration (USA) in March 2015.

Developing countries also generated and approved novel biotech plants. In 2013, Indonesia ratified the environmental certificate for cultivation of drought-tolerant sugarcane (*Saccharum* spp.). In Brazil, a virus-resistant bean (*Phaseolus vulgaris*) was approved in 2011 and is due for commercialization in 2016 and a GM *eucalyptus*

Table 2. Global status of GM technology: GM crops approved for commercialization in at least one country

Commercial trait	GM trait	GM Plant
Abiotic Stress Tolerance	Drought stress tolerance	Maize
		Sugarcane
Altered Growth/Yield	Enhanced photosynthesis/yield	Soybean
	Volumetric wood increase	Eucalyptus
Disease Resistance	Black spot bruise tolerance	Potato
	Viral disease resistance	Bean
		Papaya
		Plum
		Squash
		Sweet pepper
		Tomato
Herbicide Tolerance	Glufosinate herbicide tolerance	Argentine canola
		Cotton
		Maize
		Polish canola
		Rice
		Sugar beet
	Glyphosate herbicide tolerance	Cotton
		Creeping bent grass
		Maize
		Polish canola
		Potato
		Soybean
		Sugar beet
		Wheat
	Isoxaflutole herbicide tolerance	Soybean
	Mesotrione herbicide tolerance	Soybean
	Oxynil herbicide tolerance	Argentine canola
		Cotton
		Tobacco
	Sulfonylurea herbicide tolerance	Carnation
		Cotton
		Flax
		Maize
		Soybean
Insect Resistance	Coleopteran insect resistance	Maize
		Potato
	Lepidopteran insect resistance	Cotton
		Eggplant
		Maize
		Poplar
		Rice
		Soybean
		Tomato
	Multiple insect resistance	Cotton
		Maize
		Poplar
Modified Product Quality	Altered lignin production	Alfalfa
	Non-browning phenotype	Apple
	Modified oil/fatty acid	Argentine canola
		Soybean
	Phytase production	Argentine canola
		Maize
	Modified flower color	Carnation
		Petunia
		Rose
	Modified amino acid	Maize
	Modified alpha amylase	Maize
	Delayed ripening/senescence	Melon
		Tomato
	Delayed fruit softening	Tomato
	Modified starch/carbohydrate	Potato
	Reduced acrylamide potential	Potato
	Anti-allergy	Rice
	Nicotine reduction	Tobacco
Pollination control system	Fertility restoration	Maize
	Male sterility	Argentine canola
		Chicory
		Maize

Note. Source: ISAAA GM approval data base

(*Eucalyptus* sp.) in 2015 (James, 2014; www.isaaa.org/gmapprovaldatabase). FuturaGene, owned by the Brazil–based Suzano Pulp and Paper company and the second largest producer of *eucalyptus* pulp globally, developed the transgenic *eucalyptus* that contains a gene encoding an *Arabidopsis thaliana* protein that facilitates cell wall expansion and accelerates growth. According to FuturaGene, the GM tree produces 20% more wood than the conventional variety and is ready for harvest in five and a half years instead of seven.

There is a growing interest in GM forest trees due to the increasing global trend for timber production from plantations and bioenergy applications. Since forests can be grown on marginal lands, competition with land resources suitable for agricultural production can be avoided. At the same time, the increased productivity from bioengineered forests will provide an option to protect native forests.

A few GM forest trees have been produced commercially. In China, poplar (*Populus* sp.) trees are cultivated for uses in furniture, boat making, paper and chopsticks, because of their flexibility and close wood grain. (ISAAA, 2015). Since 2000, China produces GM poplars to fight Asian longhorn beetle that devastated 7.04 million ha of poplar. Three clones of *Populus nigra* were developed with the *Bacillus thuringiensis (Bt)* gene *cry1Aa* and a hybrid white poplar (*Populus alba*) was transformed by fusion of *cry1Aa* and the gene coding for a proteinase inhibitor from *Sagittaria sagittifolia*. In the transgenic poplar plantations, the fast spread of the target insect pests was inhibited effectively and the number of insecticide applications was significantly reduced. The performance of the *Bt* black poplar plantations is significantly better than that of the clones deployed locally, resulting in a substantial 90% reduction in leaf damage. In 2014, GM poplar was cultivated in 543 ha in China (James, 2014).

ArborGen Inc. (Ridgeville, SC, USA), a tree seedling company, has developed a GM loblolly pine (*Pinus taeda*) cultivar with enhanced density. Loblobly pines are used for lumber, plywood, and paper (ISAAA, 2015). As none of the inserted genes are derived from plant pests, the USDA deregulated the GM loblolly pine that can be cultivated without undergoing environmental studies (http://www.capitalpress.com/Timber/20150128/usda-cannot-restrict-gmo-pine).

Near-term innovations

Regulatory constraints, with delaying approvals and increasing costs, have discouraged biotech innovations, except in big corporations. The cost of discovery, development, and authorization of a new biotech crop or trait has been estimated to be approximately US$ 136 million (Prado *et al.*, 2014). Notwithstanding, good Research and Development projects continue to be pursued both in developed and developing countries. A wide variety of plants are being generated for resilience to biotic and abiotic stresses, increased water or nitrogen use efficiency (NUE), and nutritional improvements (Ricroch and Hénard-Damave, 2015). The major multinational agribusiness corporations often collaborate with public institutions, private entities, and philanthropic organizations in the least developed countries, particularly in Africa. Other relevant innovations for non-food purposes, such as pharmaceutical, biofuel, starch, paper and textile industries are being pursued in developed countries.

Sustainable trait management

Management of several sustainable biotech traits is quickly becoming available. The main multinational seed corporations continue to develop GM traits directed to broad-spectrum herbicides and resistance to chewing insects on a wide range of species. Most of these innovations are related to stacking different HT and/or IR genes. Gene stacking simplifies and enhances pest management as demonstrated by IR and weed HR based on a single gene technology (Que *et al.*, 2010).

Nonetheless, research continue to focus on other kinds of sustainable agronomic traits and several traits and crops in the pipeline resulting from both private and public endeavors that target the developing world are about to be commercialized. Some case studies are listed below.

Water-Efficient Maize for Africa (WEMA)

Agriculture requires more water than any other human activity. Drought is a threat to farms around the world and in Africa drought is one of the major factors that prevent good yields. The Food and Agriculture Organization of the United Nations estimates that by 2025 approximately 480 million Africans could be living in areas of water scarcity. To face this challenge, plant scientists are developing drought-tolerant traits. The WEMA project is a public-private partnership that aims to improve food security and livelihoods for small farmers in Sub-Saharan Africa by finding ways to double the maize yields. In this project, GM and non-GM technology, including marker-assisted breeding, are combined to generate hybrid maize seeds with increased water use efficiency and resistance to insect pests. To this end, the *Bt* gene will be stacked with the drought-tolerance biotech trait (MON87460) that expresses the *Bacillus subtilis* cold-shock protein B (cspB), licensed from Monsanto. (http://wema.aatf-africa.org).

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN)

In Mexico, the biotech maize CIEA-9 was developed with enhanced adaptation to severe drought and extreme temperatures. The antisense RNA expression was used for silencing trehalase in the popular maize inbred line B73 (derived from Iowa Stiff Stalk Synthetic). This biotech maize requires 20% less water, endures high temperatures (up to 50°C), and the seeds germinates at 8°C, demonstrating their ability to withstand cold at early development stages (Ortiz *et al.*, 2014). In 2012, the Government of Mexico granted 4 ha for experimental release of CIEA-9 in Sinaloa (Mexico). This permit was the first delivered to a Mexican public research center since the biosafety law was authorized (Wolf and Otero, 2015).

Centro Internacional de Mejoramiento de Maíz y Trigo (CYMMYT; International Maize and Wheat Improvement Center)

Over the past five years, this Mexican center has analyzed experimental releases of genetically engineered drought-resistant wheat (*Triticum* sp.). All the different events tested in experimental trials on 0.1-ha plots at the Tlaltizapan Morelos site were drought resistant (Wolf and Otero, 2015).

ArborGen Inc.

This Brazilian company developed a GM *eucalyptus* tree that can withstand extremely low temperature. It contains a cold-inducible promoter driving a C repeat-binding protein from *A. thaliana.* This biotech tree combines the fast-growing and highly desirable fiber quality characteristics of a known Brazilian eucalyptus variety that can withstand freezing temperatures. Transgenic freeze-tolerant eucalyptus can grow up to 52.4 feet (15.97 m) at 16.8oF (-8.4°C), compared to the control trees that grew only 0.3 feet (9 cm) (Hinchee *et al.*, 2011). This freeze-tolerant tropical *eucalyptus* product (AGEH427) is currently going through the government review process for deregulation in the USA (www.arborgen.com).

Arcadia Biosciences Inc. (Davis, CA, USA)

The NUE trait contributes to improve yields in N-limited environments and reduces fertilizer costs and N fertilizer pollution (Hirel *et al.*, 2011). Among the various genetic engineering strategies for NUE enhancement in crops, the overexpression of the gene coding for alanine aminotransferase that increases N uptake at early growth stages is a very promising candidate for commercialization. The intellectual property associated with this invention has been licensed to Arcadia Biosciences Inc. The company possesses the rights to use this gene technology in major cereals, such as wheat, sorghum (*Sorghum bicolor*), rice, maize, and barley (*Hordeum vulgare*), as well as in sugarcane. Field trials have been executed for rice in China, for rice and wheat in India. Its value for maize and rice is being assessed in Sub-Saharan Africa through private-public partnerships. Rice with NUE/water use efficiency and salt tolerance (NEWST) is on field trial in Uganda. The National Agricultural Research Organization (NARO), African Agriculture Technology Foundation (AATF), and Arcadia Biosciences cooperate on this research (Ortiz *et al.*, 2014; James 2014).

Laboratorio Nacional de Genómica para la Biodiversidad at CINVESTAV

The National Laboratory of Genomics for Biodiversity at the Irapuato campus (Mexico) and a private Mexican company are developing GM plants that will be able to absorb and optimize the use of phosphorus. The GM plants absorb phosphites rather than phosphates and so improve the use of fertilizers and weed control that compete for the phosphorus element. According to the developers, the trait can reduce the required amount of fertilizer by 30% to 50%, eliminates or reduces the use of herbicides, and is harmless to humans and animals. The group is developing a GM tobacco as first crop and, if successful, the trait will be introduced into maize for Africa in the near future (Wolf and Otero, 2015).

Examples of transgenic plants resistant to fungal disease

(1) Late blight of potato, one of the most devastating diseases caused by a pathogen similar to fungi, *Phytophthora infestans*, accounts for 20% of potato harvest failures worldwide, translating into 14 million tons and valued at EURO 2.3 billion (Ortiz *et al.*, 2014 and references therein). Several lines of transgenic potato containing R genes identified in wild relatives with high resistance to late blight have been produced (such as resistant genes from the wild Mexican relative *Solanum bulbocastum*, was used to breed the Fortuna cultivar and the *Rpi-vnt1.1* gene isolated from *Solanum venturii* had been introduced into the potato variety Désiree). As these R genes had been identified in wild potato species, the use of the so-called cisgenic technology facilitated the rapid transfer of these genes into cultivated potato varieties without linkage drag. These plants have been shown to be resistant to late blight in several years of field tests (Gaffoor and Chopra, 2014 and references therein; Ortiz *et al.*, 2014, Jones, 2015).

(2) In wheat, one of the most damaging fungal diseases is powdery mildew. Transgenic wheat lines harboring different versions of a powdery mildew resistance gene (*Pm3 R*) have gone through field tests. Two years of field trials have revealed that the GM plants were more resistant to powdery mildew than the nontransgenic control plants (Gaffoor and Chopra, 2014).

(3) The chestnut blight fungus secretes several toxic compounds, such as oxalic acid that lowers the pH of the surrounding plant tissue, with death of the infected tissue as a consequence. Plants transformed with a wheat gene encoding oxalate oxidase were able to detoxify the oxalic acid, thereby starving the fungus and restricting it to the bark of the tree (*Castanea* sp.). These plants were tolerant to the disease and have undergone rigorous laboratory testing and several years of successful field trials (Gaffoor and Chopra, 2014).

(4) Banana (*Musa* sp.) plants have been engineered to control a bacterial disease *Xanthomonas wilt*, better known as BXW. The transgenic plants containing genes from sweet pepper (*Capsicum annuum*) encoding a hypersensitive response-assisting protein (Hrap) or a ferredoxin-like protein (Pflp) were evaluated over two successive crop cycles in a confined field trial in Uganda (Tripathi *et al.*, 2014). Approximately 20% of the 40 Hrap lines and 16% of the 26 Pflp lines, for a total of 11 transgenic lines, showed 100% resistance and retained the resistance in the ratoon crop. As elicitor-induced resistance is not specific against particular pathogens, this transgenic approach may also provide effective control of other bacterial diseases of banana, such as moko or blood disease in other parts of the world. Nearly 15 million people either rely on bananas for their income or consumption, making it an important food and cash crop in the Great Lakes region of East Africa. Food security studies revealed that in Uganda, Rwanda, and Burundi, bananas constitute >30% of the daily per capita caloric intake, rising to 60% in some regions (Tripathi *et al.*, 2014).

Other ongoing biotech crop research activities for sustainable management that are on field trials in Africa include: (i) IR cowpea (*Vigna unguicula-*

ta) in Burkina Faso (L'Institut pour l'Etude et la Recherche Agronomique, AATF, Network for the Genetic Development of Cowpea, and The Commonwealth Scientific and Industrial Research Organization), Ghana (AATF and Savanna Agricultural Research Institute), and Nigeria (AATF and Institute of Agricultural Research); (ii) virus-resistant cassava (*Manihot esculenta*) in Nigeria (National Root Crops Research Institute), Kenya (Kenya Agricultural and Livestock Research organization [KALRO], International Institute of Tropical Agriculture [IITA], Danforth Plant Science Center [DDPSC], and Masinde Murilo University of Science and Technology), and Uganda (NARO, DDPSC, and IITA); (iii) Fungal resistance and drought/salt-tolerant wheat in Egypt (Agricultural Genetic Engineering Research Institute); (iv) Virus resistant sweet potato *Ipomoea batatas*) in Kenya (KALCRO and DDPSC), (vi) IR sweet potato in Uganda (NARO and DDPSC); and (vii) nematode-resistant banana (NARO and University of Leeds, UK) (James, 2014).

Output traits for food and feed

Nutritionally enhanced food crops

A few *nutritionally enhanced food crops* have undergone safety approval, namely maize with increased lysine content and canola and a number of GM soybeans with improved fatty acid profile, including high stearidonic acid, an intermediate of omega-3-Fatty Acid. However, the last decade witnessed great progress in R&D to generate nutritionally improved biotech food crops specifically for targeting low-income families. Addressing nutritional deficiencies by gene engineering would lead to decreased healthcare costs and increased economic performance. Biofortified staple crops harboring essential micronutrients to benefit the world's poor and new functional GM food crops for enhancing human health are under development. Several of these GM crops are currently being tested in developing countries. Some relevant examples are given below.

(1) Golden Rice, named for its golden color due to its high β-carotene content, is one of the first examples of a GM staple crop that was specifically designed to combat malnutrition and vitamin A (VitA) deficiency, because it is an essential nutrient needed for the visual system, growth, development, and a healthy immune system. Golden Rice was generated by the research group of Ingo Potrykus (ETH Zürich, Switzerland) (Ye *et al.*, 2000) to offer a viable solution for eye damage of three million preschool-aged children due to VitA lack. The GM rice (GR1) was engineered with two genes from other organisms (daffodil [*Narcissus poeticus*] and the bacterium *Erwinia uredovoia*) that reconstitute the carotenoid biosynthetic pathway within the rice genome (Tang *et al.*, 2009). The current Golden Rice version, known as GR2, utilizes genes from two distinct proVitA pathways, including the maize phytoene synthesis gene instead of the analogous daffodil gene used in the GR1 rice. Golden rice can produce β-carotene amounts that were up to 35 μg/g dry rice. Bioavailability testing has confirmed that Golden Rice is an effective source of VitA in humans (Hefferon, 2015 and references therein).

(2) Transgenic biofortified rice has also been engineered to combat iron and folate deficiency, with improved mineral bioavailability, and with high content to essential amino acids, such as lysine (Blancquaert *et al.*, 2015; Hefferon, 2015).

(3) The BioCassava Plus (BC+) program genetically engineered cassava with increased levels of iron and proVitA. Retention and bioavailability of transgenic cassava are similar to the findings on conventional biofortification research. The first field trials for a proVitA-biofortified cassava began in 2009, followed by trials for high-iron cassava, and delivery of the biofortified crops is expected in 2017. Additional traits included in BC+ are increased shelf life, reduced cyanide levels, and improved disease resistance (Tohme and Beyer, 2014). The National Root Crops Research Institute of Nigeria is performing field trials with proVitA-rich cassava (James, 2014).

(4) Transgenic bananas with proVitA and iron are being developed by the NARO Uganda and the Queensland University of Technology. The per

capita consumption of bananas is estimated to be 0.7 kg per day in Uganda. Scientists applied the pro-Vitamin A genes used in Golden Rice to a popular local variety. Bananas with up to 20 ppm proVitA have been generated and trials have started in Uganda. The ProVitA bananas are expected to be released in 2020. A human bioavailability study began in late 2013 (Waltz, 2014).

(5) Sorghum biofortified with VitA and bioavailable zinc and iron is tested by the Africa Harvest and Pioneer Hi-Bred in Nigeria (in collaboration with the National Biotechnology Development Agency) and in Kenya (in collaboration with KALRO) (James, 2014).

(6) Nutritional fatty acids associated with reducing coronary heart disease risks can be introduced into oilseed crops to improve human health. So far, 10 transgenes that have led to the accumulation of high-value fatty acids in plants (Ortiz *et al.*, 2014). High oleic acid GM soybeans produced by Pioneer Hi-Bred International, Inc. (Pioneer), a DuPont Company (Johnston, IA, USA), was the first biotech soybean product of this kind (Plenish™). RNAi technology was used to decrease the expression of the endogenous soybean gene encoding fatty acid desaturase (*gm-fad2-1*) that produced seeds with an increased concentration of oleic acid (C18:1) and a correspondingly reduced concentration of linoleic acid (C18:2). The purpose of this change in fatty acid profile is to provide a stable vegetable oil that is suitable for frying applications without the need for hydrogenation (De Maria, 2013).

(7) To synthesize Omega-3 long-chain polyunsaturated fatty acids found routinely in fish oils, scientists of the Rothamsted Research Institute (Harpenden, UK) have metabolically engineered camelina (*Camelina sativa*) plants. The metabolic pathway to produce this fatty acid was reconstituted in camelina by substituting synthetic versions of up to seven genes from marine algae (Betancor *et al.*, 2015). The levels of eiosapentaenoic acid and docosahexaenoic acid o*bt*ained were economically reasonable, thus representing a tangible success. Therefore, GM oilseeds can be a novel source of this essential oil. Omega-3 long-chain polyunsaturated fatty acids are of great interest due to their dietary benefits, such as improvements to brain function and development as well as for cardiovascular health. The camelina plants with a high content of these omega-3 oils in the laboratory/glasshouse are being evaluated for their performance in the field. Other beneficial fatty acids have also been made in plant seed oils, including γ-linolenic and stearidonic acid, as well as arachidonic acid (Hefferon, 2015).

(8) Transgenic tomato (*Solanum lycopersicum*) fruits with threefold enhanced hydrophilic antioxidant capacity have been obtained through metabolic engineering. The "purple" tomato contains genes from two snapdragon (*Antirrhinum majus*) transcription factors Delila and Rosea1 that control anthocyanin biosynthesis (Butelli *et al.*, 2008). Anthocyanins, compounds found in blueberries (*Cyanococcus* sp.) and cranberries (*Vaccinium* sp.) are believed to fight cardiovascular diseases and exhibit anti-inflammatory properties. Tomatoes were chosen because they are quite affordable antioxidant sources. The GM tomato with an as much as 30% significantly extended life span in the cancer-prone mice (*Mus musculus*), is currently being tested on heart patients in Britain (Hefferon, 2015). A recent study shows that the purple tomato not only is more healthy, but also has a longer shelf life and is more resistant to diseases than not GM tomatoes (Zhang *et al.*, 2013).

(9) Transgenic tomato plants that accumulated trans-resveratrol and trans-resveratrol-glucopyranoside have been obtained by transformation with the stilbene gene from grape (*Vitis vinifera*). These GM tomato lines showed a significantly increased antioxidant capability and ascorbate content. The GM tomato extracts were able to counteract the pro-inflammatory effects of phorbol ester in a culture of monocyte-macrophages (Hefferon, 2015).

Nutrionally enhanced feed crops

GM feed crops have been developed to improve the nutritional value of animal feed as well as to produce more environmentally friendly manure. Biotech crops engineered with increased levels of amino acids are an alternative to the direct addition of supplemental amino acids in animal diets. Examples of these types of crops include GM maize with enhanced production and accumulation of free lysine in the corn kernel; protein-enriched GM soybean with more digestible lysine, methionine, threonine, and valine; high-methionine GM lupine (*Lupinus* sp.); high-tryptophan GM rice; and GM alfalfa with increased levels of cysteine, methionine, aspartate, and lysine (ISAAA, 2012; Hefferon, 2015).

GM feed crops with phytase enzyme have been shown to improve phosphorus availability. Non-ruminants cannot efficiently absorb phosphorus stored in plants as phytate salts. The undigested phosphates excreted by these animals can accumulate in the soil and water, leading to phosphorous pollution and organic matter accumulation. In addition, phytic acid forms insoluble salts with zinc and other cations that reduce the bioavailability of trace minerals. GM corn, soybean, canola, and wheat expressing phytase transgenes have shown a positive effect on performance, phosphorus retention, and excretion. Other antinutritive factors that have been tackled by plant gene engineering include GM soybeans with reduced levels of the antinutritive oligosacharides raffinose and stachyose and GM cotton seeds with low contents of the phenolic pigment gossypol (ISAAA, 2012).

Production of pharmaceuticals in biotech plants

Plants can be genetically engineered to harness endogenous metabolic pathways and the protein biosynthesis machinery to produce complex small-molecule compounds and recombinant biologicals. A number of plant species have been genetically engineered in several metabolic pathways to produce defined secondary metabolites of high pharmaceutical value, including paclitaxel, tropane, morphine, and terpenoid indole alkaloids either as whole plants or cultured organs/cells. Several advances are being implemented in terms of quality, purity, and yield, as well as procedures to meet regulatory requirements to move from these products from proof-of-principle to commercial production (Fisher *et al.*, 2015).

One of the key features of plant-based production platforms that distinguish them from other biological manufacturing concepts is the lack of a single biotechnological basis or a standardized platform. The technologies encompass stable transgene integration and transient expression in plants by means of bacterial, viral, or hybrid vectors (Chen and Lai, 2015). The platforms range from plant cells or simple plants, growing in bioreactors containing fully defined synthetic media, to whole plants growing in soil or in hydroponic environments. Whereas transient expression can produce very large amounts of the protein of interest within a short time, transgenic plants are preferable when the transgenic seed production is needed. Many pharmaceutical products can be improved and made in a shortened time or on an enlarged scale in plant-based systems. These features are relevant when products can be produced with a superior quality and/or with plant specifications or when production scale and costs are important factors.

The production of recombinant pharmaceutical proteins by means of using GM plants, often described as molecular farming, originated from the need for safe and inexpensive biopharmaceuticals in developing countries. Plants synthesizing expressing vaccine proteins can be grown using local farming techniques, only need to be partially processed, are easily transportable, and do not require refrigeration. Vaccines produced in food or feed crops effectively elicit an immune response to a particular pathogen when consumed fresh, dried, or lyophilized into a powder and reconstituted as a juice when needed. Therefore plant made vaccines could be easily available at low costs at remote regions of the planet (Hefferon, 2015).

These developments open interesting opportunities for low-income countries and investment in manufacturing pharmaceuticals in plants increases globally. When production needs to be scaled up, the capital investments on plant-manufacturing platforms in special molecular farming are expected to be considerably lower than with mammalian cell culture platforms. Companies in the USA and Europe have invested in the establishment of new currently good plant-manufacturing practice facilities (Lössl and Clarke, 2013).

In 2012, an important breakthrough was achieved when the first plant-made pharmaceutical product was approved for use in humans, namely ELELYSO® (taliglucerase alfa) (Pfizer, New York, NY, USA), a recombinant form of human glucocerebrosidase produced in transgenic carrot (*Daucus carota*) root bioreactors for the treatment of the lysosomal storage disorder Gaucher's disease (Stoger *et al.*, 2014). Another product gained global attention because of its role in an experimental Ebola therapy. The monoclonal antibody ZMapp, developed by Mapp Pharmaceuticals (Mountain View, CA, USA), was produced in tobacco plants at Kentucky Bioprocessing, a unit of Reynolds American. The drug was first successfully tested in humans during the 2014 West Africa Ebola virus outbreak, but has not yet been subjected to a randomized controlled trial (Zhang *et al.*, 2014). This spectacular example of molecular farming proved it to be a fast and cheap way to produce novel biologicals.

Besides these success stories, a number of plant-derived pharmaceutical products are currently on the market or undergoing clinical development for several clinical applications, including antibiotic-associated diarrhea, inflammatory bowel disease, osteoporosis, HCV HSV/HIV, vaccine, anti-caries antibody, and microbicide (Sack *et al.*, 2015). Moreover, several pharmaceutical companies with plant-based production facilities established commercial platforms for nonpharmaceutical products, such as cosmetics, veterinary pharmaceuticals, technical enzymes, research reagents, and media ingredient, as a manner to generate revenue during costly clinical studies (Sack *et al.*, 2015).

It is important to be aware that, as for all medical interventions, safety and legal issues are required for production and usage of plant-made pharmaceuticals. Depending on the plant production system, different biosafety rules apply. Metabolites produced in cell suspension cultures based on medicinal plants are treated as natural products, whereas recombinant proteins produced in plants are considered products of GM organisms and, therefore, follow different regulations. The development of plant cell suspension cultures as a platform for plant-made pharmaceuticals have been encouraged, partly because of the lack of a coherent regulatory framework for whole plant-derived pharmaceuticals (Fisher *et al.*, 2015). Consequently, the first plant-derived recombinant pharmaceutical protein approved for human use was produced in plant cells. Notwithstanding, there are impressive efforts to incorporate the latest regulatory innovations of industry-like platforms into whole plant-based manufacturing processes and to define updated guidelines (Fischer *et al.*, 2015). With innovative and optimized production processes that can be scaled up and appropriate regulatory and biosafety frameworks, plant-derived recombinant proteins may offer high-volume and cost-effective delivery systems for many medical applications in this century (Mangan, 2014).

Examples of veterinary pharmaceuticals produced in feed include GM seeds for antibiotic replacement in animal farming, such as rice grains with human lactoferrin and/or lysozyme as antibacterial and immunity-stimulating agents in chickens and pigs (Humphrey *et al.*, 2002; Hu *et al.*, 2010). Recently, *Arabidopsis* seeds have been transformed with an antibody against enterotoxigenic *Escherichia coli* and used as a proof of concept for a passive oral immunization-based approach for piglets (Virdi *et al.*, 2013).

Plant biotechnology for industrial applications

Innovations on output traits aiming at supporting sustainable processes in the chemical and fuel industry are lagging behind other plant biotech developments. To our knowledge, the only product approved for commercialization is the Amflora potato produced by BASF Plant Science (http://www.sciencemag.org/news/2013/12/eu-court-annuls-gm-potato-approval). This GM potato produces starch composed almost exclusively of amylopectin because the gene coding for starch synthase, involved in the synthesis of amylose had been switched off by RNAi strategy. As for certain industrial uses of starch only the thickening properties of amylopectin are required, the gelling amylose component is undesirable in many products and can interfere with certain processes. The chemical modification or separation of these two components is associated with increased consumption of energy and water. The European Commission approved the Amflora potato for industrial use in 2010 and cultivation started on a small scale in the Czech Republic, Sweden, and Germany. However, in January 2012, BASF Plant Science decided to stop marketing the Amflora potato in Europe due to lack of acceptance of GM crops in Europe and relocated its headquarters from Germany to the USA. In 2013, the European Union annulled the approval for BASF's Amflora potato.

Potato has also been engineered to produce high-amylose starch by suppression of the starch-branching enzyme SBE1 and SBE2 through RNAi. Still at R&D stage, the production of high-amylose starches can be used in the production of packaging material as well as film and coating from natural resources (Menzel *et al.*, 2015).

Other biochemical pathways for the production of molecules for the chemical industry are actively engineered, but most are still at R&D stage, including the tailoring of oil composition for use as biofuel and bio-based lubricants in camelina and *Jatropha curcas* (Kim *et al.*, 2014; Kim *et al.*, 2015); altered lignin content and composition to develop more efficient biofuels and biomaterial conversion processes in poplar, sorghum, and sugarcane (Fu *et al.*, 2011; Bottcher *et al.*, 2013; Van Aker *et al.*, 2014). Sugarcane has also been transformed with microbial genes that produce cellulose-degrading enzymes to produce self-processing plants (Harrison *et al.*, 2011).

Plant biotechnology for phytoremediation

There are a rapidly increasing number of scientific publications relating to phytoremediation and an expanding number of ways in which plants can be used for effective remediation of contaminated soil, sludge, sediment, ground water, surface water, and wastewater. Several case studies have demonstrated that GM technologies have successfully enabled phytoremediation to be tailored towards specific pollutants. Examples include model plants developed to degrade 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), trichloroethylene (TCE), and polychlorinated biphenyls (PCBs) (Rylott *et al.*, 2015). Focus is now turning from model plant systems to the transfer of this technology into plant species suitable for remediation in the field. One example is the transfer of rabbit cytochrome P450, 2E1 into poplar trees (Doty *et al.*, 2007), based on the pioneering approach of expressing a single human 2E1 in tobacco for increased degradation of TCE, vinyl chloride, carbon tetrachloride, chloroform and benzene (Doty *et al.*, 2000; James *et al.*, 2007).

Conclusion

Biotechnology provides to many of the challenges that our world faces today, from feeding and fuelling a growing population, tackling a worldwide epidemic of neglected and chronic diseases, to mitigating the environmental impact of modern human societies. Plant biotechnology with focus on seed-varietal improvement, such as GM technology and molecular-assisted breeding, has generated products that help agriculture to achieve enhanced yields in a more sustainable manner. GM technology has brought significant improvements to earned income, life quality, and per acre productivity. The global value of transgenic seed alone has been estimated at US$ 15.7 billion, representing 35% of the approximately US$ 45 billion commercial seed market (James, 2014), which is a formidable achievement, considering the very limited number of commercialized crops and traits. Relevant is also that farmers in developing countries touched approximately 50% of the economic gains of the GM technology and that GM crops generated a provisional benefit of US$ 68.21 billion between 1996-2013 (Brookes and Barfoot, 2015a) for growers of which 94.1% or more than 16.9 million were smallholder and resource-poor farmers from developing countries (James, 2014).

Although impressive, these figures are less remarkable when challenged with the statistics of 800 million people around the world, or 78% of the world's poor people, who live in rural areas and rely on farming, livestock, aquaculture, and other agricultural work for their subsistence (www.worldbank.org/en/news/feature/2014/11/12/for-up-to-800-million-rural-poor-a-strong-world-bank-commitment-to-agriculture) and for whom the GM technologies do not satisfactorily reach the needs in the least developed countries. Although more than half of the global GM crop area is located in developing countries, the major GM crops commercialized today, i.e. soybean, maize, and canola, except cotton, are grown on large farms in Latin America and do not match the interests of most smallholder farmers in the least developed countries. Crops of relevance to marginal environments, such as millet (*Pennisetum glaucum*), groundnut (*Arachis* sp.), cowpea, common bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), cassava, yam (*Dioscorea batatas*), and sweet potato, to name a few, have been mostly ignored by GM technology.

Because of their restricted trade, these so-called neglected underutilized crop species (NUCS) present little economic interest for commercial seed companies, but they have the potential to play an important role in the improvement of food security by contributing to food quality and dietary diversity. NUCS may also increase sustainability of agriculture, because they are believed to be well adapted to niche-specific environments, such as marginal and harsh lands, and to need a low input. As such, NUCS can help mitigate the impact of climate change on food production. However, these crops have been abandoned by researchers and farmers in favor of major crops that are sometimes promoted even in less suitable areas (Chivenge *et al.*, 2015). Moreover, the limited information on the genetic potential, agronomy, water requirements, and nutrition of NUCS remains a hindrance to their development and competitiveness. Therefore, actions have to be taken to overcome the constraints and obstacles for the cultivation of NUCS in regions where the uncertain climatic future can hamper food security, including acceleration of research to improve genetics and management as well as cultural acceptability and marketing.

Biotechnology tools can quicken the genetic improvement of NUCS. The GM approach can be used to introduce directly the desired sustainable management and the valuable output traits into varieties well adapted to local growing conditions. A major technological constraint is plant transformation that is critical for the development of biotech crops, for which GM techniques, such as transgenics, cisgenics, or by precision breeding, are required in the developmental process. The lack of efficient transformation protocols and breeding programs for geographical niche crops is in blatant contrast with the continuous striving for simpler, more robust, and more efficient transformation protocols for crop species for intensive agriculture.

There have been significant advances in the development of GM crops that can deliver food with health benefits beyond basic nutrition and in targeting small-market crops and a few NUCS for quality traits. These so-called second-generation traits will soon reach the market. The innovations coincide with an increasing consumer demand for healthy and nutritious food. The public sector shares a great deal of the research done in this field and public-private partnerships excel in translating the proof-of-concept to a marketable product.

Plant-made pharmaceuticals have become a major focus point since 2010, when realistic opportunities for commercial development emerged. Plant-manufacturing platforms for pharmaceuticals or molecular farming open interesting prospects for low-income countries, where large quantities of medicines need to be provided on a regular basis. Cost-effective local focus and needle-free deployment can be of great help for the treatment of tropical diseases.

In the industrial sector, plant biotechnology has the potential not only to generate more productive biomass feedstocks and minimize inputs, but also to develop more efficient biofuels, chemicals, and bio-material conversion processes. A number of nonfood crops improved with sustainable management have gone through the regulatory process. Additionally several biochemical pathways are currently being explored for the development of quality traits for the chemical industry and for phytoremediation (Ricroch and Hénard-Damave, 2015).

Of the greatest technological gaps in the commercialization of second-generation biofuels along with chemicals are the conversion processes that are costly, environmentally threatening, and time consuming. Advanced nonfood feedstocks have to be developed that can grow on marginal lands and simultaneously can decrease the costs of lignocellulosic biomass pretreatments. Numerous projects are under consideration that aim at engineering lignin content and monomer composition to optimize lignin degradation (Harfouche *et al.*, 2014).

Examination of the fast uptake of biotech crops on millions of hectares globally and of the current R&D pipelines impacting numerous plant species indicates that plant biotechnology will be a major tool to overcome the challenges of sustainability and development. Developing and emerging economies have taken the lead in terms of adoption of biotech crops and also in approvals of new transgenic crop varieties (James, 2014). As more actors become involved in R&D and more technologies are adapted and applied to new regions and local crops, the more developing countries will play a leading role in agricultural biotechnology. In the near term, most of the developing world will continue to rely on development assistance and innovations, as well as on technology partnerships and joint ventures with companies from developed countries that look for access to large developing markets. However, as research capacities increase, public sector institutes and private firms in emerging and low-income economies are likely to develop new biotech crops on their own. In the not too distant future, agricultural biotech research in developed countries could be surpassed in the same manner that production has already been.

The opportunities offered by plant biotechnology have never been greater, but neither have the challenges been, among which the most daunting is public perception and its influence on the regulation of biotech crops. All GM crops are submitted to a rigorous battery of tests and regulatory scrutiny prior to commercialization. Typically, the properties of the GM crops are compared to those of the corresponding non-GM variety with respect to various potential risk factors. Such comparative analyses include agronomic, molecular, compositional, toxicological, and nutritional assessments. Regulatory systems must ensure that all steps are in place to guarantee biosafety, but they must also ensure that none of these steps is unnecessary. Currently, the biggest constraint to commercialization of transgenic products is the regulatory delay, including, among others, test repetition, slow review time, and requests by regulators for additional information, often not necessary to demonstrate safety, and lack of clarity with respect to the regulatory requirements. Another source of delay is political interference in the biosafety regulatory process that hampers technologies developed by public-sector institutions or small private firms that, compared to large multinational corporations, have less financial flexibility to absorb the costs until the regulatory authority finally renders its decision (Bayer *et al.*, 2010). Thus, the extensive time needed to complete a regulatory file may significantly reduce the net benefits of GM products.

The costs of compliance with biosafety regulation also deter low-income and emerging economies from considering GM technologies as a solution to agricultural problems. Biotech developers must take into account not only the countries where the cultivation of the new biotech crops could take place, but also where the consumption of such crops might ultimately occur. So, an emerging country that wants to export GM food to the developed world is confronted with regulatory frameworks that do not give it much latitude. Moreover, low-income and emerging economies will not be able to keep pace with the ever-changing regulatory requirements of the developed world and will clearly restrict their decision to apply GM technology.

Public perception of GM crops and food is influenced by numerous factors, including access to information or misinformation, commercial actions by corporations, moral and ethical beliefs, and perceptions of personal benefit from the technology. Anti-GMO activists diffuse misinformation to uphold the belief that harm will come to those who consume foods made up of GM ingredients, heightening anxiety with the mass public as well as with public authorities (Blancke *et al.*, 2015). This concerted opposition to GM crops resulted in a number of complex legal and regulatory issues that have halted cultivation and stymied plant research in Europe with disastrous consequences to the development of new crops varieties and their introduction to markets worldwide. The best example is Golden Rice that has still not been approved for release in spite of its urgent need and readiness for well over a decade. Should concerns of this nature persist, R&D efforts will probably be restricted to large agribusiness corporations that will continue to focus on major intensive agriculture crops.

Nevertheless, there is no time to waste. The world's overpopulation and the pressures on the Earth system require all the ingenuity human beings can deliver. To ensure that the biotechnologies live up to the expectations, they will have to focus on the priorities that could slow, limit, or halt research and development, including negative public opinion and the lack of regulatory harmonization. Needless to say that markets and technology alone cannot promote the sustainable development of human societies. A deep transformation of societal values in a holistic manner will be required that can only be achieved with strong political will.

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