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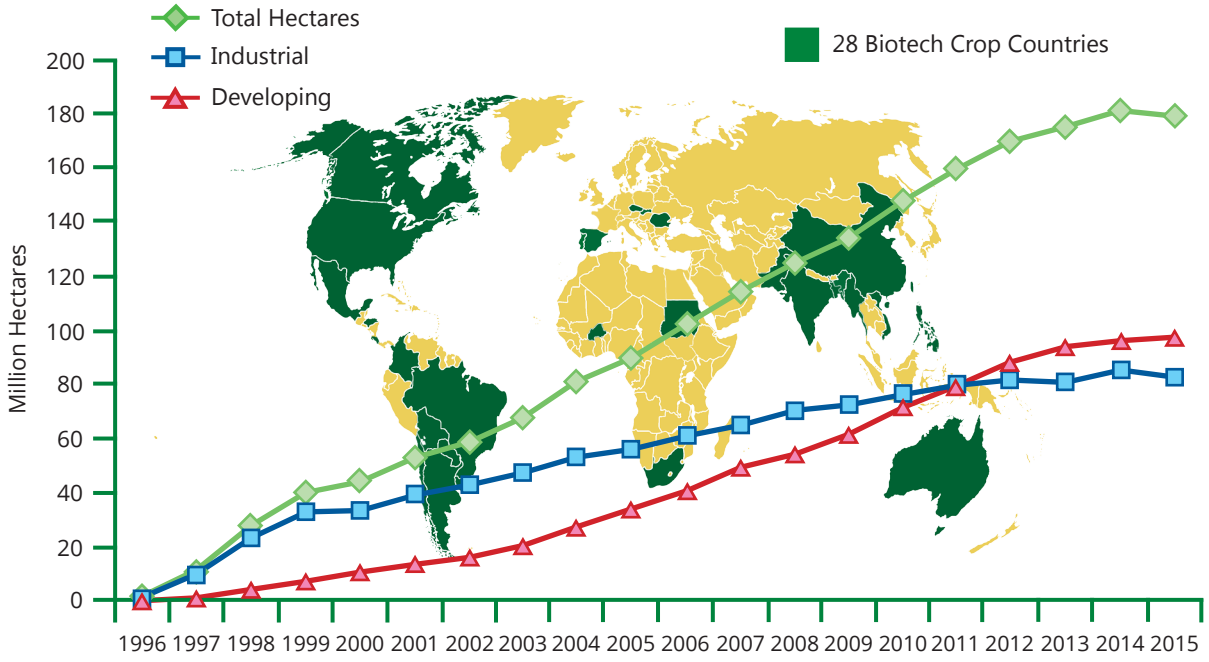
INTERNATIONAL SERVICE FOR THE ACQUISITION OF AGRI-BIOTECH APPLICATIONS

BRIEF 51

Invitational Essays to Celebrate the 20th Anniversary of the Commercialization of Biotech Crops (1996 to 2015): Progress and Promise

Clive James • Paul Teng • Mahaletchumy Arujanan
Rhodora R. Aldemita • Richard B. Flavell • Graham Brookes • Matin Qaim



Up to ~18 million farmers, in 28 countries planted 179.7 million hectares (444 million acres) in 2015, a marginal decrease of 1% or 1.8 million hectares (4.4 million acres) from 2014.

Source: Clive James, 2015.

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ISAAA prepares this Brief and supports its free distribution to developing countries. The objective is to provide information and knowledge to the scientific community and society on biotech/GM crops to facilitate a more informed and transparent discussion regarding their potential role in contributing to global food, feed, fiber and fuel security, and a more sustainable agriculture. The author takes full responsibility for the views expressed in this publication and for any errors of omission or misinterpretation.

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Invitational Essays to Celebrate the 20th Anniversary of the Commercialization of Biotech Crops (1996 to 2015): Progress and Promise

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FOREWORD

The ISAAA 2015 Brief celebrates the *20th Anniversary of the Global Commercialization of Biotech Crops (1996 to 2015) and Biotech Crop Highlights in 2015*. It features the achievements, and trends in the global commercialization of biotech crops in the 20 year period, 1996 and 2015. It embraces the highlights and economic benefits as well as the future prospects of crop biotechnology. The 2015 Brief is complemented by a collection of five invitational essays which address different and complementary aspects related to biotech crops, by authors who are knowledgeable and directly involved in crop biotechnology.

Dr. Paul S. Teng, a professor at Nanyang University in Singapore, and chair of ISAAA, offers a general commentary on the broad role of biotech crops in meeting food security challenges. The presentation covers the challenges related to food security, its scope, and the interconnections of the global food system. The state of food security/insecurity is reviewed as well as a discussion of opportunities to improve food security and the role that biotechnology can play in meeting the challenges.

Dr. Mahalectumy Arujanan of the Malaysian Biotechnology Information Center (MABIC) and Dr. Rhodora R. Aldemita of ISAAA, trace the evolution of conventional crop improvement technologies, the role of the new breeding technologies (NBT) and more specifically the potential of the fast evolving new genome-editing technologies such as CRISPR and its applications in crop improvement.

Dr. Richard B. Flavell, Chief Scientific Advisor of CERES Inc., an agricultural biotechnology company, based in California, U.S.A., examines the contribution of biotech crops in the first twenty years and the role of new biotech applications in the future. Importantly, he presents a forward-looking strategy that would enable the adoption of appropriate regulation that will facilitate the use of new evolving biotech crop applications such as CRISPR. The essay proposes a new vision of a strategy involving the troika of transgenes, genome editing and novel microbes (the use of plant microbiomes as a new source of additional genes to modify plant traits). The strategy also calls for science-based regulation and its harmonization internationally. It concludes that failure to utilize the new technologies such as CRISPR and implementation of appropriate regulation, will deny and seriously compromise future plant breeding initiatives and their vital contribution to feeding the world of tomorrow.

Dr. Graham Brookes of PG Economics, U.K. presents an analysis of the global, economic and environmental impact of GM crops, during the period 1996 to 2013. He presents the economic and environmental gains derived from a combination of technical advances and the role of the technology in the facilitation and evolution of more cost effective and environmentally-friendly farming practices.

Dr. Matin Qaim of Georg-August-University of Goettingen, Germany, offers an essay that presents the results of a meta-analysis (1996 to 2015) he conducted and draws some broader conclusions about the average effects of GM crops at the global level, and also the reasons for deviations in specific situations. He provides evidence on the global impacts of GM crops and their contribution to economic, social and environmental benefits.

ISAAA is pleased to share this collection of essays to celebrate the 20th anniversary of the commercialization of biotech crops. By sharing this information it is hoped that this knowledge will

facilitate informed "decision-making" about biotech crops in general and a better understanding, acceptance and adoption of the new biotechnology genome–edited applications such as CRISPR which will in turn contribute to increased crop productivity and importantly the alleviation of poverty and hunger, and a more just and peaceful world.

Clive James
Founder and Emeritus Chair of ISAAA

Meeting Food Security Challenges in an Inter-connected Global Food System

Paul Teng
Professor, Nanyang University, Singapore

Background: Challenges to Food Security

Because of the 2007-08 global food crisis and more recent high and volatile food prices, food security has become a major concern among many countries and is now firmly back on the agenda of regional blocks in Asia and Africa, and of international development agencies. Indeed, many governments, especially those in developing countries, are well aware that food security is an issue of national security (Desker et al., 2014). Emerging trends occurring globally and regionally are changing the food security landscape and threatening the countries' ability to feed themselves. These trends include, but are not limited to the following: population growth and urbanization, the declining performance of agriculture, natural resource constraints, degradation of land and water resources, climate change, high and volatile food and oil prices, the increased production and use of biofuels, increased market speculation, and the rapid transformation of supply chains (Teng and Escaler, 2014). At the same time, food production and supply systems are rapidly changing in response to many factors. Food demand and the channels for accessing food are shifting as per capita income increases and urban populations swell. Land competition in rural zones is becoming more acute as biofuel and other non-food or partial-food production increases. These challenges are also being faced in an era where consumers have become more discerning about the sources of their food, how food is produced and also with increased awareness about environmental conservation. All of these have resulted in intense discussions at both regional and domestic levels, and have forced countries to revisit their food security policies to try to protect the more vulnerable sectors of society.

The Scope of Food Security

Food security has been described in the 2013 State of Food Insecurity Report as a "complex phenomenon" (FAO, UN 2014), characterized by many interacting variables. All these have, in recent years, led to changes in thinking about food security, from a uni-dimensional concept to a multi-dimensional one (i.e. from focus mainly on the supply/production dimension to include additional dimensions of physical availability, economic availability/food affordability, food nutrition, food safety, and stability). There is also increased recognition that food security at the country level can only be adequately addressed if there is a multi-sectoral approach which involves both the government and private sectors, and civil society or informal groups (Teng and Escaler, 2014).

The post "Green Revolution" experience has shown that food security has to include an individual's ability to access and secure good quality and nutritious food. This realization led to the need for an expanded definition of "food security" (Teng and Oliveros, 2015). In 1996, the FAO moved away from the initial focus of food availability and redefined food security as a condition "when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (FAO, 1996). This definition may be interpreted to suggest that food security can only be achieved if the following four basic dimensions

are simultaneously met: "availability", "physical access", "economic access" and "utilization" (Teng and Escaler, 2010). The FAO often adds a fifth dimension, "stability", to emphasize the importance of the stability of the four dimensions over time. While each dimension is necessary for overall food security, they may weigh in differently in a rural setting as compared with an urban setting and even across countries with different incomes and net food trade balances. These four dimensions are explicated in the following sections.

The 'food availability' dimension encompasses all aspects of food supply, whether through primary production of crops and animals, reserve stock, food imports, overseas contract farming or foreign food aid. Raising agricultural productivity is a key part of this dimension, particularly for countries that are more dependent on their own agriculture. Imports and reserves play a larger role in net food-importing countries such as Singapore and Hong Kong. While food availability is necessary, and often the focus in most discussions, it is not sufficient on its own to ensure food security at the household level. The 'physical access' dimension is about 'farm to table' systems and supply chains, and in the case of vulnerable or marginalized households, being able to physically reach food supplies. At a country level, success of this dimension means having adequate transport infrastructure and logistical support to move food in a safe manner. This dimension is affected by war and conflict, as well as natural disasters (Teng and Escaler, 2014). 'Economic access' to food simply means the ability of a household to buy the food it requires and is a concern for both developed as well as less developed countries. Poorer consumers commonly spend a higher proportion of their household budget on food than more well-to-do consumers. Employment and income security, macroeconomic policies and market prices affect economic access to food, as shown by the two food indices discussed later in this section. The 'food utilization' dimension includes both nutrition and food safety. While a household may have the capacity to purchase all the food it needs, it may not always have the ability to utilize that capacity to the fullest. Factors that can influence this dimension include the quantity and quality of food, general child care and feeding practices, food preparation, food storage, food safety generally and health status of individuals. Among all the food security dimensions, 'stability' underpins the others and implies no disruptions in any of the previous four dimensions leading to insecurity.

Challenges to Food Security

Food security is continually challenged by a variety of factors that include population growth and urbanization, the declining performance of agriculture, natural resource constraints, climate change, high and volatile food and oil prices and the rapid transformation of supply chains (Teng and Escaler, 2012). Some of these will be discussed here.

As noted by Teng and Oliveros (2015), Asia is expected to contribute the lion's share to global population increases, between now and 2050, an increase of 2.4 billion, from the current 6.9 billion to 9.3 billion. At the same time, the population living in urban areas is projected to gain 2.9 billion, passing from 3.4 billion in 2009 to 6.3 billion 2050 with most growth concentrated in the cities and towns of the less developed regions. Asia, in particular, will see its urban population increase by 1.7 billion with China and India alone accounting for about a third of the total increase. One predictable outcome of this massive population shift is urban poverty. Already, Asia accounts for over half the world's slum population. Africa has some of the fastest population growth rates in its developing countries. Today, Asia has eleven megacities, cities with over 10 million inhabitants. By 2025, when the number of megacities is

expected to reach 29, Asia would have gained another five. Urbanization in combination with rising incomes will increase food demand and accelerate the diversification of diets. As incomes rise, diets will come to include more resource-intensive food products, such as meat, dairy, eggs, fruits and vegetables thus unleashing a rapid increase in demand for raw agriculture commodities.

Agriculture's performance in the region has declined over the last few decades, with its share of gross domestic product (GDP) falling from 43 to 18% between 1961 and 2009 in South Asia, for example (Fan, 2011; World Bank 2011). The number of people working in agriculture has also steadily declined from 70 to 55% between 1980 and 2010, and is projected to further fall to 49% in 2020 (FAO, 2011). In terms of farm size, while smallholder agriculture continues to dominate Asian farming systems with 87%of the world's 500 million small farms (less than 2 hectares) in the region, farm sizes in the region are getting smaller as a result of population growth and inheritance-based fragmentation (Thapa and Gaiha 2011). A more worrying trend is the fact that annual growth in productivity, measured in terms of average aggregate yield has been slowing over the years (Trostle, 2008). Global aggregate yield growth of grains and oilseeds averaged 2.0% per year between 1970 and 1990, but declined to 1.1% between 1990 and 2007. Yield growth is projected to continue declining over the next ten years to less than 1.0% per year. Asia's agricultural sector is also facing a new challenge in the fact that farmers are also growing older. For example, according to the Japanese Agriculture Ministry, 70% of Japan's three million farmers are 60 or older (Fackler, 2009). Lastly, concomitant with the changes in the age profile of farmers is the gender-relatedness of the farming community in countries like China which has seen massive rural to urban migrations. A study conducted in three South-western China provinces showed that the average age of active farmers was around 50 years old and women composed 78% of the total agricultural labor force (Song et al., 2009). This contrasts with Africa, which demographically is a younger continent.

Many of the world's agro-ecosystems being used as food production systems are already showing worrying signs of degradation. According to the Millennium Ecosystem Assessment, 60% or 15 out of 24 ecosystem services examined are already being degraded or used unsustainably. The use of two of these systems, capture fisheries and fresh water, is now well beyond levels that can be sustained even at current demands, much less future ones. Climate change will put additional pressure on natural resources and food security through higher and more variable temperatures, changes in precipitation patterns, and increased occurrences of extreme weather events (Nelson et al. 2010). According to recent projections by the International Food and Policy Research Institute (IFPRI), Asia's production of irrigated wheat and rice will be 14 and 11 percent lower, respectively in 2050 than in 2000 due to climate change.

Going forward, some challenges will require science and technology solutions while others are more likely to be ameliorated through management and policy. Farm productivity and production, as well as climate change effects are among those which lend themselves to possible solutions using biotechnology.

The Inter-connectedness of the Global Food System

In today's connected world, national food security is strongly linked to regional and global food security though many over-lapping supply chains and inter-linked food systems (Teng and Oliveros,

2015). The F.A.O. considers Food systems to include all the people, institutions and processes by which agricultural products are produced, processed and brought to consumers; including the public officials, civil society, researchers and development officials who design policies, regulations, programs and projects that shape food and agriculture (FAO 2013). Food systems therefore also have features of a complex system, in which changing any aspect of that system inevitably causes changes in other parts of the system or the entire system. Analysis of food systems, such as those in the Rice Bowl Index, have shown that, on a recurring basis, the food surplus parts of the world are in the Americas and Australasia, while Africa, and Asia have been food deficit regions and expected to remain so going towards 2030 (Frontier Strategy Group and Syngenta, 2014).

Asia currently is a major importer of the world's surplus production of key food commodities, accounting in the trade year 2014/15 respectively, for 77% of global soybean exports, 37% of global corn exports, 31% of global milled rice exports, and 25% of global wheat exports (Table 1). Africa is a relatively smaller importer of soybean, corn and wheat but is surpassing Asia in rice imports. Given population projections and Asia's demand for key commodities such as wheat, rice, corn and soybean in recent years, the import of these key commodities is likely to increase further in the next two decades.

These trade figures show how dependent Asia and Africa are on inflows of the key agri-food commodities from the Americas and the relative inefficiencies of Asian and African agriculture to produce the same four commodities. For example, while Southeast Asia is the region with the most rice production and exports, it is also the region which imports the most.

Intra-Asian trade beyond the above four food items, in recent years has become an important source of food supply to many countries to either make up for their own shortages in production or to add to the diversity of food demanded by the growing middle classes (Teng and Morales, 2013). This is also a reflection of the growing importance of food supply chains, which through various trade mechanisms, ensure the movement of food from agriculture surplus production areas to agriculture deficit production areas of the world. The rural landscape in Asia is therefore no longer the sole source of food in most countries for their people living in the cities. Rather, many cities now import food from a distance.

Of notable interest in comparing food surplus regions with the food deficit regions is the use of technology, such as biotech crops, in these regions. The major corn and soybean exporting countries are adopters of biotech crops, e.g. the U.S.A., Brazil and Argentina; while major importing countries like China and Indonesia in Asia, and Nigeria in Africa, have yet to grow biotech staple food crops. Deeper analysis reveals that differences in the average yield levels of corn, soybean and rice are also evident between these two regions, with visibly higher yields in the Americas (Fischer et al., 2013).

The State of Food (In)Security

Today's reality with respect to food (in)security is that there is a world food system that leaves close to one billion people hungry, a further billion suffering from micronutrient deficiency and another billion that are overweight or obese (FAO, IFAD and WFP, 2014). Although there has been discernable improvement in the reduction of hunger in many regions of the world, there remains in 2012-14, about 227 million hungry people in Africa and 520 million in Asia (FAO, IFAD and WFP, 2014); for the latter, it

Table 1. Production and imports of surplus food by Asia and Africa

Crop	Item	1000 MT		Percentage of Global Import/ Exports (%)	
		2013/2014	2014/2015	2013/2014	2014/2015
Corn	**Global Production**	**989,038**	**991,291**		
	Global Imports	**122,956**	**111,760**		
	Asia Imports	44,069	41,240	35.84	36.90
	Sub-Saharan Africa Imports	2,215	2,390	1.80	2.14
	Global Exports	**129,695**	**116,890**		
	Asia Exports	6,705	4,735	5.17	4.05
	Sub-Saharan Africa Exports	2,865	2,865	2.21	2.45
Soybean	**Global Production**	**283,736**	**315,055**		
	Global Imports	**110,854**	**113,661**		
	Asia Imports	83,434	87,887	75.26	77.32
	Sub-Saharan Africa Imports	25	25	0.02	0.02
	Global Exports	**112,723**	**117,181**		
	Asia Exports	416	522	0.37	0.45
	Sub-Saharan Africa Exports	15	15	0.01	0.01
Rice (Milled)	**Global Production**	**477,080**	**474,556**		
	Global Imports	**40,540**	**39,968**		
	Asia Imports	12,717	12,560	31.37	31.43
	Sub-Saharan Africa Imports	12,845	12,930	31.68	32.35
	Global Exports	**42,920**	**42,237**		
	Asia Exports	34,669	33,682	80.78	79.75
	Sub-Saharan Africa Exports	265	265	0.62	0.63
Wheat	**Global Production**	**716,137**	**725,034**		
	Global Imports	**158,598**	**156,600**		
	Asia Imports	43,288	39,000	27.29	24.90
	Sub-Saharan Africa Imports	19,065	20,050	12.02	12.80
	Global Exports	**162,135**	**160,120**		
	Asia Exports	8,601	5,595	5.30	3.49
	Sub-Saharan Africa Exports	1,055	1,155	0.65	0.72

Source: http://www.fas.usda.gov. Accessed 4 April 2015

is ironical to have this number in a region that is the most economically dynamic in the world and with the most millionaires.

Globally, the world is represented by a wide spectrum of high, middle and low income countries with varying food and nutritional needs. Some continue to experience widespread hunger and poverty, with the rural poor still dependent on subsistence agriculture and the urban poor exposed to hunger due to unaffordable food (i.e. lack of economic access). As well as undermining gains in poverty alleviation and food security, the periodic spike in food prices has led to macroeconomic instability in a number of countries, including both net food importers and exporters. Other countries are undergoing rapid transformation resulting in changes in food demand and diet diversification. At the other extreme, some countries with higher per capita incomes have consumers demanding healthier diets and more sustainable food-production systems. Countries also differ in the role that agriculture plays in their economies. In the more highly developed countries, agriculture accounts for commonly less than 5% of GDP and the labor force while in others, it accounts for as high as 20 to 30% of GDP and 38 to 54% of the labor force (Teng and Oliveros, 2015).

Low incomes and the proliferation of poverty negatively affect food utilization, which refers to proper sanitation and safety as well as general diversity and nutritional value of food. Price increase and volatility push millions of people beyond the poverty threshold, forcing them to live on inexpensive but low-nutrition substitutes. FAO estimated a 75 million increase in chronic hunger in 2008, which brought the number of undernourished people mostly in developing nations in Asia-Pacific and Sub-Saharan Africa to a staggering 923 million (FAO, 2008). Similarly, the study of Anriquez, Daidone and Mane (2010) found that soaring prices of staple food compelled the most vulnerable sectors to lower both the quantity and quality of their food as well as trim down on other equally important non-food expenditures such as health and education. This has long-term repercussions on physical and mental development, particularly of the younger population.

Many efforts have been made to assess how food secure a country may be, taking into consideration the F.A.O. multi-dimension definition of the term (FAO, 1996). Two notable ones are the Dupont-Economist Intelligence Unit (EIU)'s Global Food Security Index (GFSI) (EIU 2014) and the Frontier Strategy Group – Syngenta's "Rice Bowl Index (RBI)" (FSG & Syngenta, 2014) which currently has only been applied to Asia. The GFSI uses a list of indicators organized into three sets – affordability, availability and quality and safety – and while African countries generally rank among the bottom of the list in food security, many Asian countries are in the top fifty in terms of food security, with Singapore achieving a rank of no. 5 globally and the most food secure country in Asia. The EIU's GFSI describes the state of food security under prevailing conditions and favors those countries which have access to uninterrupted food supplies and can afford to purchase these supplies. The second index, the RBI, is based on the concept of "Food security robustness" (Teng and Morales, 2013) and is an assessment of a country's ability to withstand perturbations to its food security system, the latter characterized by a set of factors grouped into four – farm level factors, demand and price, policy and trade, and environmental factors. The latest RBI results (FSG and Syngenta, 2014) show that generally, Asian countries still lack the ability to be food secure in the face of disruptions in food availability, and remain vulnerable to price hikes and any threat which reduces the supply of food to the region. Both these indices do affirm that farm level production of food is an important contributor to making food available, and hence any factor which improves farm productivity and consequently overall production, positively affects food security. One

such factor is farming technology, including new crop varieties which can withstand adverse weather phenomena like drought and flooding, or protect crops from insect pests and diseases.

Opportunities to Improve Food Security in Inter-connected Food Systems

The environment affects food production through abiotic and biotic factors. The abiotic environmental factors include water availability, soil moisture, land/soil quality, as well as climate variability (e.g. temperature and weather). Biotic factors are exemplified by insect pests, diseases and weeds. Food production also leads to environmental pollution. Food production involves chemical inputs such as fertilizers and pesticides which potentially can alter food and water quality that may lead to land and water contamination as well as health problems. Energy consumption for food production and transport leads to CO2 emissions which contribute to climate change (Teng and Oliveros, 2015). Environmental degradation and the occurrence of environmental stressors such as water stress, floods, droughts, loss of soil moisture, land degradation, loss of biodiversity, and deforestation as well as climate variability related shocks, higher temperature, extreme weather, erratic rainfall (recurrence dry spells) have continued to cause losses in food production.

Looking towards the future, the Inter-governmental Panel on Climate Change (IPCC) has partially published the final version of the Fifth Assessment Report (hereinafter IPCC AR5) on global climate change and has suggested that extreme climate and weather events are likely to reduce crop yields. No food security dimension can escape from the impact of climate change because all food systems are potentially affected, including food production, access, utilization, and price stability (Teng & Oliveros, 2015).

The evidence clearly shows that having high levels of farm production improves food availability for the countries producing the food and also for those who rely on imports. With the anticipated and currently experienced effects of the growing environment on food crop production, it is important that means be deployed to reduce such effects on crop production. In this, biotechnology crops have proven to be an important contributor.

Role of Biotechnology in Food Security

In the preceding sections, the scope of food security and the forces influencing food security, were discussed. In the context of biotechnology's role, the main biotechnology application in food security has been through biotech crops and their impact on making more food and feed available, as noted previously. More specifically, biotechnology can address the following issues in food security:

- Provide surplus food production: more with less; price stability
- Provide labor saving production technology
- Reduce negative externalities, e.g., pesticide pollution
- Reduce effects of environmental stress (drought, floods)
- Reduce effects of biotic stress (pests, diseases)
- Improve nutritional and safety value of food, and
- Increase trade in food (Teng, 2014)

The role that biotechnology plays to address each dimension of food security (availability, physical

access, economic access and utilization) also varies. Key parameters influencing food availability through production, with their corresponding biotechnology crop trait, are as follows:

- Crop yield: Bt/herbicide tolerance, improved photosynthesis, disease resistance
- Access to technology: herbicide tolerance, conservation tillage
- Competing uses for biofuel: Bt/herbicide tolerance
- Competing uses for animal feed: phytase
- Environmental factors such as frequency of severe weather: drought tolerance, submergence tolerance, heat stress tolerance
- Degraded land: tolerance to salinity
- Nutritional quality: enhanced nutritive value of biotechnology crop such as high iron, and
- Freshness: delayed senescence.

Stresses such as those arising from environmental and biotic stresses cause reductions in crop yield; the traits in biotechnology crops that reduce the effects of stresses on agricultural production are among the most beneficial to food security. By reducing crop losses due to stresses, any surplus production would enable the countryside to feed those living in cities.

Assuring physical access to food requires that food be transported from farm to consumer via a supply chain involving logistics and infrastructure. In developing countries where modern cold-chain management to preserve freshness of perishable foodstuffs is lacking, biotechnology crops can provide traits through delayed senescence genes, which prolong shelf life of fruits and vegetables. Traits such as Bt have also been shown to reduce the level of mycotoxins in corn during transport. In times of conflict or when there is delay in transport of food, such traits can mean the difference between having edible food and unsafe food (Teng, 2014). Another aspect of physical access to food is through trade. Increasingly, biotechnology tools like detection kits or polymerase chain reaction (PCR) allow traders to comply with international agreements, such as the Cartagena Protocol on Biosafety, since most of the corn and soybeans grown in the world are varieties of biotechnology crops. Asia especially imports most of the world's surplus corn and soybean to feed animals in response to the increasing demand for protein (see earlier section, above). Biotechnology-based detection kits facilitate trade and movement of biotech crops, without which Asia will not be able to meet its protein demand.

Economic access to food is strongly influenced by the price for key food commodities such as soybeans, corn and some vegetables. Biotechnology crops, by assuring stability and surplus in production, have generally kept prices down except during extreme weather events that cause significant crop loss. The situation could have been worse since the 2007–2008 food supply disruption crisis, with occasional price hikes. Traits like drought tolerance in corn and submergence tolerance in rice have ensured some level of agriculture in spite of extreme weather. In the case of vegetables, biotechnology traits such as virus-resistance have prevented losses, which disrupt supply resulting in lowered economic access to food. Studies have shown that any rise in food prices increases the proportion of the populace who go hungry.

Food utilization is strongly dependent on the nutritive value of the food and its safety for consumption. Biotechnology traits, such as increased levels of omega–3 fatty acid and vitamin A, have both been designed to improve foodstuffs such as soybean and rice. Delayed ripening and Bt traits assure safety through freshness while detection kits allow rapid tests for certain bacteria.

Concluding Remarks

Modern biotechnology encompasses a range of technologies, which may be grouped according to their application domains: crops, aquaculture, livestock, environment and natural products (Teng, 2014). Biotechnology applications in crops have included plant tissue culture, biopesticides, biofertilizers, biodiagnostics, marker-aided selected crop varieties, and biotechnology (genetically modified) crops. At the farm level, apart from new biotech crop varieties, other inputs such as fertilizer and water, and proper management practices also need to be present in order for crops to express their full potential. Agriculture, however, remains an important area of impact for biotechnology due to the importance of assuring food security.

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Evolution of Agriculture and the Crop Technologies*

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Introduction

For some, rural farming tends to embrace a romantic context of agriculture and farming but reality is quite different. Former US president, Dwight D. Eisenhower said ***"farming looks mighty easy when your plow is a pencil, and you're a thousand miles from the corn field."*** This mistaken perception has given rise to opposition and activism against molecular technologies which have great potential for developing new crop varieties/hybrids which can make an essential and important contribution to food security, alleviation of poverty and for mitigating the negative effects of climate change.

Another popular public mis-perception is that all foods developed by conventional breeding and now consumed by humans are "natural and safe", and, unlike GM crops have not been modified by human intervention. In order to gain a better understanding of "natural" foods and agriculture, it is imperative to briefly review how agriculture and more specifically crop improvement, has evolved in the past ~10,000 years.

Cross-breeding (crossing between the related plant species) was the first method used by farmers and breeders early-on to modify plants. This early crop improvement was followed by hybrid breeding, mutation breeding and tissue and cell culture in the ~1930s. Genetic modification (GM) of crops, which are currently widely adopted on ~180 million hectares was the subject of intensive R&D in the 1980s and commercial adoption started in 1996. Today the most promising new breeding technologies (NBT) include genome editing which is advancing rapidly. It is noteworthy that there is no one single technique that may be a silver bullet for solving all the challenges that we face in crop production. These various techniques and different farming practices are all complementary and an integrated approach should be embraced.

Plant Domestication, Selection and Evolution

Genetic modification of our foods began when man started domesticating plants, and evolved from a nomadic hunter-gatherer life-style. The early farmers started saving seeds from plants. They carefully selected food with ideal traits such as size, taste, resistance to pest and diseases, and yield. This is often called "artificial selection", where plants suited for cultivation and possess desirable traits are selected and saved. The selected crops were then further enhanced by cross-breeding with related species which resulted in changes in the plants' genome. Thus, none of the food that we eat today existed before humans intervened. All the food that we eat today has very little resemblance to the food consumed by our ancestors millions of years ago.

Many are not aware of the origin of the foods and their wild relatives. Wild plants have traits that are not entirely suited for cultivation or consumption. For example, grain crops have a natural tendency to shatter their seeds to ensure their survival. This trait makes "harvesting" difficult or impossible

and results in "wastage" of grain. Obviously, alteration to the plant was needed to ensure access and storage of more food. The wild or ancient maize was a grass, called Teosinte that originated in Southern Mexico. Teosinte is made up of about a dozen seeds in hard shells which are not palatable. Today's maize that feeds the entire world's livestock and poultry is the successor of Teosinte. Without continuous selection and breeding processes, modern maize would not have evolved.

Wild potatoes do not look anything like the Idaho potatoes that we are consuming today. Potatoes originated from South America and looked like thin finger-like growths, with a bitter taste. Our ancestors picked up the largest and tastiest tubers and selectively bred them to produce the modern potato that we have today.

Cauliflower, broccoli, kale, Brussels sprouts, and cabbage are products of breeding programs from a wild mustard plant in the Northern Mediterranean region. Many mistakenly believe that kiwi originated from New Zealand. However, kiwi, originally known as Chinese gooseberry, originated from China. Thus, genes and crops have moved around the world continuously over thousands of years, undergoing genetic modification, through all possible means, and resulted in "modified wild plants" suitable for human consumption.

Conventional Breeding Techniques

Once the science of genetics was better understood, plant breeders used what they knew about the genes of a specific plant to select for specific desirable traits to develop improved varieties (ISAAA, 2013). Conventional breeding has been practiced for hundreds of years and today it features an increasing number of molecular tools and techniques.

Just like "genetic modification techniques", conventional plant breeding also results in changes in the genetic makeup of the plants. The difference between conventional and GM is that genetic modification is more precise and only a few genes are involved. Another difference is that in conventional breeding, genes of interest can only be incorporated from related plants and not from plants in another taxonomy group. For example, commercial rice can only be bred with another type of rice and not with any other plant species. However, in genetic engineering, the taxonomic group does not pose a barrier. Genes from any species can be inserted into other species or organism. This is a significant advantage as some genes are not available within the same species. For example, rice cannot be fortified with iron through conventional breeding because the global conserved rice germplasm does not contain the gene for high iron. Another popular myth is that only genetic engineering prevents farmers from saving their seeds for the next season. Since the 1930s when maize hybrid seeds were introduced on a large scale, farmers had to purchase seeds for every planting seasons, because after the first generation, hybrid maize seeds lose their comparative advantage in terms of higher productivity.

Hybrid Seed Technology

This technology is employed to improve yield, resistance to pests and diseases, and time to maturity. Hybrid seeds are developed by hybridization or crossing of parent lines that are 'pure lines' produced through inbreeding. Pure lines are plants that "breed true" or produce sexual offspring that closely resemble their parents. By crossing pure lines, a uniform population of F1 hybrid seed can be produced

with predictable and improved characteristics (ISAAA, 2013). Producing hybrids with improved productivity can take up to 20 years.

In the US, the optimal and complete adoption of maize hybrids, coupled with improved cultural practices by farmers, has more than tripled maize grain yields over the past 50 years from an average of 35 bushels per acre in the 1930s to 115 bushels per acre in the 1990s. This notable achievement, resulting from the combination of utilizing improved hybrid breeding and agronomy, is an exemplary model of global significance.

Hybrid rice technology helped China increase its rice production from 140 million tons in 1978 to 188 million tons in 1990. Research at the International Rice Research Institute (IRRI) and in other countries indicates that hybrid rice technology offers opportunities for increasing rice varietal yields by 15-20%. Many cultivars of popular vegetables or ornamental plants are F1 hybrids.

Mutation Breeding

During the same time frame, (1930s), similar to the development of hybrid technology, researchers realized that variations or mutations could also be created by exposing plants to X-rays and chemicals, which became more popular in the 1940s. Plants were exposed to gamma rays, proton, neutrons, alpha particles, and beta particles to induce mutations that give rise to useful traits. The other way of inducing mutation is by using chemicals such as sodium azide and ethyl methanesulphonate.

These mutation methods, and crops derived from them are still used today. Of the 2,252 officially released mutation breeding varieties, 1,019 or almost half have been released during the last 15 years. Wheat, rice, barley, potatoes, soybeans and onions are examples of plants produced via mutation.

It is important to note that none of these techniques or plants developed using these techniques are generally subject to onerous regulations that apply to GM crops.

Marker-Assisted Selection

Traditionally, plant breeders have selected plants based on their visible or measurable traits, called the phenotype. This process can be difficult, slow, influenced by the environment, and costly.

Today, plant breeders routinely use marker-assisted selection (MAS) which helps them to identify specific genes. The markers that the breeders seek are actually a string of genes that are located near the desired genes. If the markers are present, it confirms that the desired genes are also present and the plants will show the desired phenotype.

Tomato breeders and farmers have benefited significantly from this technology because tomatoes are extremely vulnerable to many pests and pathogens including diseases caused by bacteria, fungi, virus and nematodes. Making crosses and backcrosses using conventional technology to develop resistant tomatoes takes a long time, whereas molecular breeding is less time consuming and hence provides a significant comparative advantage. More than 40 genes that confer resistance to major classes of tomato pathogens have already been mapped, cloned, and/or sequenced (Grube, et al. 2000). Currently,

tomato breeding through MAS has resulted in a large selection of varieties with resistance or tolerance to one or more specific pathogens.

Biotech/Genetically Modified Crops (Recombinant DNA Technology)

In the 1990s, we witnessed the rapid adoption of biotech crops, also known as genetically modified crops (GM). GM crops proved to be controversial by some sectors of society, because they wrongly perceived that the technology was unsafe and that it would benefit only the large farmers in industrial countries, and not small farmers in developing countries. Evidence shows that the reverse is true with developing countries planting more (54%) than industrial countries (46%) in 2015. Furthermore, of the 28 countries which planted biotech crops in 2015, 20 were developing and only 8 were industrial countries. Of the 18 million farmers who planted biotech crops globally in 2105, 90% were small resource-poor farmers in developing countries. The area of biotech crops increased more than one hundred fold from 1.7 million hectares in 1996 peaking at 181.5 million hectares in 2014, and making a marginal decrease to 179.7 million hectares in 2015. them the fastest adopted crop technologies in recent history and in the same class as the Green Revolution of the 1960s.

The first biotech crop (GM) was approved for commercial planting in 1996. This year, 2015, marks the 20th anniversary of adoption of biotech crops which have resulted in significant and multiple benefits particularly for small resource-poor farmers in developing countries. While the technology has contributed towards food security, alleviation of poverty and sustainable agriculture, it is still perceived by critics as an unnatural phenomenon, and hence unacceptable.

A breakthrough research conducted at the University of Ghent and the International Potato Institute (CIP) found that all sweet potatoes from all over the world naturally contain genes from *Agrobacterium* (Kyndt, 2015) - in the past *Agrobacterium* (ISAAA, 2013b) was often used for transforming some GM crops and deemed "un-natural" and unacceptable by critics. However the researchers discovered the foreign DNA sequences while searching the genome of sweet potato for viral diseases. The presence of this "foreign" DNA in sweet potato categorizes it as a "natural GMO". The sequences appeared to be present in each of the 291 tested sweet potato cultivars and even in some wild related species. Different research methods confirmed the same conclusion: the specific sequences are not due to contamination, but are part of the sweet potato genome.

Sweet potato serves as an excellent example to illustrate that transfer of genes across species happens naturally and the techniques, as used by scientists, only contribute to more precision and speed, and less cost.

In summary, breeders will not resort to genetic modifications unless they encounter limitations with conventional breeding methods. If genes of interest cannot be found within related species, it is impossible to breed using conventional methods. Breeders then look for the desired genes in other species and these genes can only be transferred to the crop using recombinant DNA techniques or genetic modification. Genetic modification and other various molecular techniques will undoubtedly become one of the mainstream practices in the future improvement of crops that will be required to overcome abiotic and biotech stresses as well as to improve nutritional deficiencies. The new challenges

related to climate change will require the use of an array of molecular tools to develop resilient crops capable of high productivity under both stressed and unstressed conditions.

New Breeding Technologies: The Start of Gene Revolution 2.0

The Green Revolution for wheat and rice, that took place in the 1960s increased crop productivity dramatically, especially in the developing world. The Green Revolution was based on conventional breeding techniques to produce semi-dwarf varieties of wheat and rice, and was pioneered by the Nobel Laureate, Norman Borlaug who saved a billion people from hunger. 1996 marked another historical event in global crop production with the first commercial adoption of genetically modified (GM) crops: GM crops are also referred to as biotech crops, which is the terminology used in this Brief. Some observers have characterized the introduction of biotech crops as an evolution from the green revolution to the gene revolution. While sceptical critics claimed that biotech crops had the potential to benefit only farmers in industrialized countries, after 20 years of commercial planting, biotech crops have delivered significant benefits to over 16 million small resource-poor farmers in developing countries; in fact, biotech crops have become the fastest adopted crop technology in recent times, in the same class as the green revolution of the 1960s. 2015 marks the 20th anniversary of the adoption of biotech crops, which were successfully planted by millions of risk-averse farmers on 2 billion accumulated hectares (equivalent to twice the total land mass of the US or China), and generating an increase in farm income of US$150 billion during the period 1996 to 2015. Today, a generation of new crop breeding technologies (NBTs) are emerging that utilize new knowledge of crop genomes such as include genome editing and RNA interference (RNAi).

Genome editing of Plants

One of these NBTs is known synonymously as genome editing, gene editing, site-directed mutagenesis or genome engineering. For consistency genome editing is the preferred term used in this Brief. Genome editing is conducted by inserting, replacing, removing or disrupting DNA sequences often using artificially engineered nuclease enzymes, often called "molecular scissors". Genome editing can be used in two distinct ways. One is to simply make a cut in the DNA of a target gene which the cell then attempts to repair using its natural DNA-repair mechanisms. However, these are error-prone and sometimes add or delete small sections of DNA as they join the cut ends together. This is the same error-prone process that generates random mutations during repair of DNA damage from normal environmental mutagens or during mutation breeding with more highly mutagenic chemicals or radiation. Significant from a regulatory point of view, the mutations resulting from genome editing are indistinguishable from those that result from random natural mutations or those generated by mutation breeding. An alternative, more advanced approach to genome editing is to also supply a 'repair template' or other additional DNA into the cell at the same time as making the cut in the target gene. The repair DNA can be designed either to make small edits to the gene as it re-joins or to insert a whole transgene into a pre-determined site in the genome. Another refinement is to add a pair of molecular scissors targeted to the beginning and end of a pre-determined gene sequence. This double-cut strategy results in the excision of a functional part of a specific gene and an altered plant trait.

There are major differences between genetic modification (GM)/genetic engineering and genome

editing. When used in its simplest mode, genome editing makes small insertions or deletions at the target site but leaves no foreign (GM) genes in the plant. These mutations can result in minor modifications to the sequence or the complete knockout of gene function. In conventional GMOs, the DNA insertions occur essentially at random and the desired insertions must be selected from many individual events. However, a specific form of genome editing can be used to purposefully direct large segments of DNA including complete transgenes to pre-determined locations in the genome where it is known to function correctly and not interfere with the operation of neighbouring genes.

Genome editing offers a powerful tool for molecular biologists working in all areas of biotechnology – agriculture, medical and industrial sectors because it overcomes some of the limitations of genetic engineering. Genome editing tools can be used to make small but highly significant edits to correct inherited errors or to make other improvements to gene function. They offer more precision, options, speed and cost-effectiveness and have an enormous added advantage in plant breeding, especially if they also do not require the onerous and very costly regulatory process that applies to GM crops containing foreign genes. They also offer a more precise way of targeting foreign genes into pre-determined, so-called safe harbour sites and thus overcome some of the limitations of conventional genetic engineering.

Genome editing is expected to be a part of an integrated solution, when employed in combination with conventional breeding practices and genetic engineering to improve crops, including climate resilient crops, and crops with higher productivity to feed 9.6 billion people in 2050. Similarly, genome editing technologies can be used to remove allergens, toxins or provide more nutritious (fortified) food to malnourished population, and alleviate poverty among resource-poor farmers in developing countries.

Just like genetic engineering applications that are used to develop a multitude of recombinant drugs for the medical sector, genome editing also has its roots in the medical field where it has been used as a therapeutic approach for genetic disorders such as sickle cell anaemia or cystic fibrosis.

Genome editing technologies are the new tools in the plant breeders' toolbox that are expected to become a mainstream technology in due course, just like the advent of genetic engineering in the 1990s. For example, the number of published papers in genome editing in the last 10 years is a testament to the potential power of this technology. A check on the citations in PubMed shows that more than 480 papers were published on genome editing in 2014 alone. This probably signals the start of the new Gene Revolution 2.0 in crop production that could contribute to improved crops necessary to feed a growing world population that will climb to an alarming 11.2 billion by the turn of the century in 2100.

The Mechanism of Gene Editing

Genome editing to improve an organism's genome, first emerged when scientists attempted to harness the organisms' own DNA repair mechanism. Eukaryotic organisms undergo DNA double-stranded breaks (DSB), and one important pathway in their cells responsible for the repair of DSBs is referred to as non-homologous end-joining (NHEJ) (Davis and Chen, 2013). Once DSB has been completed, the cells start their own "natural" repair mechanism and seals back the broken DNA.

Figure 1. Overview of artificially engineered nuclease induced gene editing process (Punwar, 2014)

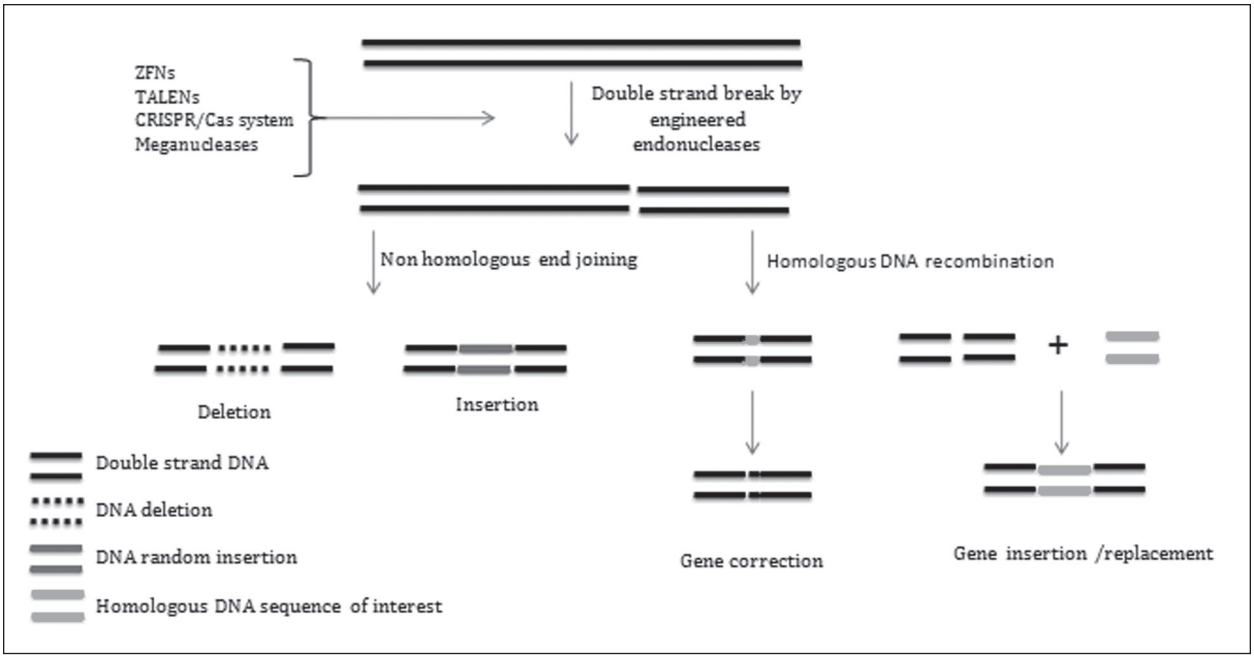


Figure 1 depicts how scientists use the cell's ability to repair DNA damages. Scientists realized the same DSB and NHEJ mechanisms could be used to insert, replace and delete DNA sequences to develop desired traits.

New Tools in Plant Breeders' Toolbox

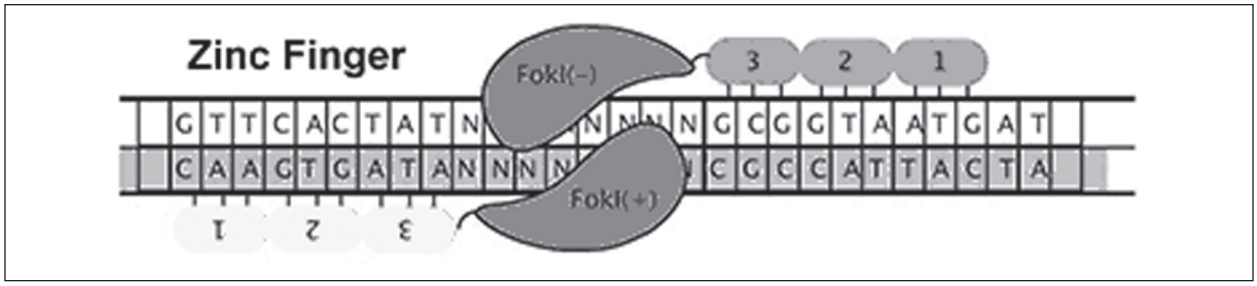
In the interest of presentation, a selection of current genome editing tools, have been categorized into the following four classes

i) Zinc Finger Nucleases (ZFN) technology
ii) Transcription Activator-like Effector Nucleases (TALENs)
iii) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
iv) Oligonucleotide Directed Mutagenesis (ODM)

Zinc Finger Nucleases (ZFN). Zinc finger nucleases (ZFNs) are chimeric proteins engineered to facilitate genome editing by introducing a double-stranded break (DSB) at specified locations (Carol, 2011). ZFNs consist of DNA-binding domain and DNA-cleaving domain. Each finger recognises 3-4 bases of sequence, and researchers can target their desired sequence that needs to be cleaved by assembling a number of fingers together (with some limitations).

The DNA-binding domain is made of a chain of 2-3 finger modules and recognizes a unique hexamer (6 bp) sequence of DNA, whereas the DNA-cleaving domain is made of an endonuclease domain of Fok1 (a type of enzyme that cleaves the DNA sequence) (Figure 2). Both together make up a highly-specific pair of genomic scissors. Once the DSB is induced, NHEJ mechanism is employed to edit the genome.

Figure 2. Schematic drawing of ZFN showing a 3-finger ZFN bound to their target cleavage site together with the Fok1 endonuclease to initiate a DSB (www.addgene.org)



Since the discovery of this technique in 1991 (Pavletich & Pabo, 1991), it has been used in genomic manipulation of plants, mammalian cells and in therapeutic applications.

Transcription Activator-like Effector Nucleases (TALENs). TALENs are made by fusing transcription activator-like (TAL) proteins which are secreted by Xanthomonas bacteria and Fok1 endonuclease. Just like ZFNs, by assembling an array of TALs and fusing them with Fok1 genome can be cut at specific sites. When DSBs are induced, the DNA sequence is then repaired to include sequence alterations – by knocking out some genes or inserting new ones (Figure 3). TALENs are more predictable to design than ZFNs and provide a simpler tool for scientists to edit any genome.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). This technology is based on the immune mechanism of prokaryotic cells first discovered in *E. coli*. The palindromic repeats refer to a sequence of DNA that has the same nucleotides bases when read from either end. Imagine the word RADAR – it reads the same way even if read backwards.

Figure 3. Schematic drawing of TALENs where two TALENs and the Fok1 domains bind to a specific site to induce DSB (www.addgene.org)

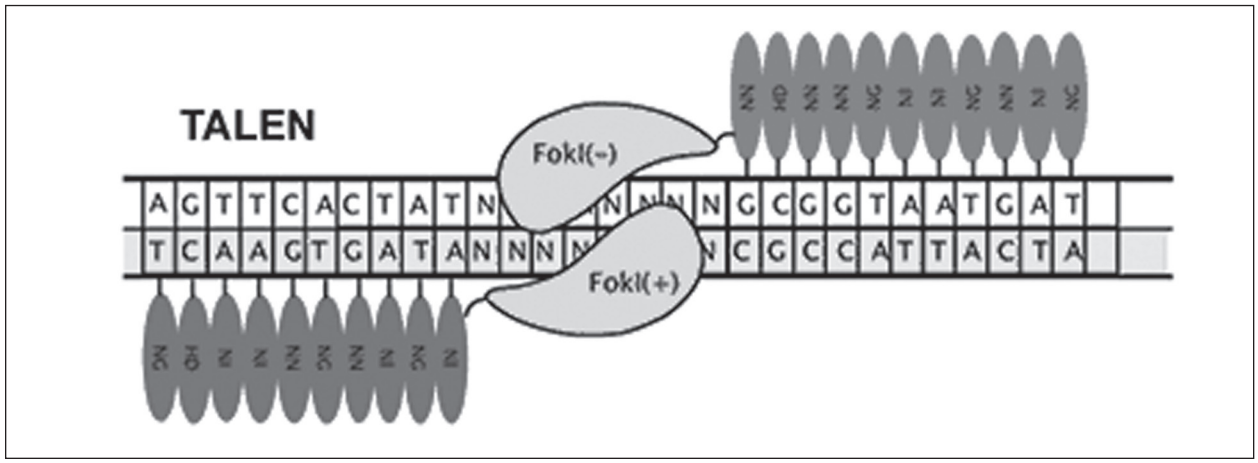
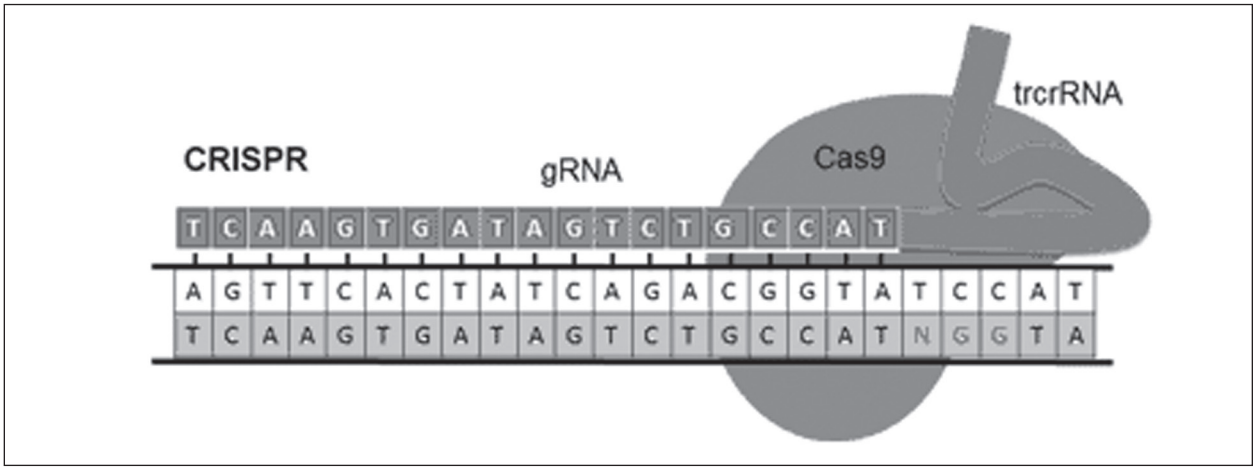


Figure 4. Schematic drawing of CRISPR-Cas9 that shows gRNA bound to Cas9 and additionally to trans-activating crRNA (trcrRNA) that enhances Cas9 specificity towards the cleavage site.



CRISPR system in bacteria incorporates DNA sequences from viruses that attack them in between their own sequence to build a memory bank that will enable them to recognize the same virus if they are attacked again. The bacteria then synthesizes a restriction enzyme known as CRISPR-associated protein nuclease (Cas9) that destroys the attacking virus by slicing its DNA.

CRISPR-Cas9 now serves as a powerful tool to the scientists to target and edit DNA sequences in a genome. Cas9 is the endonuclease at work here to cleave the specific site on a DNA sequence. The enzyme is guided by a short RNA, called the guide RNA (gRNA) which recognizes the specific site. This system allows scientists to design the gRNA to suit their target cleavage site.

Once the site-specific cleavage is done, scientists can then insert new genes or delete existing genes. CRISPR-Cas9 model is considered to be the most powerful of all gene editing techniques.

Oligonucleotide Directed Mutagenesis (ODM). This technique uses oligonucleotides to induce targeted mutagenesis in plant genome. The method allows introduction of new mutation, reversal of existing mutation or the induction of short deletions (Lusser et al. 2011). The oligonucleotides are designed to be homologous to the target sequence in the host genome but not to the nucleotide(s) that needs to be modified. These oligonucleotides are usually around 20-100 nucleotides long and are delivered into the plant cells using methods such as particle bombardment of plant tissue or electroporation of protoplasts.

The introduced oligonucleotides target the homologous sequence in the plant genome and create one or more mismatched base pairs. This then allows the cell's own gene repair mechanism to induce correction activities. The advantage of this method is that the introduced oligonucleotides degrade in the cell while the induced mutations are stably inherited.

Applications of Gene Editing for Crop Improvement

To date, there is only one product of gene editing that has reached the market:

SU Canola™ (Sulfonylurea Tolerant). Canola tolerant to sulfonylurea or SU Canola™ was developed by Cibus Global, a company based in San Diego using non-transgenic breeding through precision gene editing. It was approved by the US and Canadian regulators in 2014 and first grown commercially on 10,000 acres (4,000 hectares) in the USA in 2015. The crop did not go through the same regulatory process prescribed in the US for GM crops as it was deemed to be a product of mutagenesis.

SU Canola™ is a non-GM crop where the company employed precision genomic editing to perform target mutagenesis. The technique used is termed Rapid Trait Development System (RTDSTM) where Cibus generates whole plants from protoplasts. Mutation is targeted at acetohydroxyacid synthase (AHAS) gene using gene-repair oligonucleotide (GRON) which is distinct and precise. The protoplasts are then cultured into microcalli and whole plants. SU canola™ is expected to offer more weed control options to farmers.

To Regulate or Not to Regulate?

The first genome-edited crop to be commercialized is Cibus' SU Canola™ planted on 10,000 acres (4,000 hectares) in the USA in 2015. According to Jones (2015), the case-by-case review of the first few examples of genome-edited crops conducted by the USDA, has concluded that these crops are not considered as GMOs. In the EU, a GMO is defined as "an organism altered in a way that does not occur naturally by mating and/or natural recombination." However, crops developed through mutagenesis are not regulated in the EU. The US regulatory position on genome-edited products is already indicated in its approval of SU Canola™ but the EU position and that of other key countries like China is pending. It is noteworthy that China already has a strong R and D in genome editing and has already developed and reported a genome-edited wheat resistant to the important disease powdery mildew of wheat (Wang, 2014). An unnecessary and costly regulatory requirement will not only pose serious impediment to innovation in this area but will also keep genome editing out of reach of public sector institutions and small developing countries where the need is greatest.

Limitations of Genome–Edited Biotech Crops

Just like their GM predecessors, the genome-edited techniques have their limitations. For example, it is technically challenging to design and assemble the ZFN modules necessary to target a specific DNA site. It requires the design of many candidates and extensive screening process to acquire a ZFN with the desired DNA binding specificity. This makes the commercial ZFNs costly and the specialist experience necessary is retained largely in-house by the leading developers of the technology Sangamo Inc., Ca. USA. TALENs are easier to design than ZFNs and there is more open source support of their use. However, construction of new TALENs for each different target site also requires intensive work to optimize and is costly.

Early results using CRISPR–Cas9 in both plants and animals look promising. The use of a short guide RNA as the targeting molecule makes the construction of new vectors much easier and cheaper than

either ZFN or TALENs and offers huge potential. It is like a magic wand with extreme precision and ease of handling.

However, it is not yet clear exactly how precise the targeting to the pre-determined DNA site actually is and all these techniques need to be evaluated for the likelihood of off–target effects. Research in overcoming potential constraints is moving at an unprecedented pace worldwide and there is good probability of defining the shortcomings and developing some solutions in the near-term.

Terminology and the Future of Genome-Edited Crops

Recognizing that definitions and terminology are necessary but rarely perfect, ISAAA consciously decided from the outset to use the term Biotech Crops rather than GM crops, because all crops have been genetically modified over time, and hence the term GM crops is not the most appropriate. An option is to use Biotech Crops, or better still Bio Crops, as a generic term to cover all the crops developed with molecular tools, of which one set of tools is transgenic, another tool would be genome-edited, leaving room for other tools to be included and added to the toolbox that will undoubtedly expand over time. The further development of genome editing tools is probably inevitable given our necessary pursuit to "sustainably intensify" crop productivity whilst negating environmental footprints. Genome-edited tools hold much promise particularly if the products are not subject to the onerous and costly regulatory regimes required for GM crops developed through genetic engineering techniques.

Gene Silencing for Genetic Improvement

Scientists as early as the 1920's have protected plants from a severe virus by prior infection with a mild strain of a closely-related virus – a mechanism called cross protection. Later, it was also found that transforming plants with virus-derived transgenes gave protection against the challenge viruses even when no transgene protein was produced. The work by Beachy et al, in 1986 on coat protein (CP)-mediated resistance to tobacco mosaic virus introduced the concept of pathogen-derived resistance (PDR).

The term gene silencing was also coined early on as a molecular process that involves the down regulation of specific genes which finds application in genetic defense system against viruses and invading nucleic acids. Gene silencing (GS) was found to occur through post transcriptional gene silencing (PTGS) or RNA interference (RNAi), transcriptional gene silencing (TGS), and virus-induced gene silencing (VIGS). All these pathways play an important role at the cellular level, affecting differentiation, gene regulation and protection against viruses and transposons. Since most of GS phenomena were found to be related to RNA activity within the cell, the term RNA silencing is now often used to describe GS and comprise all mechanisms by which RNA sequences regulate gene expression. Genetic and biochemical studies have confirmed that the mechanisms of RNAi, co-suppression, and virus-induced gene silencing are similar.

RNA Interference/Post Transcriptional Gene Silencing

The discovery of RNAi started when Napoli and colleagues (1990) attempted to produce more intense purple colored petunias by introducing additional copies of a transgene encoding chalcone

synthase (a key enzyme for flower pigmentation). However, instead of a darker flower, petunias were either variegated or completely white. They termed this phenomenon co–suppression since both the expression of the existing gene (the initial purple color) and the introduced gene/transgene (to deepen the purple) were suppressed. Then in 1998, Fire, Mello, and colleagues, published their breakthrough study on the mechanism of RNA interference which showed injecting annealed sense/antisense RNA and neither antisense nor sense RNA alone into *Caenorhabditis elegans* can cause silencing. Furthermore, only injections of double stranded RNA (dsRNA) led to an efficient loss of the target mRNA. They called the phenomenon RNA interference. The co-suppression pathway in petunia and virus-induced gene silencing revealed that all these processes led to the accumulation of dsRNA. Thus, it was clear that co-suppression in plants, quelling in fungi and RNAi in nematodes all shared a common mechanism. Further work showed that this effect was even more widespread, occurring in fruit flies and mammals.

The RNAi mechanism pathway is initiated by the enzyme dicer, which trims long double stranded (ds) RNA, to form small interfering RNA (siRNA) or micro RNA (miRNA). These processed RNAs are incorporated into the RNA-induced silencing complex (RISC), which targets messenger RNA to prevent translation. The target mRNAs cannot accumulate in the cytosol, although they remain detectable by nuclear run-on assays. In certain instances, the DNA expressing the target mRNA also undergoes methylation as a by-product of the degradation process. The natural function of RNAi and its related processes is the protection of the genome against invasion by mobile genetic elements such as viruses and transposons as well as orchestrated functioning of the developmental programs of eukaryotic organisms.

PTGS or RNAi could be initiated not only by sense transgenes but also by antisense transgenes and biochemical evidence suggests that similar mechanisms might operate in both cases. In addition, multiple-site integrations, aberrant RNA formation, repeat structure of the transgenes and others lead to the formation of dsRNA which initiated PTGS.

Transcriptional Gene Silencing (TGS)

Gene expression was once believed to be affected by DNA methylations and chromatin remodeling and they play a major role in transcriptional gene silencing (TGS). In TGS, silenced transgenes coding regions and promoters are densely methylated which promotes protein binding following methylated cytosine recognition. This then leads to the formation of heterochromatin that prevents binding of transcription factors and protein translation – similar to developmental control and aging.

Further studies showed that dsRNA can be originated from viruses and transgenes that induce both TGS and PTGS; an alternating but not exclusive routes of regulation. RNA silencing was also found to be associated with de novo DNA methylation in plants. The fact that almost all DNA and histone methylation events are confined to transposons and repeats suggests a role for RNAi as a targeting mechanism for specific sequence chromatin modeling or TGS.

Virus Induced Gene Silencing

RNA interference has been regarded as a natural antiviral mechanism for protecting organisms from

viruses. It blocks infection by RNA viruses especially in plants and lower animals because many plant viruses produce dsRNA replication intermediates and very effectively cause RNA silencing VIGS (Virus-induced gene silencing). When viruses or transgenes are introduced into plants, they trigger a post transcriptional gene silencing response in which double stranded RNA molecules, which may be generated by replicative intermediates of viral RNAs or by aberrant transgene coded RNAs. They then follow the usual RNAi pathway where dsRNAs are then digested into 21-25 nt small interfering RNAs or siRNAs. The siRNAs subsequently assemble into a nuclease complex called RNA-induced silencing complex (RISC), then bind and destroy homologous transcripts. Viral RNAs not only trigger PTGS, but they also serve as targets. Cleavages of viral RNA results in reduce virus titers in local and distant leaves and the plant consequently recovers.

Various GM crops have been developed through genetic engineering methods of introducing transgenes. With the use of RNAi, new GM plants were developed that kill insects by disrupting their gene expression, which is a step beyond existing GM crops that produce toxic proteins. These new crops target particular genes in particular insects hence, will be far more targeted and less likely to have unintended effects than other genetically modified plants.

Crops generated through gene silencing, co-suppression and antisense technology have been approved for cultivation in many countries including the USA, Europe, Canada, Australia, New Zealand and Brazil – a listing can be found in Appendix 6 of the Full Brief. Hence, through RNAi, the ability to reduce gene expression that is highly sequence specific, technically manageable, economical and efficient is important in agriculture to enable nutritional improvement of plants and the management of various plant diseases. Innate™ potato and Arctic® apple improved through this technology are presented below.

Innate™ Potato. Innate™ potato was developed using RNAi technology to silence different proteins. This biotech potato was approved for cultivation in the USA in 2014 and was developed by J.R. Simplot Company. The word Innate™ was used to specifically indicate that there is no added foreign/protein in Innate™ potato.

The silenced proteins are:

i) Polyphenol oxidase (PPO): this is an enzyme that is released when cells are damaged or bruised. PPO interacts with different compounds that result in unsightly browning of the flesh of the tuber, which is a major consumer concern. This is easily noticed when potatoes are peeled and left at room temperature. PPO is only silenced in the tuber and not in the leaves.
ii) Asparagine amino acid: When potatoes are subjected to high temperature (as in the preparation of French fries), the sugars react with asparagine to form acrylamide, which is a potential carcinogen. Innate™ potato has up to 70% less acrylamide.

Subsequent to the approval of Innate™ potato, Simplot has been given approval by USDA to deregulate Generation 2 Innate™, with more traits including further reduction of acrylamide, resistance to late blight, and reduced levels of sugar (derived from starch) when stored at low temperatures. Importantly, Generation 2 Innate™ does not have a foreign gene – the gene used is Rpi-vnt1 from a wild potato plant native to South America, which allows Generation 2 Innate™ potato to qualify as a non–GMO in some countries (www.biofortified.org).

The benefits of Generation 2 Innate™ potato are multiple, not only to consumers and the environment but also to farmers and the food industry:

i) Reduced risk of potential cancer associated with significantly lower levels of acrylamide
ii) Reduction of waste in the food industry due to bruising, the number one concern of consumers
iii) Reduction in sugar content (derived from starch) when stored at low temperatures
iv) Reduced number of fungicides applied by growers to control late blight worldwide; the disease is the most important disease of potatoes worldwide causing losses estimated at US$7.5 billion annually
v) Reduced need for additional chemicals used during storage to prevent shrinking, sprouting and bruising. Currently 20% of potatoes produced in the US are rejected due to high sugar content which leads to shrinkage

It is estimated that if all fresh Russet potatoes in the US were Generation 2 Innate™ potato, the savings would be 400 million lbs of potatoes annually, and this would contribute to sustainability. More specifically, this translates to a saving of US$90 million in producer costs, 60 million lbs of CO_2 emission, 6.7 billion gallons of water, and 170,000 acres (68,000 hectares) less hectares requiring pesticide spraying (Entine, 2014).

Arctic® Apple. When apples are cut and exposed to oxygen, two compounds (PPO and phenol) interact and combine to form a brown pigment. Sliced apples, which quickly turn to an unsightly brown, is not only a problem to food service companies but also to homemakers who pack lunch boxes for family members. Sliced apples turn brown in no time and become unattractive and unpalatable. Okanagan Specialty Fruits, a small biotech company in Canada has overcome the browning issue by silencing the Polyphenol Oxidase (PPO) enzyme that causes browning. The company employed RNAi technique to silence PPO and reduced it by 90%.

Just like the Innate™ potato the DNA in RNAi apple is not from a foreign source but from Apple's own genome. Arctic® Apple which has a kanamycin antibiotic marker is expected to be planted for the first time in the US in 2016 – it is also already approved in Canada. Artic® Apple will be the second fruit to be approved after PRSV resistant papaya that was approved in both the US and China. It is approved for export into Japan.

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Crop Improvement Using Transgenes, Genome Editing and Microbes: A Forward-looking Essay to Celebrate 20 Years of Transgenic Crops

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Summary

Summary

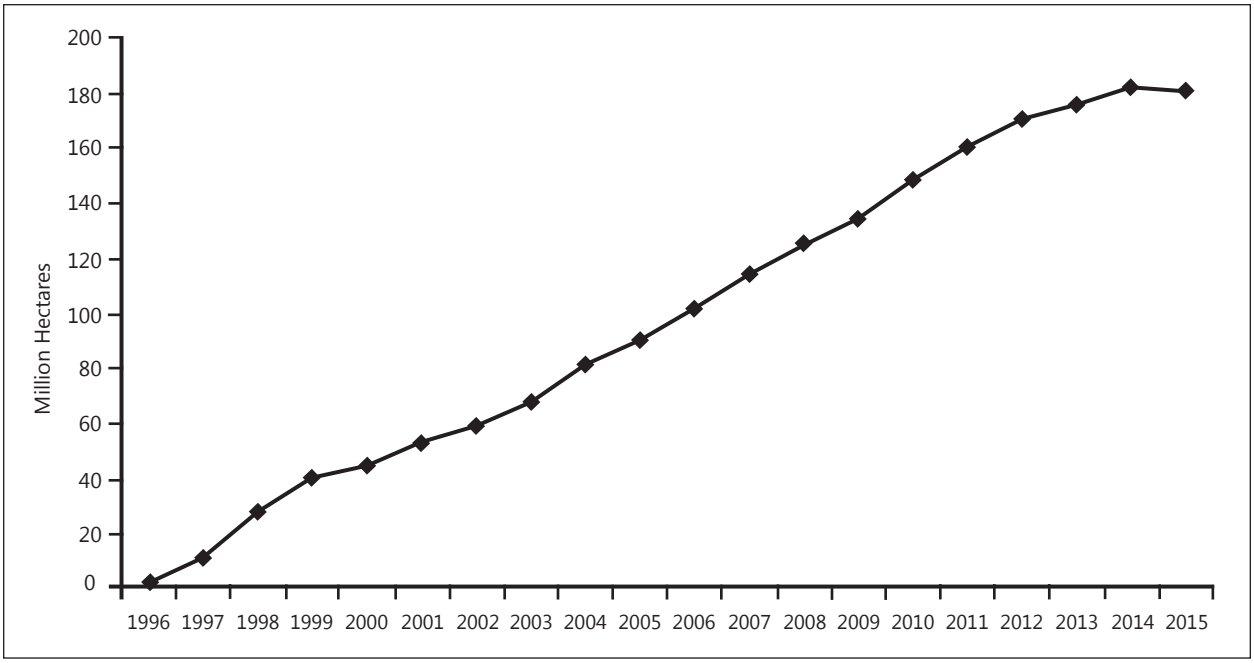
The acreages of transgenic crops across the world, year-on-year since 1996, record the fastest adoption ever of any major agricultural innovation. The knowledge and understanding of plant biology and crop improvement has similarly been undergoing the fastest growth of all time. This essay takes a look back at the development of transgenics from the frontiers of plant science of the 1980s. Ways of bringing new genes into plants are now undergoing another radical change with the development of genome editing. Genome editing offers many more opportunities in plant breeding and helps unite genetics, trait improvement and breeding. Plant microbiomes are also discussed as sources of genomes to enhance crop traits. The trio of technologies – transgenes, genome editing and novel microbes open up a new vision for plant breeding. How they can contribute over the coming twenty years is outlined. Discovery of trait-enhancing genes for key crops remains a research priority. Requirements for the new technologies to help food security, especially in developing countries are discussed, based on learnings from the introduction of transgenes in the 1990s. Amongst the many important changes required, establishing the right science-based regulations is critical. These regulations need to be harmonized across the world. Without appropriate regulations that inspire investment and agricultural improvements, future contributions based on the new methods of modern plant breeding will be severely compromised, with severe losses for mankind and the planet.

Introduction

It is some 20 years since the commercialization of so-called transgenic, "Biotech" or "GM" crops became firmly established. They were developments from the frontiers of biosciences. While ISAAA has been detailing the acreages of transgenic crops across the world, year-on-year, recording the fastest adoption ever of any major agricultural innovation (Figure 1), the knowledge and understanding of plant biology and basis of crop improvement has similarly been undergoing the fastest growth of all time. This has also been due to the exploitation of the techniques and logic of biotechnology. The result is a series of innovations for plant improvement which make it possible to analyze and create routinely in crop plants what was beyond us just a few years ago. They provide remarkable opportunities for plant breeding, just as transgenes did in the 1990s. The new products may fall outside the biosafety regulations as currently written for transgenes in some countries. There are also new opportunities emerging to exploit for crop improvement the microbes that live in plants. These are a source of genetic variation not formally explored in most plant breeding programs. Thus, the future scientific and regulatory options of genetically altered crops are different from the past. We can be confident that progress will continue to make out-of-date today's knowledge and arguments for and against innovations in plant breeding. Scientists and commentators on plant improvement therefore need to continue to look not at today's science but at tomorrow's. This is especially true for developing countries where adoptions of innovations often come later but where the most efficient food production gains need to be made, and will surely be demanded.

The urgent needs for and challenges associated with food security for all are well known. This essay seeks to draw attention to the new knowledge and breakthroughs that will influence the future of

Figure 1. Global Area of Biotech Crops, 1996 to 2015 (Million Hectares)



Source: Compiled by Clive James, 2015.

plant breeding and its products for farmers and societies worldwide. It includes a review of the past 20 years to reveal learnings from the first transgenic plants and the issues they raised. Then follows a review of recent innovations in targeted genetic changes and in the exploitation of endophytes that will determine what industrialized and non-industrialized countries could create, or adopt, to enhance yields of food, feed, fiber and bioenergy crops in the next 10-20 years. To take advantage of the new technologies it is necessary to continue discovery of genes to be modified for targeted plant improvements and continue the building of easily searchable databases. Sections are devoted to these. The essay ends with the updated vision of plant breeding that embraces the new technologies and what needs to be done to increase the adoption of targeted genetic modifications and microbes in plant breeding, to meet world objectives in food and nutrition security. Many important items are included for institutions and countries to consider. Of critical importance is the establishment of fit-for-purpose, science-based regulations, without which many potential benefits from the new technologies will be lost. It is concluded that developing countries need to determine their own destiny with respect to the new genetic technologies in plant improvement and not rely on the decisions or regulations of others who may have different interests.

The Early Years of Transgenic Crop Plants

The first fertile transgenic plants were created and the transgene shown to be inherited in a Mendelian manner in 1982 and as series of noteworthy papers published in 1983. These experiments were carried out by three groups based in five laboratories: Monsanto in St. Louis, USA, (Fraley et al, 1983) the Plant Breeding Institute, Cambridge, England in collaboration with Washington University, St. Louis, USA (Bevan et al, 1983) and the University of Ghent in collaboration with the Max-Planck-Institut für Züchtungsforschung in Köln, Germany (Herrera-Estrella, et al, 1983). The motivations were based on the need to improve traits in plant breeding and the clear demonstrations in bacteria, yeast, drosophila and mammalian cell lines of what would be possible. It was recognized that transgenic traits would be attractive because they are genetically dominant and easily managed in a breeding program compared with the complex inheritance patterns of traits determined by collections of genes. The plant experiments were similar in each of the laboratories. All exploited the effectiveness of the soil Agrobacterium to infect tobacco and the ease of regeneration of tobacco plants from genetically transformed protoplasts and calli in culture. The approaches were grounded on the brilliant discoveries that genes on a piece of DNA (TDNA) are transferred from a plasmid in Agrobacterium into plant chromosomes in nature, as part of a complex system that links plant and bacterial biology. Each laboratory joined a gene encoding drug/herbicide resistance, as a selectable marker, to other genes to find and select the plant cells that had received the new DNA and integrated it into its chromosomes.

The first transgenic plants to enter agriculture were in China in the early 1990s. These were tobacco plants containing a gene specifying a viral coat protein that provided resistance to the virus. The first approval for commercial sale of a genetically modified product for food use was in the USA in 1994 when Calgene marketed its "Flavr-savr™" delayed ripening tomato, an RNAi product. By the end of 1995, applications had been granted to commercially grow at least nine transgenic crops: soybean, corn, cotton, rapeseed, squash, tobacco, carnation, potato and papaya (James, 1995). By the end of 1998, there were 56 transgenic crop products based on nine crops approved for commercialization in at least one country, with corn having the most products (James, 1998). The leading transgenic traits were resistance against glufosinate, bromoxynil, sulfonylurea, glyphosate or sethoxydim herbicides,

virus resistance, seed quality (modified oil) and resistance to lepidopteran or coleopteran insects by various genes from *Bacillus thuringiensis* (Krattiger, 1997). In 2014, GM crops were grown on 181.5 million hectares in 28 countries by some 18 million farmers (James, 2014) with many additional genes and species in transgenic trials in some countries. The acreage of transgenic crops in developing countries became greater than in industrialized countries in 2011 (Figure 1). The statistics reveal the huge value in monetary terms to agriculture (>$133 billion, James, 2014), in farmer satisfaction and, in addition, benefits to the environment due to reduced pesticide usage and low or no-till agriculture associated with use of post planting herbicides.

What was happening during the years 1982 to 1996 before the first major launches of transgenic crops? It was necessary to learn how to introduce DNA into the major crops using Agrobacterium or a gene gun, to build better systems to transfer any genes of choice into plant cells, to regenerate fertile plants in many crop species, to find and redesign genes that could confer an important new trait in a crop, to select plants that carried only the T DNA and not unwanted parts of the T-DNA plasmid, and also to perfect the level of gene expression using regulatory DNA sequences (promoters) such that the trait was fully functional throughout the plant or in specific tissues. For herbicide resistance this meant having enough protein product of the transgene in all parts of the plant to ensure there was no plant death when the herbicide was applied in farmers' fields. Many transgenic plants were created with the same transgene to find the one that was optimal, as frequently transgenes became silent. We did not know why then (Finnegan and McElroy, 1994; Flavell, 1994). To achieve all these technical advances, introgress the favored transgenic event into a range of cultivars, evaluate them in a range of environments and pick those that were economically valuable was extraordinary progress, because nothing had been done like this before.

Genes for herbicide resistance and for *Bacillus thuringiensis* (Bt) proteins toxic to insects were not chosen by accident to make early products. They were chosen because the traits were valuable, addressed known needs, and single genes to achieve the traits were obtainable relatively easily from bacteria. In the case of Bt there had been an established industry using the bacteria for some 50 years (Krattiger, 1997; Sanchis, 2010). A large amount was known about various strains and their ability to target different insects. It was also known that resistance was due to a single protein. Similarly, much was known about the modes of action of commercial herbicides on plants and how to find genes to provide resistance to them. The speed of early scientific progress and the very high rates of commercial adoption of many transgenic cultivars in multiple crops are testimony to the correctness of these choices for early products. Looking back on what we knew then compared with what we know now, the transgenes were remarkably well designed but we knew little about the complexities of putting genes into new positions into chromosomes in relation to gene regulation, stability and chromatin conformations. We should remember no plant genomes had been completely sequenced.

The outstanding adoption rates of transgenic crops were due not only to the innovative molecular genetics and the breeding of effective transgenes into many cultivars for diverse environments but also to the development of the biosafety regulatory systems, initially within the EU and US and subsequently in many key countries. These were essential to enable the transgenic crops to be trialed, evaluated and accepted as safe by regulatory authorities. Without the spreading of sound knowledge about transgenes and an intense focus on generating biosafety laws there would not have been such widespread adoption. It is these laws and government decisions that also enable biotech crops and

their products to be exported and imported around the world. The introduction of new regulations was not so much that scientists believed that, for example, the initial herbicide and insect resistance genes would cause plants to be toxic to human health but that there needed to be systems in place to guard against unhealthy gene products entering the food chains as the scale of plant genetic modification grew to involve large numbers of less well known genes and gene products. Regulations have evolved over the years, including the Cartegena Protocol on Biosafety (see below).

In summary, it was the combination of outstanding science, plant breeding, farmer adoption, enhanced environmental safety, poverty reduction, new national and international laws, intellectual property protection and wealth creation, all laced together by great strategic thinking that made this a truly extraordinary story of achievement, as well as one of great controversy (Lemaux, 2008, 2009). The transgenes and the products that were the outcome of these strategies were all proprietary and nearly all the early products came from industries in the USA or Europe. The combination of establishing the industry on the basis of 1) proprietary genes and enabling technologies, 2) new biosafety criteria that are very expensive to fulfill and 3) products for which farmers were willing to pay substantial additional payments, helped drive the investments to products rapidly and successfully in the richer countries. However, these same items have led to stifling of commercial innovations from public sectors, small companies and developing country initiatives. These are serious issues.

ISAAA's mapping of the story of adoption of transgenic crops across the world since 1996 (James, 2014) has revealed year-on-year increases but the same traits, herbicide and insect resistances, predominate. This may give the impression that transgenic breeding has been static. This is not the case. More genes for insect tolerance have been added and stacks of genes for the same trait are now becoming routine to provide better resistances and also to combat the emergence of insect resistance and herbicide resistances in weeds (see James, 2014). Breeders have also been learning how to stack genes effectively. There is the simple approach based on the crossing of parents that have different transgenes within them. While this is easy in principle, when many transgenes are needed to support many traits effectively, combining of all the right transgenes in elite germplasm breeding programs is a major challenge. It is therefore appearing more attractive to combine the genes into one vector and introduce all, or at least some, of the transgenes linked together in stacks. Designing multiple adjacent genes such that they do not influence each other adversely by increasing or decreasing activity is complex. Furthermore, any necessity to go back and re-deregulate every new stack containing the same genes is costly, time consuming but necessary, because most current regulations require new deregulation of all new events. Here lies a conundrum between the best scientific solutions and the regulations.

Improvement of Existing Plant Traits by Transgenes

It was assumed by most plant scientists during the 1990s that by now many plant-based traits would be enhanced by the addition of transgenes designed to overcome the deficiencies of specific genotypes/alleles/allelic combinations. The transgenic plants would have higher yields and greater tolerance to abiotic and biotic stresses, for example. This was expected because it was not difficult to find genes that when increased or reduced in expression enhanced a trait in Arabidopsis or rice, for example. However it has not happened. Why? There are many interacting factors behind this and it is important to understand them to gain the right perspective of future options.

Introduction of a transgenic trait depends on the efficacy of the genetic change to improve the trait, the cost of the research and its deregulation through the relevant regulatory authorities (not only for the country where the trait is first introduced but also the countries into which the crop will be imported), the size of the market and potential profitability. Public and market acceptability of the new products are additional, dominating factors. An example of the influence of the latter in the US was when McDonalds declared they would not market any products made from transgenic potatoes. Such decisions severely reduce commercial incentives to produce crop products, in spite of the opportunities and major need to improve crops. Where there is not open acceptance of the transgenic product then segregation of the transgenic and non-transgenic crops with separate marketing could be considered but this is expensive, runs the risk of cross contamination and there are plenty of organizations looking for any mis-use of transgenes. Thus, in summary, many factors are considered before a transgene(s) is driven down the development chain towards a product. Many of these factors are so critical that they have been responsible for the lack of proliferation of transgenic traits much beyond herbicide resistance and insect resistances, even when suitable genes are available.

The public sector literature is filled with clear demonstrations of how new versions of plant genes can change plant traits for the better. There have been huge investments into such manipulations in Arabidopsis and rice, as well as over 40 other plant species (Rensink and Buell, 2004; Buell and Last, 2010). Positive results covering every trait explored have been demonstrated including drought tolerance, salt tolerance, flowering time, tillering, grain size, architecture, uptake of nutrients, disease resistance and nitrogen use efficiency. Many different genes when increased or decreased in activity have been shown to improve each trait, indicating the diversity of genetic systems underlying complex traits. This is an extraordinary compilation of knowledge from a massive discovery investment over the past 20 years. It has revolutionized plant science and our understanding of plant processes.

The findings in Arabidopsis and rice provided long lists of genes with which to enhance traits in plants. Also, the making of transgenic plants and comparing the effects of adding a single gene has been routinely used to prove the role of a gene and the processes underlying traits. However, it has turned out that to demonstrate improvement in a model or non-improved crop plant is much easier than improving a commercially relevant elite crop variety using single transgenes. This is illustrated well by the attempts in corn where large sums of money have been committed to the goal in large and small plant breeding companies. On the one hand, single genes have enabled major changes to be made to biosynthetic pathways to create added value in oil composition, provitamin A levels and for other end product, biochemical traits not previously selected (James, 2014). On the other hand, for the traits that breeders have selected hard over decades, the creation of economically significant trait increases manifested in many environments and genetic backgrounds has been exceptionally difficult using single or few plant transgenes that are designed to change the activities of the plant gene, although the use of a bacterial gene to enhance drought tolerance in commercial corn hybrids by Monsanto should be noted (James, 2014). Furthermore, in corn breeding inadequate traits are overcome by making hybrids and selecting for heterosis, whose basis optimizes traits using multifactorial systems. Heterosis-based breeding is therefore likely to make further improvements in complex systems by the addition of single genes even more difficult. These challenges, therefore, contrast with the introductions of herbicide tolerance and insect resistance where the traits did not previously exist in the crop and the transgenes used came from the bacterial kingdom. Such conclusions coming from very large investments into corn biotechnology are profound and should be considered carefully in future transgenic breeding programs all over the world.

When trait improvement is known to be based on reduction of a gene product this can be achieved by inserting a transgene designed to make an RNA that is antisense to the sequence of the gene. This RNAi approach has been used from the beginning of plant biotechnology and the first Flavr Savr™ tomato contained such a transgene to reduce the levels of polygalacturonase in the fruit. As illustrated by this product, a tissue-specific promoter can be used to reduce the gene activity only in the tissue required to achieve the trait. In those days the precise mechanism by which such genes achieved their effects was unknown. Now we know that small RNAs, single or double stranded, play major roles in trait regulation (Chen, 2012). They regulate mRNA survival and can recognize complementary DNA sequences to program extents of DNA methylation and chromatin structures that influence gene expression.

In summary, it is readily possible to design transgenes and get them expressed in all plants. Where the trait is genetically simple, for example fungal resistance (Jones et al 2014), then single transgenes can often confer major changes to a trait, even in elite germplasm. Many need to be exploited. Genes which change major regulatory molecules such as hormones also usually have potent effects. However, for more subtle and genetically complex traits where breeders have already selected very hard then the probability that changing the expression pattern of a single gene will improve the trait sufficiently in many elite genotypes appears to be low. Equivalent genetic variation has probably already been selected. These sorts of conclusions fit with what is predicted from genetic analyses of plant traits. In retrospect, it appears that many biotechnologists have been too naive in expectations about what single transgenes can do for many genetically complex plant traits in elite, commercial germplasm. Use of non-elite model crops has been extraordinarily valuable for science but apparently less so for predicting improvements in complex traits in crops. But what of crops relatively neglected by breeders and grown predominantly in the developing world? It is likely that transgenes could play a larger role in trait improvement programs than in corn, for example, because selection has not been so intense for so many years and complementation of genetic deficiencies via heterosis is not the routine.

Improvement of Existing Traits by Crop Genome Editing

One of the major motivations for embarking on the insertion of transgenes in the 1980s was the hope that it would be possible for breeders to be able to swap alleles for more useful alleles to enhance existing traits. This is a "holy grail" for breeders. To put this into practice demands knowing which alleles are good and which are poor.

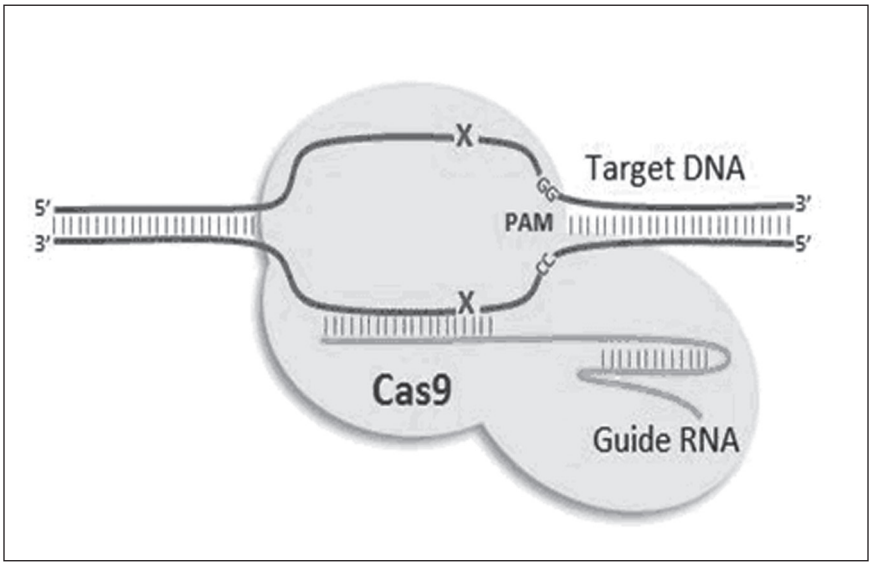
Full genome sequences combined with genetic analysis are beginning to provide the resolution within genomes required to define the effects of single alleles and therefore define which alleles should be introduced to achieve plant improvement (Cook et al, 2012). This represents excellent progress but still suffers from the need to undertake extensive backcrossing programs with the associated problems of having deleterious alleles linked to the ones being introduced. Thus these approaches, although valuable, do not meet breeders' dreams of being able to swap easily one allele for another or create new alleles at will.

In the last few years the challenge of modifying defined genes to alternative alleles has leapt ahead with the developments of truly ground breaking technologies that will change plant science and breeding (Kumar and Jain, 2014; Voytas, 2013; Ran, 2014; Doudna and Charpentier, 2014). There are various

versions but all are based on deliberate cleavage of chromosomes at specific target sites and then modification of the DNA during repair of the broken sites. The different techniques all enable gene-specific "editing". In simplest form they enable mutations to be introduced into any gene to knock out its function, create a modified protein product or vary the expression of the product. All create genetic diversity with precision and good efficiency.

So-called Zinc Fingers Nucleases (Voytas, 2013) were the first widely used gene editing tools and EXZACT™ Precision Technology is available in commercial form (Rudgers and Sastry-Dent, 2014). These are proteins built up of two units. The proteins are designed to target and bind to a unique 18-24 bp sequence with a 5-6 bp gap between them and the associated nuclease then cleaves the DNA between the protein fingers. A drawback to their widespread use was the difficulty in creating designs to target uniquely the genes to be modified. The discovery of transcription activator-like effectors, TALEs in *Xanthamonas* bacteria, led to these being fused to an endonuclease to generate TALENS (Voytas, 2013). These are much easier to design to recognize a given DNA sequence where cleavage was desired. They therefore became the preferred approach. However, now the preferred method of cleaving DNA and modifying a gene sequence is the CRISPR/cas9 system emanating from Clustered Regularly Interspaced Short Palindromic Repeats in bacteria and DNA cleavage by the cas9 nuclease (Figure 2; Doudna and Charpentier, 2014; Ran, 2014; Kumar and Jain, 2014). The DNA nuclease is guided to the right gene by a complementary short RNA that is easily made in the laboratory from a genome sequence or in the plant from complementary DNA sequence (Svitasha et al, 2015).

Figure 2. The CRISPR/cas9 system for changing any gene sequence. The cas9 nuclease (grey-blue) is localized specifically to a given chromosomal DNA sequence by the guide RNA that contains a 20 base sequence complementary to DNA sequence to be cleaved. The cleavage sites are given by the red crosses. More details are given in Kumar and Jain (2014) and Ran (2014).



After DNA cleavage by any of these technical approaches the cell's own DNA repair activities work on the break. The repair systems, which join non homologous ends together are error prone, are found in all organisms. The errors in the repair lead to loss of nucleotides or variations in the repaired DNA sequence. This then leads to loss of the protein, generation of an altered protein or altered regulation if the DNA being repaired has regulatory functions. Additionally, and importantly, if extra copies of a modified gene are supplied at the time of cleavage (by inserting the gene into the cells at the same time as the CRISPR/cas9 molecules), then insertion of this new copy can occur by homologous recombination while repair is taking place (Svitasha et al, 2015).

Because no protein engineering is required to target the cleavage enzyme and because targeting is achieved simply through RNA/DNA base pairing, CRISPR/cas9 has emerged as the system of choice. It is much easier to use, much cheaper, much better suited to multiplex gene targeting, and for high throughput genome-wide gene editing with greater efficiencies than TALENs or Zinc Fingers (Doudna and Charpentier, 2014).

In summary, the cas9 technology can be used to efficiently promote the alteration of individual or multiple gene sequences, gene families or homologous genes in polyploids and/or delete chromosome segments of various sizes. Also, very importantly, it is straightforward to produce plants that do not contain the selectable markers that have been one of the points criticized by many in transgenic crops. Because the genetic changes are targeted, genome editing also overcomes some of the other difficulties associated with transgenes introduced at random into chromosomes, namely gene silencing due to methylation/chromatin structure variation. The CRISPR/cas9 technology has been used successfully to achieve defined changes in Arabidopsis, *Nicotiana benthamiana*, rice, wheat, corn, canola, orange and sorghum (Kumar and Jain, 2014; Svitasha et al, 2015; Li et al, 2013; Shan et al, 2013; Wang et al,2014).

The system has been deployed to activate or repress genes, so illustrating its potential to replace transgenic approaches with the same objective. In addition inactive cas9 has been fused with different effector repressor or activator domains to recruit proteins to specific genes to alter their expression. The idea of fusions between inactive cas9 with histone modifying enzymes to introduce custom changes in a plant epigenome has been proposed, an exciting additional way of modifying the epigenetic control of plant gene activities (Kumar and Jain, 2014).

Products with changes in one, two or more homologous genes can be recovered from genome editing. One of the early examples in plants of gene editing resulted in non-functional mutations in wheat at all six recessive loci determining sensitivity to a fungus, so making the plant resistant to the fungus (Wang et al, 2014). The example illustrated the power of the technique to change several loci in one experiment, something that would be essentially impossible by conventional breeding. When this does not occur then homologous mutations recovered separately can be stacked by breeding to create the full set of null alleles.

It is most important to note that the approach of using targeted nucleases to change specific genes is dependent on knowing the genomic sequences in and around genes. This was not so easily determined before the major achievements of sequencing genomes that have emerged over the past 20 years. Thus, not only was the targeting of nucleases to specific genes unknown when transgenic biology took

off in plants but also if the targeting systems had been known, the molecular genetic information to exploit them was unavailable.

A comparison of the major similarities and differences between addition of transgenes and genome editing are given in Table 1. Both approaches will continue to be used in the near future. However, major advantages to genome editing, includes the fact that, any transgene can be introduced into a plant genome at a specific locus by homologous recombination. There is every reason therefore to believe that many, if not most, defined genome changes sought in the future will not be via transgenics, as we have known them, but by the genome editing techniques, which will evolve rapidly as more experience is gained. Also, noteworthy amongst the advantages of genome editing are the absence of a selectable marker in the products, the natural gene locations are retained in the species, the process can scale to allow unlimited numbers of gene changes (Table 1), and there is a strong case for the technology not to be regulated like transgenes are. It should also be noted that most transgenics made by the research communities over the past 20 years involved changing the level of expression of an existing gene with or without other changes in the coding sequence of the gene. These sorts of changes can also be made by genome editing techniques. The CRISPR/cas9 system is being actively developed by large numbers of scientists covering all the common research organisms, including man, so many far reaching technical developments will appear rapidly (Weinstock, 2013). This adds to the conclusion that the technologies for making targeted genome edits of any kind and in many genes at the same time is assured.

Table 1. A comparison between features of making genetic changes by adding transgenes versus genome editing.

Transgenes	Genome Edits
Any gene foreign or not	Any gene, foreign or not
Up or down regulate any gene	Up or down regulate any gene
Can add multiple genes simultaneously	Can edit many genes simultaneously
Endogenous alleles remain	Endogenous alleles altered
Chromosomal position not selected	Chromosome position precisely targeted
Selectable marker usually retained	No selectable marker retained
Gene stacking difficult	Stacking modifications easy
Genes easy to select/track	Stacks more difficult to select/track
Homozygous only in two or more generations	Homozygous in one generation
Disturbs standard genetics	Retains standard genetics
Does not scale	Scales readily

Endophytes as Sources of Additional Genomes to Modify Traits.

Millions of microbes, comprising many taxa, families and strains live within every plant in apparent mutualistic harmony as endophytes (Hallman et al, 1997; Turner, James and Poole, 2013; Porras-Alfaro

and Bayman, 2011; Gaiero et al 2013). Plant traits are determined in part by interactions between plant-endophyte and endophyte-endophyte genomes. Thus, these endophytes need to be considered as part of the functional plant genome, a concept new to plant breeding programs today. The microbes associated with plants are known to aid germination, facilitate uptake of key metabolites from the soil, provide plant hormones to modulate plant development, to suppress pathogens, provide additional tolerance to stresses of many kinds and many other benefits. From surveys of the literature, it is probably sound to assume that critical traits in all plants can be modified by changing the composition of the endogenous endophyte communities even in elite cultivars. Thus, much useful genetic novelty for plants is housed within different combinations/communities of plant microbiomes. It will be complex to sort out the genetics of consortia of microbes inside crops. The metagenomics studies on large populations of endophytes in plants has begun (Sessitsch et al, 2012; Lundberg et al, 2012) but will grow tremendously over the coming years, given that development of the molecular biology of microbes is tractable. Many of the microbes cannot be cultured on defined media and may not have free living forms. Others are readily cultured. It is facile to get a plant to take up bacteria and fungi providing that they are compatible with the plant and microbial biology established through evolution and selection. Thus, large discovery projects to find strains that provide new or enhanced traits to our crop plants coupled with the means of adding them to seeds, soils or other ways are underway to provide a new and exciting addition to plant breeding. Many of the principles involved are well understood from agricultural application of rhizobia to enhance nitrogen fixation. Microbiomes may turn out to be large sources of novel genetic information available to breeders and their deployment should not suffer from many of the issues associated with targeted genetic changes to plant chromosomes. The fact that they can be added to elite cultivars and do not recombine with plant genomes means that this genetic variation can be exploited more easily. The applications may be very suitable for developing countries with poorer soils to adopt relatively rapidly and I would not be surprised if new industries based on microbes became sources of such innovations.

Agendas for the Next 20 years

Discovering the Genetic Changes to Exploit

All forms of targeted genetic modification require knowledge of DNA sequences and desirable genes that can promote trait improvements. In the 1980s, the technologies to create transgenic plants came ahead of knowing which (plant) genes were worth introducing into crops for commercial gain. Indeed, at that time, most plant genes were unknown because mass sequencing had not been initiated. Similarly, the development of genome editing technologies are somewhat ahead of knowing a large catalog of genes which need to be inactivated, modified in novel ways or replaced with new alleles to make major crop yield gains. However, the explosion of knowledge during the past 20 years of genes, their context in chromosomes, their DNA sequences, variants in different cultivars and strains, operating networks of genes, RNAs and proteins underlying traits have created a platform from which to better identify and deploy targeted gene changes. It still remains a challenge however to discover the changes that can meet commercial criteria. In this section I emphasize the value of finding the genetic basis of trait improvements via "Genome Wide Association Studies", "Genomic Selection", Quantitative Trait Loci, QTL, gene knockout analyses (Huang and Han, 2014; Mammadov et al., 2012; Cook et al, 2012) as well as in microbiome genomes. The value of continuing to find valuable genetic variants has been greatly enhanced now that it is possible to introduce them directly into elite cultivars singly and in

combinations. Discovery programs to find and evaluate relevant trait-changing genes must remain a focus of crop plant science.

This review is focused on transgenes, genome editing and microbes. Readers should assume that such innovations will be superimposed on routine plant breeding procedures of crossing and progeny selection to increase the rate of gain in yield.

Finding Commercially Relevant Transgenes

Discovery programs to find transgenes that can enhance major traits have been ongoing in public and private industries for over 25 years. In some cases, the trait improvements have been sought using genes from non-plant sources such as the insect resistance genes from *Bacillus thuringiensis*. In other cases, it has been most common to design a transgene that changes the amounts of expression of an existing gene, insert the construct into a plant and screen for changes in a trait. A large catalog of such gene-trait associations has been built up, especially using Arabidopsis and rice as model species. This catalog should be frequently scrutinized for potential sources of transgenes to modify traits as new crop species become priorities for transgenesis. This can include the many developing country crops, especially those that have not received intense selection for all the critical traits. From all the ongoing work, some new transgenes can be expected to become deregulated and widely adopted in the coming decades.

As resistance to common herbicides has emerged in weeds and resistance to Bt peptides accumulated in common insect populations, alternative transgenes have been actively sought by many groups, industrial and public. Farmers know the benefits of such transgenes and will demand them and companies have found ways to profit from them and the associated IP. New herbicide and insect resistance genes will therefore be introduced and create new opportunities for licensing and deployment either as single transgenes or more likely stacked together and with other genes (James, 2014).

Numerous transgenes conferring resistance against specific fungal and bacterial diseases are already known and surely some of these will become preferred additions to crop germplasm given the huge cost of managing diseases (Dangl et al, 2013; Jones et al 2014). These would be extremely valuable for developing countries, reducing costs and providing better yield stabilities. Amongst the most exciting research foci are searches for genes that enhance yields by more efficient photosynthesis, nitrogen use efficiency, the control of unwanted respiration and control of abiotic stresses to support a more intensive agriculture. Other transgenes that improve nutrition will emerge and could be useful for communities where diets are severely compromised. Surely, Golden Rice (goldenrice.org), containing adequate levels of provitamin A in its grain will be finally deregulated and licensed for deployment and human consumption. In 2012, the World Health Organization reported that about 250 million preschool children are affected by vitamin A deficiency, and that providing those children with provitamin A could prevent about a third of all under-five deaths, which amounts to up to 2.7 million children that could be saved from dying unnecessarily. As emphasized elsewhere, what traits emerge as deployable will depend on the value of the trait, costs, regulations and consumer acceptance, size of market, profitability, public and market acceptance and export potential. All these are critical and need to be assessed better by public as well as industrial companies.

Some of the transgenes in advanced trials today are listed in the Full Brief (Appendix 7), together with their crop and source. They include new stacks of herbicide and insect resistance genes, nutritional enhancements, nematode resistance, Asian soybean rust resistance, drought tolerance, nitrogen use efficiency and other stress tolerances and various disease resistances. They are the outputs from a very large volume of research in public and private organizations but are being trialed by very few organizations.

Finding Genes to Partially or Completely Inactivate by Genome Editing

Large catalogs of knockout mutants have been established in Arabidopsis (The Arabidopsis Information Resource, TAIR) and rice and screened for many traits. Many favorable gene-trait associations have been established. This catalog becomes additionally useful now that genome editing is established because in their simplest forms genome editing technologies provide the means of creating targeted knockout mutations in any gene. Because such model species are not routinely relevant to crops, new approaches have emerged to discover the phenotypic effects of knockout mutations in any gene. Some examples of where elimination of crop genes would be useful include removal of ricin toxins from castor bean, anti-nutritionals such as trypsin inhibitors from soybean, allergenic proteins from nuts and cereals and removal of the pathway that creates bruising discoloration in fruits (Voytas, 2013). Many others exist, but much needs to be discovered affecting more complex traits. To achieve this, TILLING (Targeting Induced Local Lesions in Genomes) technology was devised (McCallum et al, 2000; Comai et al 2004). It allows directed identification of mutations in any specific gene. It was introduced in 2000 and has since been used to find mutant genes in corn, wheat, rice, soybean, tomato, sorghum and lettuce. The method combines heavy mutagenesis with a chemical mutagen such as ethyl methanesulfonate (EMS) to create mutations in most genes with a sensitive DNA screening-technique that identifies the mutations in any target gene based on the mismatches between the mutant and non-mutant DNAs made and separated in vitro (Comai et al, 2004). The approach enables genotypes to be found that have a mutation in any known gene. This can also now be achieved one or a few genes at a time by creating knockouts of the gene of interest using gene editing approaches. The resulting plants can then be examined for the phenotypic effects of the mutation. Alternatively, starting with plants that have a desired phenotype, it is possible to find the mutant gene responsible. This is particularly useful in a breeding context. An updated system has been set up in wheat (Henry et al, 2014) in which lines of wheat have been similarly created carrying such a huge number of EMS mutations that statistically a mutation is likely in every gene. Hybridization chips have been made that carry every gene in wheat based on near-complete DNA sequencing. When DNA from the heavily mutated plant is hybridized to the chip it is possible to find which genes have been mutated. These innovations could not have been done rapidly for any gene without the full set of DNA sequences or the advanced chip technologies to find the mutated genes. Thus the sequencing of complete genomes and chip technologies built up over the past decade have revolutionized the ability to build trait-mutation associations. Now that genome editing technologies are available catalogs of trait-mutation associations will be increasingly valuable.

Finding Useful Alleles by Molecular Marker Mapping

Knowledge of desirable alleles, not necessarily carrying knockout mutations, is also emerging rapidly from detailed recombination mapping where the desirable mutations are mapped to individual

genes (Cook et al, 2012). The alleles can vary in expression, have different RNA or protein products or determine other gene regulation factors. These approaches rely on the use of high density DNA polymorphism (SNP) mapping (Mammadov et al, 2012), something that has now become routine for most crop plants. However, for the discovery of commercially important variation it may be necessary for the discoveries to be done in relevant germplasm. Where large catalogs of DNA polymorphisms are available, it is readily possible to look for associations between groups of markers and a trait improvement—so-called "Genome-Wide Association Studies" in segregating populations (Mammadov et al, 2012). From the results, "Genomic Selection" for a trait (Heffner et al, 2010) can be made on the basis of DNA polymorphisms without any knowledge of the genes involved or even the position of the DNA polymorphisms in the genome. The approach is particularly valuable where groups of particular alleles are required for trait optimization and the phenotype is difficult to measure in many environments. However, future studies on the genes around the markers will increasingly reveal the genes involved and the genetic variation to be exploited in targeted genetic changes. Surveys of recent journals show associations between defined alleles and traits (QTLs) are growing exponentially for the major crops (Cook et al, 2012). These databases of alleles and combinations of alleles that change traits will be the fountain of many genome editing approaches in plant breeding in the future. To saturate this knowledge base will take a long time. While this is a major challenge, it is a challenge that lies on the "holy grail" of plant improvement and the deployment of biotechnology for human benefit. The determining of gene-trait associations is no longer simply a matter of generating fundamental information. It is establishing the knowledge platform of where and how to introduce targeted genetic changes for plant improvement, a huge difference from the routine QTL mapping of previous decades.

As has been noted by many, now the techniques of gene mapping have been reduced to high throughput routines and with large cost reductions, it is the measurements of the traits that will limit progress. Many advanced laboratories are therefore searching easier ways of reliably and reproducibly measuring traits in the field. These will include drones carrying cameras that capture images using wavelengths that can detect variation in responses to heat, other stresses and diseases as well as canopy structures and biomass. Systems for capturing field measurements using digital web-based instruments that transfer data directly to a central server in real time for easy analysis are also being increasingly adopted. These approaches enable results to be shared across the world instantly and compared with similar experiments in other breeding programs.

Finding Microbes to Improve Traits

The microbiomes inside plants and their offspring seeds are modified in each generation by microbes taken up from the soil. Their propagation through the plant is dependent on the host plant, and microbe-microbe interactions. Thus the populations of microbial genomes are dynamic, unlike the genomes of a plant. The results of plant–microbe and microbe-microbe interactions are consequently dynamic. There are many reports that show that the effects of microbes on plants are related to the consortia of microbes present (Turner et al, 2013; Porras-Alfaro and Bayman 2011). However, there are some single bacteria and fungi that have major effects on plant growth when added to consortia in and around seeds. The *Burkholdaria phytofirmans* strain PsJN is a prominent example and appears to exert its effects across many plant species and growing conditions (Zuniga et al, 2012; Poupin, et al, 2013; Naveed et al, 2014). It promotes growth, changes in flowering time, enhances drought resistance and many other features throughout the life of the plant. Because the effects of endophytic microbes

on plant traits are likely to be dependent on host genotypes the optimal plant-microbe combinations need to be established by microbe discovery using elite cultivars. Thus, research programs involving the isolation of endophytes from soils and crops growing in various conditions that influence microbiome composition need to be developed. Testing is reasonably straightforward assuming that a relevant assay is available by which to screen large numbers of microbes. Such assays can include tests for plant growth traits, as well as abiotic and biotic stresses, given that microbiomes are well-known for being able to enhance such traits (Hallman et al, 1997; Gaiero et al, 2013; Zuniga et al, 2012; Poupin, et al, 2013; Naveed et al, 2014). Microbes suitable for routine use in agriculture also need to be stable during storage and delivery and easily applied to seeds. This is another area of important research to realize the value of plant–microbe relations.

Storing Information in Easily Searchable Databases

Defining genetic changes to create crop improvements based on plant mutations and genotypes, as described above, requires increasing access to large amounts of data. This means breeding programs will need to become much better served by bioinformatics, trained computational biologists, information capture systems, phenotyping information capture and database designers. It is not facile to create and maintain databases that are easy to use within a breeding program. However, many such databases are available to all worldwide on the web and are as accessible to small breeding programs and countries as to large countries and large breeding programs. This is a huge difference from the past for countries and crops with smaller investments in research. If plant breeding is to become much more data- driven then these databases and knowing how to use them are vital.

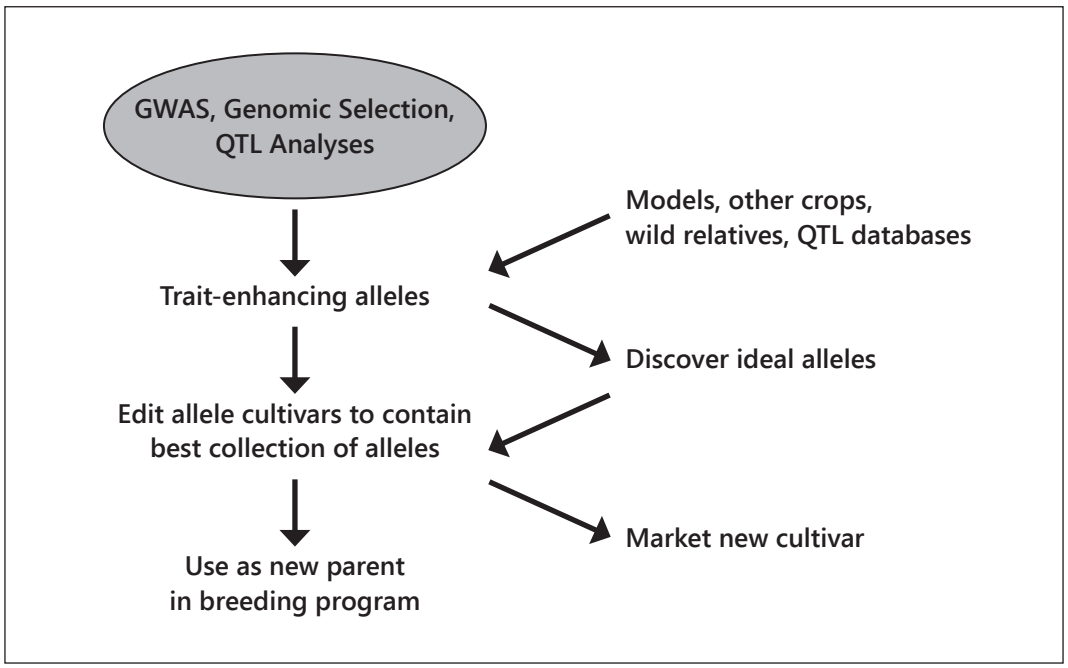
Achieving Innovative Products Based on Learnings from Transgenic Products

A new vision for plant improvement

The foregoing has illustrated that the huge developments in crop genetics, genomics and the means of changing defined genes to novel forms can move plant breeding forward throughout the world, in rich and poor countries alike. As the application of biotechnology to simple and complex traits opens up, plant breeding can increasingly be based on genomic DNA sequences (Huang et al, 2013, Cook et al, 2012) and genes of known effect and not only the trait as measured in the field. Microbes can also be deliberately incorporated to improve seeds, soils and traits.

This is a new vision for plant improvement. The breeder will no longer have to always cross in improved alleles from another elite, domesticated or wild plant with all the associated backcrossing and failures. The improved alleles can be recombined directly into existing elite lines by homologous recombination. Genes inside the regions of chromosomes that normally do not undergo crossing over in meiosis will also be able to be added into elite lines and evaluated perhaps for the first time. The numbers of such introductions over time has no limitations. Perhaps in years to come thousands of novel genes and chromosome structures will have been optimized by these approaches. I cannot emphasize this enough. If transgenes to date have been considered a luxury by some, the new vision should not be considered something small or temporary for any crop. It is very much the holistic, preferred scientific future. Figure 3 illustrates how the innovations of genetic mapping of QTL, Genome Wide Association Studies and Genomic Selection (Mammadov et al, 2012; Huang and Han, 2014, Cook et al, 2012) can

Figure 3. Integration of current gene-trait association discoveries with genome editing to produce new cultivars and better parents for crossing programs



be brought together with genome editing to create the forward-looking, integrated view of plant breeding. Multiple alleles discovered from genetic analyses that contribute to a trait improvement can be directly incorporated into an elite line, in one or more rounds of editing. A new cultivar could be the direct result and /or a better parent for the next round of conventional crossing and selection. It is a powerful vision that embraces for the first time the "holy grail" of breeders over the past 100 years. Also in this new vision of plant improvement the integration of screening genetic variation in plants in concert with populations of isolated endophytes provides an additional opportunity to produce higher yielding cultivars. Seed could be co-marketed with microbes.

Strategic, organizational, regulatory and political issues

Scientific breakthroughs have once again brought us to a new vision just as they did in 1982 when the first transgenic plants were made. What have we learnt from the development of transgenic crops that can help us with the opportunities of the next 20 years, especially for applications in developing countries? Here are some of the more important conclusions. They are mainly inspirational, organizational, regulatory, political and strategic issues because I believe these are most important, indeed essential, to get right, especially in developing countries.

A major reason that products of sound quality emerged so rapidly in the 1990s is because leaders created the right vision, comprehensive strategies to fulfill the vision and invested time and resources to overcome most of the roadblocks. They took risks because the prize was big. It was a few companies, small and large, who created and delivered the products, not public breeding programs serving the rich or poor, nor the CGIAR. To equip themselves with the right skills, the large companies reoriented

themselves internally, bought smaller companies and created strategic alliances thereby repositioning themselves to create and bring such products to market. They competed and protected investments by IP filings and opened up new issues at patent offices. They pushed for new regulations to be made so that they could sell officially deregulated products, helped establish the safety criteria that should be associated with such products and delivered the necessary documentation. In summary, a committed, comprehensive institutional approach was necessary to bring new kinds of products to market.

Yet in spite of the huge success for millions of rich and poor farmers and societies many of the opportunities created by the innovations have been lost due to:

1. Regulations being too costly to implement and/or wrongly based on the way the products were made rather than the products themselves
2. Opposition against the technology from the Green Movements and the organic lobbies because they saw opportunities to increase and/or protect their own businesses
3. Lack of leadership of politicians around the world to favor science-based regulations
4. Lack of harmony in the regulations between countries and continents
5. Europe failing to adopt science-based regulations and being a poor advocate, thereby influencing other countries not to adopt the products
6. Reduced investment into the research and application because of public concerns

As a consequence many more have gone hungry or remained weaker and poorer, with many additional lives lost in developing countries. Plant science has been vilified in many places. Innovations into food and plant breeding have been reduced or stopped, including in Europe, because of reduced investments into approaches that embraced the new technologies. In many places, societies have become mobilized against genetic innovations in breeding.

So what should be done to enable genetic innovations to benefit societies in the future, based on these experiences of bringing transgenes into agriculture over the past 20 years? I believe that the processes of genome editing and all the developments that will occur based on these approaches are so fundamental to future plant breeding that they must not be pushed aside as some fancy technique that scientists enjoy playing with. Transgenes, genome editing and beneficial microbes are so important that they need to be embedded in a new, agreed vision for plant improvement, focused on meeting the goals of "food for all" and based on the widely held beliefs of a moral imperative. The United Nations, Food and Agriculture Organization, OECD and the like, need to take the lead. Given all the push backs against transgenic crops that have seriously wounded plant science and plant breeding worldwide it is really important that genome editing for plants is not debated as a technology but only in the contest of plant breeding and food and nutrition security for all. The ethical issues associated with innovations in plant breeding also need to be assessed against the background of what happens if societies fail to produce food that relieves poverty, ill health and crops do not cope with diseases and climate changes. Public and private organizations must pull together. The vision needs to be developed internationally in a harmonized way. International leaders need to embrace it based on the increased wealth, food and nutrition security, health and environmental gains that implementation will generate. Science and business plans need to be based on better product-relevant strategies, not on the way things have been done before the scientific innovations were made. "Business as usual" will fail, with major consequences for the poor, for the following reasons:

- Adequate gains will not be made to achieve food production levels to aid human health, outputs and to reduce poverty
- The best scientists and leaders will not be attracted
- The new innovations will be wasted
- The CGIAR and other leaders in plant breeding will not embrace the technology fast or deep enough—it will stay as a research tool filling journals but not stomachs and not a means of winning races to get products to marketplaces rapidly.
- Regulations will kill the implementation and prevent public sector institutions from testing innovations
- Necessary investments that would come only from a fully integrated, exciting vision will not materialize

Therefore "business as usual" should be rejected. Scientific and institutional leaders should clearly see what the new innovations mean for the short, medium and long terms and programs should be restructured and alliances optimized to deliver outputs that meet the right business criteria. The public sector institutions should adopt the focus, planning, urgency and efficiencies of the best private sector companies (Delmer, 2005). Almost no public sector organization has deregulated a transgenic product from its own discovery program in spite of a huge research base. That is shocking and not to be repeated, surely. Major national agricultural programs, especially in developing countries, and the CGIAR must shoulder responsibilities for establishing the vision and viable strategies in ways that they failed to do with transgenics. Strategic collaborations will be essential. The private sector should be encouraged to feed the world alone and in public-private sector partnerships. Planning to achieve a faster rate of gain in plant breeding must be done locally and globally for all the critical crops. Fully integrated planning should decide what products will bring the most benefits to customers and national and international societies. Without national or global plans we will continue to be in chaos like over Golden Rice where science and societies end up on different sides, everyone loses and people die unnecessarily.

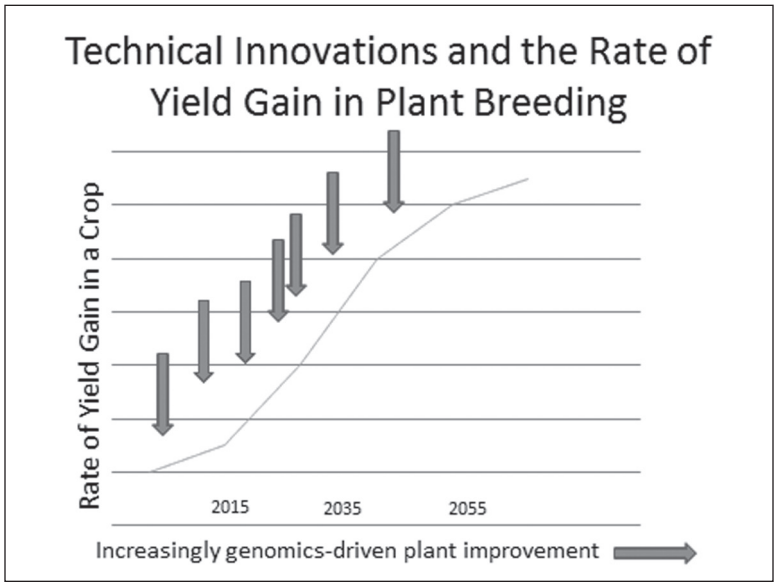
It is noteworthy that Monsanto and DuPont Pioneer are already restructuring to meet the new vision. Monsanto has put new emphasis, company purchases and strategies in place for precision seed planting, optimizing inputs and microbes with plant genotypes together with managing the large amounts of data underlying exploitation of the new sciences. DuPont Pioneer has bought the microbial enzyme company Genencor and gained an exclusive license to the intellectual property in genome editing of Vilnius University for all commercial uses, including in agriculture. It has also announced a strategic alliance with Caribou Biosciences, a company specializing in genome editing technologies, in which they will cross license IP and work together to develop the technology further. Why are these companies taking these leads? Because they know "business as usual" is not the best route to the future. Have countries reacted similarly? Has the CGIAR matched the vision and implementation strategies of the private sector to ensure equivalent services to the poorer farmers and nations? Have developing countries and the CGIAR considered the implications of the IP situation? These are very important issues relating to food security in developing countries.

How countries and the international communities respond now to the new scenarios of genome editing and use of microbiomes in plant breeding is crucial. The implications go far beyond specific instances of regulating individual products. It will determine whether or how new innovations will be

assimilated into plant improvement over many decades to come. We are at a fork in the road. This is why the issues are so important.

Consider the scenario in Figure 4. The scientific innovations being discussed here are but one of many that can and should impact plant breeding over the coming decades. These often follow an exponential curve. If societies follow the curve and benefit from a more efficient development of safe crops, then it is likely that global food and nutritional security can be realized with better local stabilities, poverty reduction and insulation from price increases. However, if we do not assimilate efficiencies in plant breeding and develop better products faster we will surely not proceed up the hypothetical of Figure 4 curve, because new innovations depend on previous ones. Societies will then have a much higher probability of having failed agricultural systems. The issues are much more crucial for the poorer countries and that is why they need to take more independent decisions and be masters of their own food destinies. The situations over transgenics in Africa and Asia surely should not be repeated with genome editing. While individuals should be able to choose what kinds of food they eat, the laws should not prevent choice and deny citizens solutions that can increase the probability of better use of land and the other resources of the planet and environmental services. In the immediate future issues relating to regulations over genome editing are critical. Much has been lost due to confusion over what transgenes, plant genetics and plant breeding entails and the resulting fears and stifling regulations. There is now perhaps a window of opportunity to work more closely with publics all over the world and create a science-based set of regulations to serve the world better, now and in the crucial decades to come (Eaglesham and Hardy 2014).

Figure 4. A hypothetical scenario relating technical innovations to the rate of yield gain in plant production. The blue arrows represent innovations coming on stream to benefit plant production. The arrows at the left could represent genomics and genome editing.



Regulations and the Future of Plant Breeding.

A recent review of international laws pertaining to plant biotechnology (Kershon and Parrot, 2013) included the following "Forecasted impact of the present regulatory systems on the future of agricultural biotechnology ranges from cloudy to devastating." Some countries are currently (re)considering their biosafety laws and regulations because they are recognized as unfit for purpose (Eaglesham and Hardy, 2014; Shearer, 2014; Schieman and Hartung, 2014, Hoffman, 2014; Kershon and Parrot, 2014). The USA and Argentina are moving to not regulate genome editing, presumably because the products cannot be distinguished from those of conventional breeding and are not made using a plant pest. Europe, however, is likely to keep them regulated because a laboratory scientist has intervened in the production of their genotype by inserting DNA into plant cells to carry out the genome editing. The Catagena Protocol on Biosafety to the Convention on biological Diversity that governs the movement of GMOs around the world is also a crucial legal instrument. It is built on the precautionary principle and allows any state to ban the import of GMOs if it believes there is inadequate evidence concerning safety. Some167 member states of the United Nations are signatories to the Protocol. These laws, as they evolve or not around the world will have the most profound effect on plant breeding, and how they can produce products for sale, export and import in all countries. One only hopes that those who are responsible for the regulations in Africa and Asia will, this time around, grasp the vision of what science can do over the coming years, as depicted in Figure 4, and the consequences of not going up the innovation curve. Genome editing, as most technologies, is neutral. It is the purpose to which they are put that counts. While there may be calls for moratoria, for good ethical reasons, on the application of genome editing to human germlines, it is likely that the reverse arguments pertain to food crops. A failure to embrace the technology to solve food, feed, fiber and fuel problems safely by plant biotechnology will surely only prolong human suffering and misery.

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The Global Economic and Environmental Impact of GM Crops

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Introduction

This paper summarizes the global economic and environmental impact[1] of genetically modified (GM) crops since their commercial introduction in 1996. It is based on two papers in the peer reviewed journal GM crops[2].

Although the first commercial GM crops were planted in 1994 (tomatoes), 1996 was the first year in which a significant area of crops containing GM traits was planted (1.66 million hectares). Since then, there has been a significant increase in plantings and by 2014, the global planted area had reached 181.5 million hectares.

GM traits have largely been adopted in four main crops; canola, corn, cotton and soybeans and in 2013, GM traits accounted for 46% of the global plantings to these four crops. In addition, small areas of GM sugar beet (adopted in the USA and Canada since 2008), papaya (in the USA since 1999 and China since 2008) and squash (in the USA since 2004) have been planted.

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The main traits so far commercialised have essentially been derived from bacteria and convey:

- Tolerance to specific herbicides (notably to glyphosate and to glufosinate) in corn, cotton, canola and soybeans[3]. The technology allows for the 'over the top' spraying of crops with the trait of these specific broad-spectrum herbicides, that target both grass and broad-leaved weeds;
- Resistance to specific insect pests of corn and cotton. This technology offers farmers resistance in the plants to major pests such as corn borers and rootworm (eg, *Ostrinia nubilalis*, *Diabrotica sp*) in corn and bollworm/budworm (*Heliothis sp*) in cotton.

Farm Income and Production Impacts

The key impact from a farm income perspective has been one of better returns for farmers, especially those in developing countries. The net economic benefit at the farm level in 2013 was $20.5 billion,

1 Farm income and production effects and environmental impacts associated with changes in herbicide and insecticide use and greenhouse gas emissions
2 GM Crops journal at www.tandfonline.com/loi/kgmc20 - GM Crops 6:1, p 1-11 Jan-March 2015 (economic impact paper) and vol 6.2, 1-11, April-June 2015 forthcoming for environmental impact paper
3 Also sugar beet in North America

equal to an average increase in income of $122/hectare. For the 18 year period (1996-2013), the global farm income gain has been $133.5 billion. Interestingly, this total farm income gain (of $133.5 billion) was divided equally between farmers in developing and developed countries.

This positive impact on farm incomes has been driven by a combination of higher yielding crops, facilitation of additional cropping in the same season (eg, of soybeans) and a reduction in costs of production for some farmers. The insect resistant (IR) technology used in cotton and corn has consistently delivered yield gains from reduced pest damage. The average yield gains over the 1996-2013 period across all users of this technology has been +11.7% for insect resistant corn and +17% for insect resistant cotton. 2013 also saw the first IR soybeans grown commercially in South America, where farmers have seen an average of +10% yield improvements. The herbicide tolerant (HT) technology used in soybeans and canola has also contributed to increased production in some countries; by helping farmers in Argentina grow a crop of soybeans after wheat in the same growing season[4], through higher yields and improved weed control. The highest yield gains were obtained by farmers in developing countries, many of which are resource-poor and farm small plots of land. Where adoption of the technology has contributed to lowering overall costs of production, this has mainly been by reducing expenditure on herbicides, insecticides and fuel used for spraying and ploughing, with these savings typically greatest in developed countries.

The adoption of seed containing biotech traits has proved to be a sound investment for the vast majority of farmers around the world who have been given the choice of using this technology. The cost farmers paid for accessing crop biotechnology in 2013 ($6.8 billion[5, 6] payable to the seed supply chain) was equal to 25% of the total gains (a total of $27.3 billion inclusive of the $20.5 billion income gains). Globally, farmers received an average of $4.04 for each dollar invested in GM crop seeds. Farmers in developing countries received $4.22 for each dollar invested in GM crop seeds in 2013 (the cost being equal to 24% of total technology gains), while farmers in developed countries received $3.88 for each dollar invested in GM crop seed (the cost being equal to 26% of the total technology gains). The higher share of total technology gains realised by farmers in developing countries relative to farmers in developed countries mainly reflects weaker provision and enforcement of intellectual property rights coupled with higher average levels of benefits in developing countries.

The higher yields and facilitation of second cropping of soybeans in a season in parts of South America has also been responsible for additional global production of 138 million tonnes of soybeans, 274 million tonnes of corn, an extra 21.7 million tonnes of cotton lint and 8 million additional tonnes of canola between 1996 and 2013. This has, therefore contributed to global food security and reduced pressure on scarce land resources as the technology is allowing farmers to grow more without using additional land. If crop biotechnology had not been available to the (18 million) farmers using the technology in 2013, maintaining global production levels at the 2013 levels would have required

4 By facilitating the adoption of no tillage production systems this effectively shortens the time between planting and harvest of a crop

5 The cost of the technology accrues to the seed supply chain including sellers of seed to farmers, seed multipliers, plant breeders, distributors and the GM technology providers

6 A typical 'equivalent' cost of technology share for non GM forms of production (eg, for new seed or forms of crop protection) is 30%-40%

additional plantings of 5.8 million ha of soybeans, 8.3 million ha of corn, 3.5 million ha of cotton and 0.5 million ha of canola. This total area requirement is equivalent to 11% of the arable land in the US, or 29% of the arable land in Brazil or 32% of the cereal area in the EU (28).

Environmental Improvements

Crop biotechnology has contributed to significantly reducing the release of greenhouse gas emissions from agricultural practices. This results from less fuel use and additional soil carbon storage from reduced tillage with GM crops. In 2013, this was equivalent to removing 28 billion kg of carbon dioxide from the atmosphere or equal to removing 12.4 million cars from the road for one year.

In terms of impacts associated with changes in herbicide and insecticide use with GM crops, there has been a net reduction in pesticide spraying (1996-2013) of 550 million kg (-8.6%). This is equal to the total amount of pesticide active ingredient applied to arable crops in the EU 27 for two crop years. As a result, this has decreased the environmental impact associated with herbicide and insecticide use on the area planted to biotech crops by 19%[7].

Concluding Comments

Crop biotechnology has, to date, resulted in improved productivity and profitability for the 18 million adopting farmers who have applied the technology to over 181 million hectares in 2014.

The economic and environmental gains derive from a combination of technical advances and the role of the technology in the facilitation and evolution of more cost effective and environmentally friendly farming practices. More specifically:

- The gains from the GM IR traits have mostly been delivered directly from the technology (yield improvements, reduced production risk and decreased use of insecticides);
- The gains from GM HT traits have come from a combination of direct benefits (mostly cost reductions to the farmer) and the facilitation of changes in farming systems. The technology, especially in soybeans has played an important role in enabling farmers to capitalise on the availability of a low cost, broad-spectrum herbicide (glyphosate) and, in turn, facilitated the move away from conventional to low/no-tillage production systems in both North and South America. This change in production system has made additional positive economic contributions to farmers (and the wider economy) and delivered important environmental benefits, notably reduced levels of GHG emissions (from reduced tractor fuel use and additional soil carbon sequestration);
- Both IR and HT traits have made important contributions to increasing world production levels of soybeans, corn, cotton and canola.

In relation to HT crops, over reliance on the use of glyphosate and the lack of crop and herbicide rotation by some farmers, in some regions, has contributed to the development of weed resistance. In order to address this problem and maintain good levels of weed control, farmers have increasingly

7 As measured by the Environmental Impact Quotient (EIQ) indicator (developed at Cornell University)

adopted a mix of reactive and proactive weed management strategies incorporating a mix of herbicides and other HT crops (in other words using other herbicides with glyphosate rather than solely relying on glyphosate or using HT crops which are tolerant to other herbicides, such as glufosinate). This has added cost to the GM HT production systems compared to several years ago, although relative to the conventional alternative, the GM HT technology continued to offer important economic benefits in 2013.

Overall, there is a considerable body of evidence, in peer reviewed literature, and summarised in this paper, that quantifies the positive economic and environmental impacts of crop biotechnology. The analysis in this paper therefore provides insights into the reasons why so many farmers around the world have adopted and continue to use the technology. Readers are encouraged to read the peer reviewed papers cited, and the many others who have published on this subject (and listed in the references section of the cited papers) and to draw their own conclusions.

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Global Impact of GM Crops, 1996-2015

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Genetically modified (GM) crops have now been grown commercially for 20 years in many parts of the world, including in developed and developing countries and by large and small farms. Most of the GM technologies used so far involve herbicide tolerance (HT) and insect resistance (IR) in crops like soybean, maize, cotton, canola, and a few others. In spite of the rapid and widespread adoption of GM crops by farmers in almost all parts of the world, public attitudes remain skeptical, especially in Europe but also elsewhere. Many believe that GM crops are risky for human health and the environment and bring no benefits for farmers and consumers. Numerous scientific studies have shown that GM crops are not more dangerous than conventionally bred crops. There is also a broad body of literature demonstrating significant economic and environmental benefits. The problem is that this scientific evidence has not really entered the public debate. Anti-biotech groups were much more successful in influencing public opinions through denying scientific results and spreading their own unsubstantiated narratives about risks and negative social impacts (Qaim, 2015).

To be sure, results of scientific studies about GM crop impacts, which were carried out in different countries and with different data and methodologies, vary significantly. Depending on many factors, some studies show higher yield effects, while others show lower or no yield effects at all. Some point at reductions in the use of pesticides, while others point at increases in the use of pesticides and other chemical inputs. Hence, individual studies should not be generalized too widely. Results always depend on the particular context. But what can we learn from looking at the existing body of literature about GM crop impacts more systematically?

A meta-analysis can help to draw some broader conclusions about mean effects at the global level, and also about reasons for deviations in particular situations. A recent meta-analysis, which we carried out, presents a clear picture: combining results from all scientific studies that compared the performance of GM and conventional crops reveals that GM technology has increased crop yields by 22% and reduced chemical pesticide use by 37% on average (Table 1). GM seeds are usually more expensive than conventional seeds, but the additional seed costs are compensated through savings in chemical pest control and higher revenues from sales. Average profit gains for GM crop-adopting farmers are 68% (Klümper and Qaim 2014).

A breakdown of GM crop impacts by type of technology reveals a few notable differences (Table 1). While significant reductions in pesticide costs are observed for both HT and IR crops, only IR crops lead to a consistent reduction in pesticide quantity (pesticides, as defined here, include insecticides, herbicides, fungicides, and all other chemical pest control agents). Such disparities are expected, because the two technologies are quite different. IR crops protect themselves against certain insect pests, so that spraying insecticides can be reduced. HT crops, on the other hand, are not protected against pests but against broad-spectrum chemical herbicides (mostly glyphosate), use of which facilitate weed control. While HT crops have reduced herbicide quantity in some situations, they have contributed to increases in the use of broad-spectrum herbicides elsewhere. The savings in pesticide costs for HT crops in spite of higher

Table 1. Mean impacts of GM crop adoption in % (meta-analysis results)

Outcome Variable	All GM Crops	Insect-resistant crops	Herbicide-tolerant crops
Yield	21.57***	24.85***	9.29**
Pesticide quantity	-36.93***	-41.67***	2.43
Pesticide cost	-39.15***	-43.43***	-25.29***
Total production cost	3.25	5.24**	-6.83
Farmer profit	68.21***	68.78***	64.29

, * statistically significant at 5% and 1% level, respectively.
Source: Klümper and Qaim (2014).

quantities can be explained by the fact that broad-spectrum herbicides are often much cheaper than the selective herbicides that were used before. Average yield effects are also higher for IR than for HT crops.

In the meta-analysis, we also differentiated between impacts in different countries, finding that farmers in developing countries benefit much more from GM crop adoption than their colleagues in developed countries. The reasons for significantly higher average yield and farmer profit gains in developing countries are twofold. First, farmers operating in tropical and subtropical climates often suffer from more considerable pest damage that can be reduced through GM crop adoption. Hence, effective yield gains tend to be higher than for farmers operating in temperate zones. Second, most GM crops are not patented in developing countries, so that GM seed prices are lower than in developed countries, where patent protection is much more common (Klümper and Qaim 2014).

Aggregating the economic effects from micro-level impact studies to the total area currently cultivated with HT and IR GM crops results in global farmer benefits of over 20 billion US dollars per year (or more than 150 billion US dollars when using the cumulated adoption rates over the last 20 years). In addition, consumers benefit through lower prices that they pay for food and other agricultural commodities. A new technology with gains in farm productivity reduces market prices to levels lower than they would be without the technology. Hence, consumers also gain from productivity-increasing technology.

GM crops have also contributed to positive environmental effects. Reductions in the use of chemical pesticides have led to benefits for biodiversity and ecosystem functions. As mentioned, pesticide reductions are particularly relevant for IR crops. HT crops have facilitated the adoption of reduced-tillage practices, thus reducing erosion problems and greenhouse gas emissions from the soil. Finally, without the productivity gains from GM crops, around 25 million hectares of additional farmland would have to be cultivated globally, in order to maintain current agricultural production levels. As is well known, farmland expansion into natural habitats is an important contributing factor to biodiversity loss and climate change.

GM crop adoption in developing countries has also led to social benefits. Especially, IR cotton is widely grown by smallholder farmers in countries like China, India, Pakistan, Burkina Faso, and South Africa. With my research group we have studied the situation in India over many years. More than 90% of the cotton growers in India have switched to GM technology. Higher yields and profits have contributed to significant welfare gains in smallholder households. Our estimates with panel data show that the

adoption of IR cotton has raised household living standards by 18% on average. Higher family incomes have also caused improvements in dietary quality and nutrition. The data suggest that GM technology adoption has reduced food insecurity among Indian cotton growers by 15-20% (Qaim and Kouser 2013). Beyond the cotton growers themselves, other rural households benefit from growth in the cotton sector through additional employment. This is particularly relevant for poor landless families. Two-thirds of all rural income gains from GM cotton adoption in India accrue to poor people with incomes of less than 2 dollars a day. Similar to these results from India, GM cotton has contributed to poverty reduction and other social benefits in the small farm sectors of China and Pakistan. These positive effects of GM cotton have increased over time.

This evidence on impacts from around the world suggests that GM crops promote sustainable development in terms of all three sustainability dimensions, that is, economically, socially, and environmentally. With HT and IR traits introduced in only a handful of crops, the range of commercialized GM technologies is still limited. The main reasons for this narrow focus are public resistance against GM crops and overregulation, leading to long and unpredictable processes for technology approval. Many other promising GM technologies have been developed and successfully tested in various countries, so far without getting the commercial go-ahead. Cases in point are GM traits such as fungal and virus resistance, drought and salt tolerance, higher nitrogen use efficiency, and higher micronutrient contents in food crops such as rice, wheat, sorghum, cassava, potato, banana, and various vegetables. The potentials of such technologies to contribute to poverty reduction and food security in developing countries are large (Qaim 2015).

This does not mean that GM crops cannot also lead to undesirable effects under particular conditions. Every technology may cause certain problems if misused or not managed properly. For instance, GM crops have contributed to a rising concentration in biotech and seed industries. More efficient regulation could help to reduce or avoid issues of market power. Several weed species in North and South America have developed resistance to glyphosate, because the same HT crops were grown year after year. Reducing resistance development requires improved agronomy, especially better crop and herbicide rotations. These problems need to be addressed, but they hardly justify banning GM crops, as some anti-biotech groups call for. For comparison, we also observe market concentration in software and internet-based industries, without banning computers and the worldwide web. And we also observe the development of resistance to antibiotics in various human pathogens, without broadly prohibiting the use of antibiotics from all medical applications. In organic agriculture, the use of copper as a non-synthetic agent to control fungal diseases can cause serious environmental problems, without calls for banning organic farming practices altogether. When we are serious about sustainable development, we need to be more open-minded and stop judging technologies with very different standards.

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