Chapter 16
Transgenic Crops: Status, Potential, and Challenges

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Abstract Since the commercialization of the first GM crop in mid-1990s, agricultural biotechnology has enjoyed remarkable growth in product development, commercialization, and global adoption. Areas planted with GM crops in the last 20 years have increased more than 100-fold, making crop biotechnology one of the fastest adopted agricultural technologies. World population is 7.3 billion today and is expected to reach 9.5 billion in 2050. To sustain this ever-growing population, we will be required to produce 70% more food than what we produce today (Headrick Res Technol Manag 59:3, 2016). Agricultural biotechnology has been and will continue to play an important role in meeting the challenge. This chapter covers a brief overview of agricultural biotechnology, starting with the development of *Agrobacterium* and gene gun-mediated transformation technologies. Input, output, and agronomic biotechnology traits are discussed with emphasis on the major crops being cultivated around the world. A brief overview of the next generation of precision transformation technologies is given with emphasis on site-specific nucleases, i.e., meganucleases, ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases), and CRISPR/Cas (clustered regulatory interspaced short palindromic repeats/CRISPR-associated). Specific examples of the use of these technologies resulting in commercially important traits are discussed. Lastly, challenges associated with further adoption of GM crops are discussed with an emphasis on risk assessment of GM crops and food, perception of risk and benefits, regulation of GM products and policy development, international trade concerns and policy decisions, and social concerns.

Keywords Transgenic crops · Transgenic traits · *Agrobacterium* · Gene gun · Risk assessment · GM regulation

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16.1 Genetic Transformation - A Brief Historic Overview

Genetic transformation is a well-established and widely used technology today with applications ranging from functional genomics to the introduction of desired traits in the plants. The journey of the development of this technology started with researchers trying to understand the mechanism of crown gall disease in plants. In their quest of knowledge, researchers discovered something very surprising which was inter-kingdom transfer of genes by the *Agrobacterium* into woody plants. This discovery became the stepping stone into the field of genome manipulation of plants that has changed the face of agriculture today.

Crown gall is a disease that forms tumors at the crown of woody plants. The disease affects the vascular system and hence interferes with normal transport of water and nutrients. Severe infections lead to death of the plant and can result in economic losses. The disease has been known for a long time; however it was in 1907 when Smith and coworkers while working on the crown gall of marguerite established *Agrobacterium* to be the causative agent of the disease (Smith et al. 1907). Initially it was thought that the irritation caused or the chemicals released by the bacterium led to the formation and growth of tumors. However a study reported by White and Braun (1942) contradicted these hypotheses by showing that although the bacteria led to the initiation of the gall, the gall has the potential to grow further even if the *Agrobacterium* is no longer present. Therefore, a new hypothesis was formed that the bacterium transforms "something" into the plant cells that continues functioning independent of the bacterium. Braun (1958) called this "something" as tumor-inducing principle (TIP); however the nature of TIP was still a mystery. Another important milestone in the field was the report of Menage and Morel (1964) which showed that plants infected with *Agrobacterium* produce opines which are used by *Agrobacterium* as a source of nitrogen and carbon. This indicated that *Agrobacterium* transferred TIP into the plant for its own advantage. Later it was established that the *Agrobacterium* has an extra chromosomal plasmid, named tumor-inducing (Ti) plasmid that provides the bacterium its tumor-forming ability (Zaenen et al. 1974). Mary-Dell Chilton et al. (1977) were the first to demonstrate that a small portion of Ti plasmid called T-DNA was transferred into the host plant genome and was responsible for producing the disease. The discovery revolutionized this field, and many research laboratories around the world further characterized the nature of this T-DNA and established it as a tool for genetic transformation.

Though *Agrobacterium*-mediated transformation has achieved widespread use, the technology has its own limitations. *Agrobacterium* does not have a wide host range to facilitate gene transfer to every genotype of crops. It was further complicated by the lack of tissue culture and regeneration protocols for a wide range of crop species. Hence there was a need to find alternate methods of transformation. John Sanford, a plant breeder at Cornell University, wanted to develop an easier method of gene transfer since it takes years to do so using cross pollination. Along with his colleagues, he developed a crude BB gun-based particle acceleration technique that was used to bombard onion cells (Sanford et al. 1991). This early version of a gene gun used 0.22 caliber bullets for acceleration of tiny, DNA-coated particles

(Klein et al. 1987). The plastic bullets used in this process were propelled toward the target tissue and were stopped by a stopping screen which had a hole in the middle. The plastic bullet stopped, and the DNA-coated particles passed through the hole, hit the target tissue, and released the DNA inside the cells. Initial experiments were crude, resulting in splashing and dying of onion cells. With some optimizations they were able to show transient gene expression in the cells. However, the use of this version of the gene gun was very cumbersome. It required frequent cleaning and used gunpowder to propel the particles. Therefore efforts were initiated to improve the technology. BioRad developed a new version of the gun called PDS-1000/He that replaced the gunpowder discharge with the blast of the inert gas helium. Further, either to reduce the cost of this device or to develop proprietary technologies, other forms of particle acceleration devices were invented, e.g., Accel gene gun (McCabe and Christou 1993), particle inflow gun (PIG) (Finer et al. 1992), etc. However despite all these innovations for the development of different types of gene guns, the earlier version of the gene gun developed by BioRad that used compressed helium to generate a blast remained most popular.

In the meantime, some other gene delivery methods were also established. However due to inherent constraints of gene transfer or due to limiting plant regeneration potential of the explants leading to poor transformation frequencies, those methods did not become very popular. Overall, *Agrobacterium*-mediated and gene gun-mediated gene transformation methods remained most commonly used.

The enablement of these gene delivery technologies coincided well with the advent of other related technologies. In vitro culture and regeneration protocols were being established for a large number of crops. Paul Berg produced the first recombinant DNA when he combined SV40 monkey virus and ʎ virus called ʎ bacteriophage (Jackson et al. 1972). This set the stage for recombinant DNA technology to join different pieces of DNA together to get desired plant gene expression cassettes. (Mullis et al. 1986) invented polymerase chain reaction (PCR) that enabled routine and easy amplification of DNA fragments in the lab. All these technologies coming together led to the dawn of the field of genetic transformation. With this continued effort, it was in 1983 when the first transgenic plant was successfully regenerated in tobacco followed by many other crops. The first transgenic plant commercially grown was virus-resistant tobacco in 1992 in China followed by FLAVR SAVR™ tomato approved for commercial cultivation in the USA in 1994 (James 1997). Since then, global GM crop acreage has made a phenomenal increase of more than 100-fold in a span of 20 years, making crop biotechnology one of the fastest adopted agricultural technologies (ISAAA 2016).

16.2 Commercial Biotechnology-Based Traits/Crops

Since the commercialization of the first GM crop in mid-1990s, GM traits have been produced in 15 crop plants (maize, soybeans, cotton, canola, alfalfa, sugar beets, eggplant, papaya, potato, pineapple, squash, apple, plum, eucalyptus, and poplar)

(James 2015). Many more transgenic crops and traits have been tested but have not yet been commercialized (Fernandez-Cornejo et al. 2014). In 2015, 18 million farmers from 28 countries planted almost 440 million acres of GM crops (James 2015).

The growth and development of commercial GM crops over the past 20 years has been based on a number of contributing factors such as potential market size of the traited crop, discovery of genes expressing the desired traits, and public and farmer acceptability of the product. Due to the high costs of developing transgenic crops, the majority of commercial products today are targeted at the largest agricultural markets: maize, soybeans, cotton, and canola in the Americas (Phillips McDougall 2011). There are several significant crops which are notably absent from GM commercialization today, in particular wheat and rice. GM traits have been developed and tested in both wheat and rice (e.g., glyphosate-tolerant wheat (Zhou et al. 2003) and Golden Rice (Ye et al. 2000)), and although they have substantial market sizes, no commercial products have made it to market yet due in part to public wariness about GM traits in crops for direct human consumption.

In addition to the commercialized GM crops, many more GM plants have been developed and field tested in the USA and elsewhere. For the years 1985 through 2013, USDA issued 17,000 release permits for testing the GM crops in field in USA (Fernandez-Cornejo et al. 2014). The four key commercial crops, maize, soybeans, cotton, and canola comprised two-thirds of the 17,000 total releases. In looking at types of traits being developed and tested, slightly more than two-thirds were for testing input traits, primarily insect-resistant(IR) and/or herbicide-tolerant (HT) traits. A snapshot of 2014 finds that there were 49 GM events being cultivated commercially and 53 more events that were in the late stages of development (Parisi et al. 2016). Of those 102 GM events, more than 75% of the traits were IR and HT.

The earliest commercial GM crop products were primarily single-trait events, meaning the product contained a single trait of interest, for example, glyphosate herbicide tolerance or lepidopteran insect resistance. Many single-trait events carry more than one gene, such as selectable markers that are used during transformation but do not confer an end-user trait in the final product, and thus are still classified as single-trait events. Increasingly, single-trait products have been combined into stacked trait products conferring two or more value-added traits to bring greater value to farmers in managing their crops. Stacked trait products are developed either by transforming more than one gene linked together in a single construct (referred to as molecular stacks) or by combining traits carried by two or more independent transformation events via breeding (referred to as breeding stacks) (Que et al. 2010). There are examples of both types of stacks among commercial products today, and nearly all GM maize, soybean, and cotton products on the market now carry more than one trait. Overall, stacked GM trait products across all crops were planted on 145 million acres in 2015, amounting to 33% of all the GM acres globally (James 2015).

For the simplicity of discussion, we have separated the types of traits into three categories: input traits, agronomic traits, and output traits. Input traits address the need for farmer inputs into the cropping system (e.g., an insect-resistant trait eliminates the need to apply insecticides in the field). Agronomic traits improve crop productivity by modifying intrinsic physiological properties of the plant (e.g.,

abiotic stress tolerance and improved yield). Output traits provide consumer-oriented benefits (e.g., enhanced nutritional quality). Some authors include herbicide, disease, and insect tolerance under the category of agronomic traits. Here we consider these traits as input traits since even though these traits improve yield performance; they do so without significant modifications to plant physiology.

16.2.1 Input Traits

The first herbicide-tolerant traits, glyphosate-tolerant Roundup Ready® from Monsanto and glufosinate-tolerant Liberty Link® from Bayer, were commercialized in the USA in the mid-late 1990s, enabling farmers to control weeds in maize, soybeans, cotton, and canola with glyphosate or glufosinate herbicides without injury to the crop plants. HT traits experienced broad farmer adoption such that by 2016, 94% of soybeans and 89% of both cotton and maize grown in the USA were genetically modified to be tolerant to one or more herbicides (USDA-ERS 2016).

In 2015, HT crops were planted on nearly 240 million acres globally with an increased value to farmers of $8 billion and a cumulative value of $63 billion for the 19 years since commercialization (1996–2014) (ISAAA 2016). However, the broad adoption of glyphosate-tolerant traits has more recently led to some weed species developing resistance to the herbicide (Heap 2014). This has created a new challenge for researchers, driving the development of a number of new herbicide-tolerant traits which began entering the market in 2016, including tolerance to dicamba (soybeans and cotton from Monsanto), 2,4-D (maize, soybeans, cotton from Dow AgroSciences), imidazolinone (soybeans from BASF and Embrapa), and HPPDs (soybeans from Bayer, MS Tech, and Syngenta). For most of these new HT traits, commercial products are stacks of multiple herbicide-tolerant traits to provide farmers with more options for managing hard-to-control weeds on their farms.

Insect-resistant traits in maize and cotton were also first commercialized in the mid-late 1990s, with the first products carrying single lepidopteran insect resistance genes derived from *Bacillus thuringiensis* (Bt). Bt is a soil bacteria that produces proteins, often referred to as Bt toxins that are toxic to specific classes of insects. A key advantage to farmers from the use of IR traits is the season long protection they provide against the target pests. With the rapid adoption of HT traits, single gene IR products quickly progressed to stacks of IR + HT traits. In the mid-2000s, Bt genes providing control of corn rootworm, a coleopteran insect pest of maize, were introduced. Following the same stacking trends, the coleopteran IR traits were rapidly converted to stacked products with lepidopteran IR and HT traits.

Depending on the geography and specific regulatory requirements, farmers planting IR crops are often required to plant a refuge, which is a portion of the crop without IR traits to serve as a refuge for insects to reproduce without selection for resistance to the IR trait (Huang et al. 2011). Refuge requirements can range from 20% to 50% of the crop area for single IR traits, with specific requirements for how the structured refuge areas are to be laid out relative to the IR field. A breakthrough

in streamlining the management of refuge fields came in the late 2000's with multiple IR mode of action stacks in maize and cotton. These stacks, often called pyramids, have two or more IR genes with different modes of action against the same key pest with the advantage of providing greater durability against the development of resistance by the insect pests (Storer et al. 2012). They also enabled advantages for farmers because with the greater durability, less refuge was required, and in many cases, it could be planted intermixed with the IR seed. For this reason, "refuge in a bag" products were swiftly adopted by farmers due to the ease in managing refuge compliance.

Commercial IR (including IR + HT) trait products were found to be very effective, even under heavy insect pressure, and thus were broadly adopted across the Americas. The percentage of maize acres in the USA that are planted to IR traits has grown from 19% in 1997 to 79% of the 90 million acres grown in 2016, while IR cotton acres have increased from 15% in 1997 to 84% of the 10 million acres grown in 2016 (USDA-ERS 2016). In Brazil, adoption has grown even faster, with IR maize introduced commercially in 2008, and by 2015 85% of the 36 million maize acres were planted to IR traits. Similarly, IR cotton in Brazil has grown to 73% of the total cotton acres since it was first introduced in 2006 (ISAAA Brazil 2017). Due to the need for some amount of refuge for IR traits, adoption rates can never reach 100%. Commercially available IR traits had been limited to maize and cotton until 2013 when IR soybean and IR eggplant (brinjal) were commercialized for farmers in Brazil and Bangladesh, respectively. Adoption of IR soybeans in Brazil has grown dramatically since launch in 2013, with nearly 40% of the 80 million soybean acres planted in 2015 devoted to IR soy. Overall, in 2014 all insect-resistant crops globally provided an increased value of $9.8 billion, with a cumulative value of $86.9 billion over the 19 years of commercialization from 1996 to 2014 (James 2015).

16.2.1.1 Major Crops with Biotechnology-Based Input Traits

Maize

The earliest commercially grown GM maize traits were single gene traits expressing either HT or IR genes separately. In 1996, Bayer launched Liberty Link® maize with tolerance to glufosinate herbicide and Mycogen Seeds introduced event 176, the first maize providing resistance to lepidopteran insect pests. One year later in 1998, Monsanto launched Roundup Ready® maize with tolerance to glyphosate as well as the first stacked IR + HT maize product, YieldGard® + Roundup Ready®. Similar IR + HT products soon followed from other GM trait developers, namely, Dow AgroSciences and DuPont Pioneer (Herculex®) and Syngenta (Agrisure®). Each of the maize trait products has slightly different expression characteristics or features depending on the expressed genes.

GM maize can be divided into three groups of products for specific markets: HT only, lepidopteran IR + HT (often referred to as aboveground IR), and lepidopteran + coleopteran IR + HT (above- and belowground IR). HT only maize is primarily

used as a refuge for IR products and in some niche markets where insect pressure is low. Lepidopteran IR + HT products target aboveground insects such as European corn borer (*Ostrinia nubilalis*), corn earworm (*Helicoverpa zea*), and fall armyworm (*Spodoptera frugiperda*), which are pests of maize globally. Lepidopteran and coleopteran IR + HT products target the aboveground pests as well as the belowground pest, corn rootworm (*Diabrotica virgifera*), which is primarily a pest in North America. Today, most of the IR products on the market are, in addition to being stacks with HT, trait pyramids with multiple modes of action targeting the key pests. Most of the current pyramid products have been achieved by creating breeding stacks that combine traits from two or more trait developers, for example, SmartStax®, developed by Monsanto and Dow AgroSciences, is a stack of four events, two from Monsanto and two from Dow AgroSciences, resulting in a product with three lepidopteran IR traits, two coleopteran IR traits, and two HT traits. Multiple IR + HT products have been developed by DuPont Pioneer (AcreMax®) and Syngenta (Agrisure®) as well, using similar stacking approaches.

GM maize was planted on 130 million or 29% of the 450 million acres of maize grown globally in 2015. The top 3 GM maize-producing countries in acres and percent adoption were the USA (82 million, 92%), Brazil (32 million acres, 89%), and Argentina (7.2 million, 70%), with 14 additional countries each producing 5 million acres or less of GM maize in 2015. Cumulative income benefits to farmers for the years 1996 to 2014 total $50.6 billion (ISAAA Crop 2017).

Soybean

The glyphosate-tolerant Roundup Ready® trait by Monsanto has been the predominant soybean trait since it was commercialized in 1996. By 2000, more than 50% of the US soybean acres had the trait, and by 2007 adoption was above 90%, where it remains today (Fernandez-Cornejo et al. 2014). This widespread, rapid adoption has been seen in nearly all of the geographies where the HT soybean has been introduced. Additional soybean traits did not arrive on the market until 2009, with a second-generation glyphosate-tolerant trait delivering improved yield over the original trait (Monsanto) and glufosinate-tolerant Liberty Link® soybeans (Bayer). In 2013, Monsanto launched the first IR soybean, Intacta™, a single Bt gene conferring resistance to lepidopteran pests and stacked with the Roundup Ready® HT trait. Key lepidopteran pests of soybean, in particular soybean looper (*Pseudoplusia includens*) and *velvetbean caterpillar* (*Anticarsia gemmatalis*), are a significant problem in South America but are not of widespread concern in the USA, and thus IR soybean has not been commercialized in the USA but has predominantly been commercialized in South America. A number of new GM traits in soybeans have been recently launched or are expected to be on the market soon, which include several new HT traits: dicamba (Monsanto), 2,4-D (Dow AgroSciences), imidazolinone (BASF and Embrapa), and HPPDs (Bayer, MS Tech, and Syngenta).

Driven by the extensive adoption of Roundup Ready® soybeans globally, in 2015 GM soybeans accounted for just over half of all the GM crop acres in the world.

The top GM soybean-growing countries with over 90% adoption in 2015 were the USA with 80 million acres, Brazil with 75 million acres, and Argentina with 52 million acres. These three countries, along with eight additional countries growing GM soybeans in 2015, amounted to 227 million acres, or 83% of the 274 million total soybean acres. The income benefit for farmers growing GM soybeans from 1996 to 2014 has been calculated to be $47.8 billion dollars (ISAAA Crop 2017).

Cotton

In cotton, IR + HT traits have become a mainstay in commercial production globally with 75% of the world's cotton acreage (59 out of 79 million acres) planted with GM traits in 2015. GM cotton is grown in 15 countries: the top three being India with 29 million acres, China with 9 million, and USA with 8 million acres in 2015. The cumulative value to farmers in the 19 years from 1996 to 2014 was $46.5 billion (ISAAA Crop 2017).

The first GM IR cotton (Bollgard®, Monsanto) was launched in the USA in 1996 with HT (Roundup Ready®, Monsanto) and the IR + HT stacked product (Bollgard + Roundup Ready®) launching a year later in 1997. Rapid adoption of GM traits in cotton led to more than 90% of US cotton acres planted to GM cotton by 2010 (Fernandez-Cornejo et al. 2014). After glyphosate tolerance, the next HT trait introduced was Bayer's glufosinate-tolerant Liberty Link® cotton in 2004, followed by two new HT traits, Xtend® dicamba-tolerant cotton from Monsanto and Enlist™ 2,4-D-tolerant cotton from Dow AgroSciences, commercialized in 2015 and 2016, respectively. As with other crops, the newer traits are being commercialized as stacked products with IR and multiple HT traits to provide farmers greater flexibility in controlling weeds and pests in their fields. The IR traits in cotton are targeted at lepidopteran pests, such as tobacco budworm (*Heliothis virescens*) and cotton bollworm (*Helicoverpa armigera*). Similar to stacking in maize, additional cotton IR traits with multiple modes of action against the key pests were developed by mid-2000 with Bollgard II® (Monsanto) and WideStrike® (Dow AgroSciences). Those products were also stacked with HT traits. Cotton products being commercialized today are breeding stacks of traits from different companies to combine even more IR traits in stacked combinations with multiple HT traits.

Canola

GM canola was planted on 21 million acres globally in 2015, comprising 24% of the world's canola acres. Canada, USA, and Australia are the primary areas of production, and the cumulative value in terms of the farmer's income benefits from GM canola was $4.9 billion (1996 through 2014) (ISAAA Crop 2017). GM traits in canola have been limited to HT and male sterility to date. Glufosinate-tolerant InVigor® canola was launched by Bayer in Canada in 1996, and glyphosate-tolerant Roundup Ready® canola was introduced by Monsanto in 1997. Bayer also

incorporated a GM male sterility system with their HT canola trait to assist in production of hybrid canola seed. New glyphosate tolerance traits and glyphosate + glufosinate stacked products are nearing launch by several trait developers (Monsanto, DuPont Pioneer, Bayer). Dicamba-tolerant canola to provide a new herbicide mode of action HT trait is in earlier stages of development in Monsanto's trait development pipeline. The important insect pests of canola do not include the lepidopteran pests that Bt traits control today, and thus currently there are no IR canola traits.

Other Crops

GM input traits have been commercialized in several smaller crops as well. Traits that confer resistance to specific diseases have been commercialized in papaya, potato, plum, and squash with mixed success. Some disease resistance traits in these smaller crops have enjoyed wide market penetration and are credited with saving particular cultivation industries (e.g., papaya ring spot virus resistance trait by University of Hawaii and Cornell Univ.); others failed to achieve significant market share (e.g., potato virus Y and potato leaf roll virus in NewLeaf® potato). Herbicide-tolerant traits in smaller crops have also been met with challenges. The USDA deregulations of Roundup Ready® sugar beets (Monsanto) and Roundup Ready® alfalfa (Monsanto) were both challenged in the courts after the products were initially launched, forcing a hold on commercial sales until those challenges were resolved. Today both traits are grown commercially and enjoy wide adoption by farmers. IR eggplant (Mahyco) was first commercialized in Bangladesh in 2014, but other key markets, specifically India and the Philippines, have been met with challenges by critics of the technology and thus have not yet approved the product for sale.

Male Sterility

Hybrid crops, such as maize and canola, take advantage of heterosis to increase crop yields but also require additional inputs to produce the hybrid seed. Production of hybrid seed requires cross-fertilization of two parental lines using approaches ranging from hand detasseling to exploiting native male sterility systems. However, more recently GM male sterility systems have been developed for these crops, the first of which was the barstar/barnase system in canola (Bayer). In this system, the barnase gene confers male sterility by preventing pollen production, and the barstar gene inhibits barnase to restore fertility. DuPont Pioneer has developed a GM male sterility system in maize, termed Seed Production Technology (SPT), which combines male sterility with a seed color marker enabling segregation of the transgenic male sterile maize from the desired non-GM fertile hybrid seed (Wu et al. 2016). Monsanto is also working on a GM male sterility system named RHS in which a transgenic plant produces non-transgenic pollen that is killed by the application of glyphosate (Feng et al. 2014).

16.2.2 Agronomic Traits

For a variety of reasons, agronomic traits have not enjoyed the same level of market penetration in major crops as input traits to date. Nevertheless, there are specific examples of successful products on the market and some compelling traits in late stages of development that are approaching launch. As technology and knowledge of plant biology overcome current challenges, it is expected that an increasing number of these types of traits make it to the market.

Starting in the late 1990s with the advent of the first complete plant genome sequence (*Arabidopsis thaliana*), significant investments were made by multiple biotechnology start-ups (e.g., Paradigm Genetics, Ceres, Inc., Mendel Biotechnology, Cereon, Crop Design, etc.) and large multinational agricultural companies (e.g., Monsanto, Bayer, BASF, DuPont Pioneer, Syngenta, etc.) in the field of functional genomics. Thousands of genes were identified and then systematically mis-expressed (e.g., overexpression or antisense expression) as transgenes in *Arabidopsis thaliana* and other model and crop species. These transgenic events were then tested for their ability to confer tolerance to abiotic stresses, improved performance under nutrient-limiting conditions, or improved growth characteristics under non-limiting conditions using a variety of approaches.

As a result of these efforts coupled with the ongoing work of academic scientists, a large number of candidate genes were identified and evaluated in crops of interest such as maize and soybean for their commercial product potential. Of these hundreds of candidate genes, several advanced far enough in company product development pipelines to become publicly known through investor presentations and scientific publications (e.g., cspB and Nfy-B drought tolerance leads developed by Monsanto and AlaT, a nitrogen use efficiency lead developed by Arcadia Biosciences). However, as shown in Table 16.1, only two have been successfully commercialized to date. One is a cold shock protein from *B. subtilis* (CspB) that is marketed as Genuity® DroughtGard® (MON87460) (Castiglioni et al. 2008). This trait was planted on 810,000 hectares in 2015 and was donated by Monsanto to the public-private partnership, Water Efficient Maize for Africa (WEMA). It is expected to be available for African farmers in select countries in 2017 (James 2015). This trait is being stacked with current IR + HT products to provide farmers with additional yield protection. The second is anendo-1,4-β-glucanase from *A. thaliana* (*cel1*) that is expressed in *Eucalyptus* spp. to increase woody biomass (Shani et al. 2003). This trait has been brought to market by FuturaGene Group and was approved for cultivation in Brazil in 2015.

As discussed in the previous section, commercialization of input traits has enjoyed a great success. However, those traits act independently without interfering in plant endogenous cellular processes (e.g., CP4 EPSPS confers tolerance to glyphosate due to decreased binding affinity for the herbicide and Bt toxins act through the formation of a pore in insect midgut epithelial cells). On the other hand, agronomic traits exert their effects through interactions with endogenous cellular processes such as nutrient utilization or stress response pathways. A beneficial

Table 16.1 Commercialized GM crops

Trait type	Crop	Trait description	Developer	Availability
Input traits disease resistance	Papaya	Virus resistance	Cornell University, South China agricultural university	Commercial[a]
	Plum	Virus resistance	USDA ARS	Not launched[b]
	Potato	Virus resistance	Simplot, Monsanto	Commercial
	Squash	Virus resistance	Monsanto	Commercial
Input traits herbicide tolerance	Alfalfa	Glyphosate tolerance	Monsanto	Commercial
	Canola	Glufosinate tolerance	Bayer	Commercial
		Glyphosate tolerance	Monsanto	Commercial
	Cotton	2,4-D tolerance	Dow AgroSciences	Commercial
		Dicamba tolerance	Monsanto	Commercial
		Glufosinate tolerance	Bayer	Commercial
		Glyphosate tolerance	Monsanto, Bayer	Commercial
	Maize	2,4-D, ‘fop tolerance	Dow AgroSciences	Commercial
		Glufosinate tolerance	Bayer	Commercial
		Glyphosate tolerance	Monsanto	Commercial
	Rice	Glufosinate tolerance	Bayer	Not launched
	Soybean	2,4-D tolerance	Dow AgroSciences	Not launched
		Dicamba tolerance	Monsanto	Commercial
		Glufosinate tolerance	Bayer	Commercial
		Glyphosate tolerance	Monsanto	Commercial
		Isoxaflutole tolerance	Syngenta	Not launched
		Mesotrione tolerance	Syngenta and Bayer	Not launched
		Sulfonylurea tolerance	BASF	Commercial
	Sugar beet	Glyphosate tolerance	Monsanto	Commercial
Inputs traits insect resistance	Cotton	Lepidopteran resistance	Bayer, Dow AgroSciences, Monsanto, Syngenta	Commercial
	Eggplant	Lepidopteran resistance	MAHYCO	Commercial
	Maize	Lepidopteran resistance	Dow AgroSciences, DuPont, Monsanto, Syngenta	Commercial
		Coleopteran resistance	Dow AgroSciences, DuPont, Monsanto, Syngenta	Commercial
	Potato	Lepidopteran resistance	Monsanto	Sales ended[c]
		Coleopteran resistance	Monsanto	Sales ended
	Soybean	Lepidopteran resistance	Dow AgroSciences, Monsanto	Not launched, commercial

(continued)

Table 16.1 (continued)

Trait type	Crop	Trait description	Developer	Availability
Male sterility	Canola	Male sterility system	Bayer	In use[d]
	Maize	Male sterility system	DuPont, Monsanto	In use, not launched[e]
Agronomic traits	Maize	Drought tolerance	Monsanto	Commercial
	Eucalyptus	Volumetric wood increase	FuturaGene group	Commercial
Output traits	Alfalfa	Altered lignin	Monsanto	Commercial
	Apple	Non-browning	Okanagan	Commercial
	Maize	Modified alpha-amylase	Syngenta	Commercial
		Increased lysine	Renessen	Sales ended
	Pineapple	High lycopene	Del Monte	Commercial
	Potato	Altered starch	BASF	Sales ended
		Reduced acrylamide	Simplot	Commercial
	Soybean	Modified oil	Monsanto	Commercial
		Modified oil/fatty acid	DuPont	Commercial
	Canola	Modified oil/fatty acid	Monsanto	Sales ended
		Phytase production	BASF	Sales ended
	Tomato	Delayed fruit softening	Monsanto	Sales ended

Data compiled from http://www.isaaa.org/gmapprovaldatabase/default.asp and http://cera-gmc.org/GMCropDatabase

[a]Commercial indicates trait is commercially available at the time of this writing

[b]Not launched indicates most of the regulatory approvals have been obtained, but product has not yet been made commercially available

[c]Sales ended indicates the trait was previously available commercially but has been removed from the market

[d]In use indicates that the male sterility system is currently in use but is not a commercial product for farmers to purchase

[e]In use, not launched indicates that the DuPont male sterility system in maize is currently in use but is not a commercial product for farmers to purchase. The Monsanto male sterility system is still awaiting final regulatory approvals prior to use

effect on crop performance in the case of agronomic traits depends on effective modification of a complex system. A primary challenge in the commercialization of agronomic traits is identifying target genes capable of consistently delivering a significant performance improvement across diverse genetic backgrounds (in elite commercial germplasm) and across diverse environmental conditions.

Much is known on the molecular, biochemical, and physiological level about plant responses to stress and about the source-sink relationships that impact yield. However, the precise system perturbations that are required to fine-tune a plant response or redirect metabolic flux onto preferred pathways, for example, without resulting in undesired changes or no change at all is not always well understood. For this reason, many of the candidate genes that show promise in model systems or

under controlled laboratory conditions do not provide consistent results when tested in different genetic backgrounds under field conditions.

From a logical point of view, improving plant performance (agronomic traits) relies on the assumption that either native plant responses to environmental conditions are not optimized to maximize economic yield or that native plants lack certain characteristics that would be beneficial to yield. That the biotechnology industry has not been more successful in delivering agronomic traits to market is not an indication that the solution is intractable. It merely indicates that technical capabilities to make molecular modifications to plants have temporarily exceeded the understanding of the biological system.

The continuous development of new methods for measuring the influence of the genome on the phenome will eventually enable more sophisticated approaches that more precisely control transgene expression or combine the effects of multiple transgenes, for example, to deliver traits with sufficient impact to be economically viable. A recent paper by Sun et al. (2017) where a potential trait gene only delivers beneficial effects when its expression is spatially restricted is indicative of the increasing levels of sophistication that will be required. In this study, the maize *PLASTOCHRON1* gene, which is involved in the regulation of cell division, was driven by a *GA2-oxidase* gene promoter, which is preferentially expressed in the growth zone where there is a transition from cell division to cell expansion of the leaf. The resulting transgenic events demonstrated increased plant height and leaf area with positive impacts on overall plant biomass and yield. However, when the PLA1 gene was expressed with a strong constitutive promoter (*UBIL*), severe developmental abnormalities ensued including failure to flower (Sun et al. 2017). Whether traits such as these are delivered using what may now be considered as traditional transgene technology or using newer gene editing tools such as zinc finger nucleases (ExZACT™) or CRISPR-Cas will depend at least in part on whether the target genes are present in the crop species. These genome-editing tools and their use will be discussed later in this chapter.

Given the diversity of environments and germplasm backgrounds that an agronomic biotechnology trait will encounter, perhaps it is unrealistic to expect the same kind of cross-crop and broad geographic penetration of particular traits that have been seen for input traits. If this limitation on agronomic traits is fundamental, their development will have to be tailored to germplasm and environment niches which will decrease the potential market size thus negatively impacting the trait valuation. Compensatory decreases in other product development costs would be needed in order to warrant investment by the agricultural biotechnology industry.

16.2.3 Output Traits

In agronomic traits multiple target genes have been identified that are involved in key physiological processes, but the precise perturbations required to deliver a quantum change in economic yield across germplasm and environments remain for the most part elusive. In contrast, output traits in most cases target metabolic

endpoints or key effector proteins in accessible and well-defined pathways. Examples include oil, starch, amino acid, and antioxidant biosynthesis as well as antigens and ripening signals. Modifications to these pathways are designed to deliver characteristics beneficial to consumers that can be grouped into several categories: enhanced nutritional content, food/feed safety, and forage quality.

While adoption of agronomic traits remains largely a technical challenge in generating products with desired effects, the delivery of output traits is primarily a market challenge. In the first instance, there is the problem of public acceptance of new GM products with the Flavr Savr™ tomato (developed by Monsanto) being a well-known example. Amflora® potatoes (developed by BASF) with a modified starch content favorable for industrial starch production also experienced a short commercial lifespan due to public concerns in the European Union. New attempts that will test public acceptance of GM produce have recently been launched including the Arctic® family of apple products featuring a non-browning trait (developed by Okanagan) that will debut in the Midwest US market in 2017 and the Innate® family of potatoes (developed by J.R. Simplot Co.) launched in 2015 that feature non-browning, black spot bruise resistance and reduced acrylamide formation potential as consumer benefits.

In commodity crops, there are a few examples of output traits that have been commercialized in "closed loop cultivation" including Plenish® and Vistive® Gold and high oleic soybean varieties from DuPont Pioneer and Monsanto, respectively. Another example is the Enogen® maize trait from Syngenta that uses an alpha-amylase enzyme to improve starch breakdown for bioethanol production (Urbanchuk et al. 2009). Many others have been developed, but not yet commercialized (e.g., high omega-3 canola (Walsh et al. 2016) and vitamin A-enriched "golden rice" (Stone et al. 2017), and some have been discontinued (Laurical™ canola, which is enriched for the fatty acid laurate and Phytaseed™ canola, which expresses an enzyme to degrade phytate developed by Monsanto and BASF, respectively). Of all the non-input traits that have reached product launch, 11 out of 13 are output traits (Table 16.1).

The National Academies report (National Academies of Sciences 2016) speculates that, "Many potential future genetically engineered traits are predicted to be output traits, engineered specifically to change the quality of a crop. Most output traits developed soon will probably not require the use of chemical agents and should not require substantial changes in agricultural practices other than the requirement for identity protection and control of gene flow."

A more fundamental problem to the industry is the potential return on investment for products that address specialty or niche markets. The return has to be weighed against the significant investments in product development and deregulation associated with bringing a biotechnology trait to market. In most cases, consumer-oriented output trait products will exist in the market place alongside traditional products and must be kept in separate distribution channels to preserve identity and value. The added effort associated with this means the market must be of sufficient size to warrant the investment. For output traits that deliver broadly recognized consumer value, it may be possible to convert the distribution channels such that the biotechnology product predominates and identity preservation is no longer necessary. However, this is unlikely to be a common occurrence.

16.2.4 Predicting Traits in the Near Future

Projecting the traits of the future is inherently challenging due to limited availability of public information relating to industry R&D pipelines. A recent paper by Parisi and coworkers (Parisi et al. 2016) outlines an exhaustive approach that relies on public databases that collect information about GM crops, databases from government regulatory agencies, information available on company websites, and an international workshop convened in 2014 with representation from key constituencies to validate and correct the gathered information. Given the limitations of the digital resources available, the vetting of compiled information by a body of industry and government representatives is the only reliable way to ensure the quality of the data. However, this approach is not easily replicated. Since it was published in 2016, it is not anticipated that significant changes in the forecast have accrued by the time of this publication. By gathering information on the biotechnology events at several stages of product development (commercial cultivation, pre-commercial, regulatory, advanced R&D, and early R&D), the authors were able to generate a prediction of the biotechnology products that may become commercialized within the next several years. Remarking on their findings, the authors state, "The number of GM events at the commercial cultivation, pre-commercial or regulatory stages has more than doubled between 2008 and 2014. Although current GM commercial varieties and the outlook for 2020 are still dominated by a few arable crops (usually for feed or industrial use) and certain [input] traits, there is a nascent growth in quality traits, with a focus on bio-fortified food and industrial applications. Also, more specialty crops are being introduced into the pipeline and bean, rice, potatoes, and sugarcane may be cultivated by 2020" (Parisi et al. 2016).

A report compiled by the National Academies of Science, Engineering, and Medicine (National Academies of Sciences 2016) offers a more circumspect tone and an important disclaimer on predicting the biotechnology crops of the future stating, "It is not possible to predict with certainty the traits that will and will not make it to market or be diffused through nonmarket mechanisms in the future. The outcome will depend on environmental challenges that need to be addressed (for example, climate change), political-economic drivers, the regulatory landscape, and the rate of scientific advances, which is in part a function of the availability of public and private science funding."

Further investment and progress in at least one category, output traits, may depend heavily on how well the new produce (apple and potato) and commodity (high oleic acid soybean) crops are received by the public. Agronomic traits that improve farm productivity may need to wait on further advancements in the understanding of how the genome exerts its influence on the phenome before we see many new products on the market. Targeted opportunities to use transgenic biotechnology to deliver disease resistance are likely to be pursued so long as the business valuation of the trait exceeds the development costs. Overall, these differences in outcome merely underscore an important point of emphasis in any discussion of biotechnology traits – the market will pick the winners regardless of

how clever, sophisticated, or well-adapted a particular technical solution may be. The costs associated with the discovery, development, deregulation, launch, and maintenance of biotechnology traits simply demand a market share or product premium that is commensurate. Traits that cannot meet those hurdles will not be commercially viable.

16.3 From Random Gene Insertions Toward Designer Crops

As we have discussed, significant progress has been made in the development and commercialization of transgenic plants in a large number of crops. However, random gene insertion has been the main approach for expressing foreign genes in plants. Multigenic products have been generated as breeding stacks where multiple transgenic events are crossed to bring all the genes together in one plant. However, since all the transgenes being combined are present in multiple events and are located at random locations in the genome, it has been challenging for the plant breeders to introgress these genes into elite varieties for product development. Moreover, random gene insertions have been used to produce desired traits in crop plants, but it is not effective in modifying any existing gene. Therefore methods for precise gene addition or modification at predetermined locations of the genome were required. Significant progress has been made in this field of study as well and a wide array of precision genome modification technologies are available today. These technologies have been demonstrated to design the genomes effectively in both plant and animal systems.

Cre/loxP is one of the earliest systems that was discovered for modifications at a single locus in the genome (Sternberg 1978). The working principle of this system is simple since it requires only Cre recombinase to initiate recombination at the pre-engineered loxP site and does not need any other cofactor for the reaction (Nagy 2000). This system has been shown to work effectively for targeted gene insertion or deletion in plant as well as animal systems (Vergunst et al. 1998; Schaart et al. 2004; Jia et al. 2006). However, mainly the system has been used for removal of selectable marker cassette from transgenic plants, such as by Monsanto and Rennessen for removal of the *nptII* gene from the high lysine maize event, LY038 (Lucas et al. 2004). Later an analogous system called FLP/FRT was discovered. It is analogous to Cre/loxP system and has similar applications (Luo et al. 2002; Li et al. 2010). Though these systems can be used for continued gene additions and removals at a pre-designed locus, these have not been able to modify the existing sequence in the genome. In addition to the above two, some other recombinase systems were also discovered which did not become very popular. A brief overview of those is nicely described by Wang et al. (2011).

A revolution in the field of precise gene modification, insertion, gene stacking, or removal came with the adaptation of site-directed nucleases, i.e., meganucleases, ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases), and CRISPR/Cas (clustered regulatory interspaced short palindromic

repeats/CRISPR-associated). Though the mechanism of all these nucleases is different, they all introduce double-strand breaks in the genome in specific targeted sequences. In response to this break, the host cell initiates its double-strand break repair mechanism. Scientists exploit this mechanism by inserting donor DNA into the cell which either gets integrated at the double-strand break site or can be used as a template for precise modification of a single or few base pairs.

The first nucleases discovered in this category were meganucleases. Meganucleases, also known as homing endonucleases, can be divided into multiple families. However, the LAGLIDADG family of meganucleases are the most studied and have been used extensively for gene targeting (Silva et al. 2011). These nucleases recognize DNA sequences ranging from 12 to 40 bp long and then insert a double-strand break. Their high degree of specificity provides higher accuracy and lower cellular toxicity. However, since a long sequence of DNA is recognized by meganucleases, it leaves very few sites in the genomes that can be modified (Rinaldo et al. 2015). The redesigning of these nucleases to read new target sequences has also been a challenge since the DNA recognition and cleavage functions of these enzymes are present in the same domain. Any changes to the DNA-binding domain may affect the cleavage activity of the enzyme (Chandrasegaran et al. 2016). Therefore, although reengineering of some meganucleases has been done to recognize new sites, largely the method has been very cumbersome and complex.

Another class of nucleases with greater flexibility are the ZFNs. These were based on the discovery of the zinc finger DNA-binding domain in a large number of transcription factors providing them the DNA-binding specificity (Diakun et al. 1986). Each finger has its own unique recognition sequence which is provided by amino acids at position −1, +2, +3, and + 6 relative to the start of the alpha-helix in the zinc fingers (Osakabe et al. 2015). Amino acids at these positions can be modified to alter its DNA recognition specificity. Therefore developing and joining multiple fingers in order to derive an array of fingers to recognize a desired target sequence became the basis of developing a DNA-recognizing protein. Further, these DNA-binding domains were combined with a non-specific cleavage domain from Fok1 restriction enzyme to generate sequence-specific cuts in the DNA. Though ZFN is an efficient method of introducing a double-strand break at the target sequence, it requires rigorous development and screening of ZFN arrays to find the efficient ones. Since their discovery, these ZFNs have been used in a large number of organisms for targeted genome modifications.

TALENs are another designed nuclease for generating double-strand breaks at target sequence. TALENS were discovered in the bacteria of genus *Xanthomonas*. *Xanthomonas* is a pathogen of crops like rice, pepper, cotton, and tomato. It secretes effector proteins (TALEs) into the cytoplasm of plant cells that binds the specific DNA sequences to modify the plant processes in order to make the plant more susceptible to infection (Nemudryi et al. 2014). The DNA-binding property of TALENs has been exploited for the development of site-specific restriction enzyme by attaching a non-specific restriction enzyme Fok1 to it. TALENs are similar to ZFNs in that they have a DNA-binding domain attached to the Fok1 domain, and both work in dimers. However the DNA-binding domain of TALENs is different

from ZFNs. TALENs consist of a series of repeated domains, each of which is about 33–35 amino acids long. Most of these amino acids are highly conserved except at position 12 and 13. Amino acids at these two positions are highly variable and are responsible for target nucleotide specificity and can be modified to change the target recognition. TALENs are comparatively easier to build as compared to ZFNs due to its straightforward DNA interaction code and the modular nature of the array. However the challenge with TALENs is their large size (about 950 amino acids for each protein) and repetitive nature of the DNA sequence due to conserved sequence of the multiple domains joined together (Baltes et al. 2014). Therefore delivery of these proteins into plants becomes a challenge. Despite the challenges, TALENs have been used for targeted genome modifications of a large number of organisms.

Recently a new RNA-guided nuclease system called CRISPR/Cas (clustered regulatory interspaced short palindromic repeats/CRISPR-associated) was developed, and it has gained widespread attention in a short period of time. The system has been adopted from bacteria where it provides acquired immunity against invading nucleic acids such as bacteriophage and plasmids (Rinaldo et al. 2015). Bacteria acquire small fragments (called spacers) from invading DNA and incorporate them into the CRISPR loci. These CRISPR repeats along with the spacers are then transcribed into pre-CRISPR RNA (pre-crRNA) which is further processed to create a restriction enzyme that consists of a spacer-based guide RNA and a Cas enzyme. The guide RNA pairs with the invading DNAs and destroys it by generating double-strand cuts with the action of Cas enzyme. This mechanism was used to create an engineered CRISPR/Cas enzyme where guide RNA is designed to recognize the desired target sequence. Target recognition by this complex requires the presence of a protospacer adjacent motif (PAM) followed by crRNA recognition sequence on the target DNA (Gaj et al. 2013; Khatodia et al. 2016). Therefore, the system's only limitation is the required presence of a PAM sequence at the target site. Since its discovery, the system has been used extensively to obtain double-strand breaks in a wide range of organisms, and the literature is replete with reports mentioning the use of this technology.

These site-specific nucleases are transformative tools and are revolutionizing the entire field of biology. These nucleases can not only be used for targeted gene insertions or stacking but also for making small changes in the genome to generate desired traits. Though traits generated with targeted foreign gene insertions would be called transgenic, traits generated by inserting small changes in the endogenous genes have the potential to be considered non-transgenic. Plants regenerated with the use of these nucleases may contain the DNA from these nucleases in addition to the intended change. However these can be easily segregated out in subsequent generations, and plants homozygous for the intended change and free of any other unintended gene integration in the genome can be obtained in the progeny plants. Though these technologies have been extensively used and a large number of reports have been published, some selected examples that have commercial and economic importance are discussed below.

Examples of developing input traits using gene editing include ALS (acetolactate synthase) herbicide resistance. Chlorsulfuron and bispyribac are some of the

herbicides that are used to control weeds in crops. These herbicides kill plants by inhibiting the activity of acetolactate synthase, an enzyme involved in amino acid biosynthesis. Transgenic lines containing mutated ALS enzyme have been generated that are resistant to these herbicides. Sun et al. (2016) took a different approach to regenerate resistant plants by editing the endogenous ALS gene instead of inserting a resistant ALS transgene into the crop plant. The researchers used the CRISPR/Cas system to edit this gene in rice callus. A donor fragment that had desired mutations in the sequence and had homology arms for homology-based repair of the endogenous gene was also transformed into the callus along with a CRISPR/Cas cassette designed to cut in the endogenous ALS gene. They successfully regenerated rice plants that showed tolerance to the application of herbicide. Leaves of the herbicide sprayed wild-type plants withered and died, while gene-edited plants showed complete resistance to the herbicide. Researchers showed the regeneration of homozygous herbicide-resistant rice plants in the T0 generation itself due to biallelic modifications created by CRISPR/Cas showing the specificity and effectiveness in recognizing the target sequence.

In a similar effort, Li and coworkers edited the rice genome to impart bacterial blight resistance. Bacterial blight is an economically important disease of rice since outbreak of this disease may lead up to 50% of the crop yield loss and may even go up to 70% in case of severe infections (Cernadas et al. 2014). OsSWEET14 is a bacterial blight susceptibility gene in rice. The effectors AvrXa7 and PthXo3 produced by *X. oryzae pv oryzae* bind to the effector binding element in the promoter of OsSWEET14 gene. This upregulates the gene which favors infection by this pathogen. Li et al. (2012) used TALENs and mutated the effector binding sites in the promoter of the OsSWEET14 gene. Inability of effectors to bind the target sequence resulted in bacterial blight resistant in the crop. Another desired trait in crop plants is the development of male sterility which is extensively used for the development of hybrids. However this trait is not easily developed in all the genotype backgrounds using traditional breeding programs. Djukanovic et al. (2013) designed a homing endonuclease to target a 22 bp sequence in the fifth exon of MS26 gene (a maize fertility gene) in corn. The enzyme led to targeted mutagenesis resulting in small deletions and insertions leading to the disruption of coding sequence. The mutation is recessive and the resulting homozygous plants for the mutation were male sterile.

Some output traits have also been generated using gene editing. Haun et al. (2014) used TALENs to disrupt the FAD2-1A and FAD2-1B genes, thus reducing the polyunsaturated fatty acid content in soybean oil. The resulting plants showed increased oleic acid from 20% to 80% and decreased linoleic acid from 50% to 4%. Reduced content of polyunsaturated fatty acids improves the shelf life of soybean oil. This eliminates the need of partial hydrogenation which is an industrial process and results in production of trans-fatty acids which are known for certain health risks. Similarly Shan and coworkers (2015) used TALENs to improve the aroma in rice grains. 2AP (2-acetyl-1-pyrroline) is the compound responsible for fragrance in rice, and BADH2 (betaine aldehyde dehydrogenase) inhibits the synthesis of 2AP. Researchers designed TALENs and disrupted the BADH2 DNA sequence. Homozygous lines in T1 and T2 generation showed increase in the levels of 2AP

from 0.35 to 0.75 mg/kg. In another similar effort, TALENs were used to improve the cold storage of potato tubers. Low-temperature warehouses are used for potato storage to extend shelf life. However cold storage induces the accumulation of reducing sugars in potato tubers. When these tubers are processed at high temperature, these sugars react with amino acids and lead to brown, bitter tasting products and even increased levels of acrylamide which is a potential carcinogen. It is known that vacuolar invertase gene (Vinv) is responsible for accumulation of reducing sugars in potato. As discussed above, transgenes have been introduced to downregulate the expression of this gene. However, Clasen et al. (2016) used TALEN technology to knock out Vinv gene. Researchers showed that the chips made from modified potato contain reduced levels of acrylamide and were light in color.

Deciphering the gene function in polyploid crops has been challenging due to the presence of multiple homoeo alleles. In order to determine the function of the gene by a typical reverse genetics approach, all of them would need to be silenced. RNAi has been used to simultaneously knock down (mRNA degradation) multiple alleles; however this technique gives variable results, and gene silencing is not always complete. Therefore a gene knockout (coding sequence mutation) strategy would be more effective as compared to knockdown of the genes. Loss of function alleles (*mlo*) of MLO locus are known to provide broad-spectrum resistance against powdery mildew to barley (Piffanelli P et al. 2004). Wang et al. (2014) used TALENs to knock out the three homoeo alleles encoding MILDEW RESISTANCE LOCUS (MLO) proteins in hexaploid bread wheat. TALENs were designed to target a conserved region on exon 2 to create a simultaneous mutation in all the three alleles. The mutations were successfully created in all three MLO genes which conferred powdery mildew resistance to the plants. Recently the same strategy was used in tomato using CRISPR/Cas technology. The MLO alleles were mutated resulting in the regeneration of a powdery mildew-resistant plant which was named as Tomelo (Nekrasov et al. 2017). This shows the efficiency of these gene modification technologies in reading a specific DNA sequence in the genome and inserting a double-strand break.

Recently a gene-edited mushroom received widespread attention. The white button mushroom is prone to browning shortly after picking which reduces its market value. Waltz and coworkers from Penn State University manipulated the genome of this fungus using CRISPR/Cas technology (Waltz 2016). They mutated the polyphenol oxidase (PPO) gene that resulted in a delay in browning. They demonstrated that the target gene had been mutated, and no other gene fragment related to CRISPR/Cas has been integrated into the genome. When they enquired about the regulatory assessment needs of the modified mushroom, the USDA stated that since it does not contain any foreign sequence, no plant-pest sequence was used to create the intended change, and no foreign sequence was present in the resulting product in addition to the change that was induced; the agency does not consider that the product needs to be regulated (USDA 2016). This is one major step that would substantially ease out the commercialization of the gene-edited crops by reducing the timeline between the discovery of traits and releasing the crops for commercial cultivation in the field. Earlier, the USDA had given a similar determination to some other gene-edited crops as well, e.g., disease-resistant rice (Iowa State University),

potato with better processing attributes (Cellectis), and reduced phytate corn (Dow AgroSciences) (Wolt et al. 2016). However, the USDA stated that when a template is inserted into the cell to repair a gene, the template may likely get integrated in the genome as well. Therefore, the regulatory requirements for any such product will be determined on a case-by-case basis. Though the USDA has opted out of the regulation of some such products, it is not known how the other agencies in the USA and rest of the world will treat the situation and what regulatory guidelines will be established to oversee such products.

Another similar development is the CRISPR-induced waxy maize developed by DuPont Pioneer. Normal field maize contains two types of starches, ~78% amylopectin and ~22% amylose, while waxy maize contains 100% amylopectin (Eriksson 1969). To develop waxy maize, scientists at DuPont Pioneer knocked out the Wx1 gene that encodes granule-bound starch synthase responsible for synthesis of amylose. As the USDA stated that CRISPR-/Cas-edited mushroom did not need regulatory approval, waxy maize edited by a similar methodology may also receive a similar finding of nonregulated status and may reach the market earlier.

16.4 Challenges Associated with Further Adoption of GM Crops

In the Americas, the USA, Brazil, Argentina, and Canada are the major producers of GM crops, including soybean, maize, cotton, and canola, representing 80% of the total global GM crop production. Elsewhere in the Americas, countries such as Paraguay, Uruguay, Bolivia, Mexico, Colombia, Honduras, and Costa Rica have each planted one or more of the major GM crops (James 2010). In Asia, Bt cotton is grown in India and China, accounting for the largest GM crop hectare plantings in the region, and GM papaya is widely adopted in southern China (James 2010; USDA FAS 2016a). GM cotton and canola have been adopted in Australia since 2008 (USDA FAS 2016b). Bt maize with stacked traits is grown in the Philippines for commercial use, and Bt brinjal/eggplant, the first locally developed GM crop which was developed through the USAID-ABSP support project, is poised for future commercialization (USDA FAS 2016c). GM maize with stacked insect resistance and herbicide-tolerant traits was planted for the first time in Vietnam in 2015 (ISAAA 2016). In Africa, Egypt, and Burkina, Faso joined South Africa in the adoption of GM crops by planting GM maize and Bt cotton, respectively, in 2008 (James 2010; USDA FAS 2015). In Europe, Bt maize, the only GM crop approved for cultivation, enjoyed low, but nevertheless, stable level of adoption, primarily in Spain (USDA FAS 2016d). The four major countries cultivating GM crops are also the leading exporters of soybean, maize, cotton, and canola. These nations trade internationally with the major destinations including China, EU, Japan, Mexico, and Southeast Asia depending on the products (USDA 2017).

Despite the economic, social, and environmental benefits of GM crops to global society (Qaim 2009; Anderson 2010; Carpenter 2013; Qaim and Kouser 2013;

Barfoot and Brookes 2014; Brookes and Barfoot 2015), adoption of GM crops in large parts of the world, such as Africa and Europe, remains compromised. This opposition is derived from a multitude of complex and intermingled concerns which have persisted ever since the adoption of GM foods and has as much to do with social and political values as with concerns about health and safety (WHO 2005). Some of the underlying concerns to general acceptance have themselves become a driving force for GM crop regulations and policy development. This section highlights risk and benefit perception of GM crops and food, regulatory and political development, international trade protection, and social concerns.

16.4.1 Risk Assessment of GM Crops and Food

Regardless of the method used (traditional breeding or recombinant DNA techniques) or the traits developed (herbicide tolerance, insect resistance, yield improvement, and/or improved nutritional value), for any crop with new traits, the potential exists for safety risks. Risks associated with GM crops, when the introduced genes and traits are safe, are no greater than conventionally bred crops. The process used to introduce genes into crops, with a history of safe use, is unrelated to risk (OECD 1986; White House OSTP 1986; US NAS 1987). While new varieties of conventionally bred crops are not usually subject to regulatory scrutiny for potential safety concerns prior to marketing, GM crops undergo risk assessment with extensive toxicological and nutritional evaluation.

Risk assessment, risk management, and risk communication are three components of risk analysis (Codex 2003a). Two international regulatory instruments, Cartagena Protocol on Biosafety (CBD 2000) and Codex principles and guidelines on foods derived from modern biotechnology (Codex 2003a, b), cover environmental safety of living modified organisms and GM food safety, respectively. The concepts and principles outlined in the Cartagena Protocol on Biosafety and in Codex (2003a, b) are intended to provide international consistency in the assessment of environment and food safety of GM crops. One of the risk assessment principles laid down in the Cartagena Protocol on Biosafety is that risks should be considered in the context of the risks posed by the nonmodified recipients or parental organisms in the environment. The concept of "familiarity" incorporated in environmental assessment of GM plants facilitates risk/safety assessments. The term "familiar" in this context expressly identifies the means to having enough information to be able to make a safety or risk judgment (CBD 2000).

The assessment approach to GM food safety is based on the principle, referred to as "substantial equivalence," that the safety of foods derived from new plant varieties, including GM plants, is assessed relative to the conventional counterpart having a history of safe use (Codex 2003a, b). Risk assessment of GM food is designed to identify whether a hazard (nutritional or other safety concern) is present, and if present, to determine its nature and severity. The safety assessment includes a comparison between the food derived from GM crop and the conventional

counterpart, taking into account both intended and unintended effects. If a new or altered hazard is identified by the safety assessment, the risk associated with it is characterized to determine its relevance to human health.

GM crops, released for commercial use and traded on the international markets, have demonstrated that they are as safe and nutritious as conventional counterparts. To date, there has not been a single confirmed case of an adverse health issue for humans or animals due to consumption of approved GM products. To create awareness and to emphasize the safety of GM crops, in June 2016, 123 Nobel Laureates signed an open letter to the leaders of Greenpeace, the United Nations, and governments around the world supporting the efficacy and safety of GMO food products. In the letter, they reiterated conclusions made by scientists and regulatory agencies around the world that assert that crops and foods improved through biotechnology are safe.

16.4.2 Perception of Risk and Benefits

Over the years, various surveys across geographies have been conducted in an attempt to gain insights into public perception and acceptance of GM food. It was widely interpreted and generally accepted that public opposition to GM crops and food is due to a general misperception of potential risks (Gaskell et al. 2004; DeFrancesco 2013). Attempts to address the disconnect between real and perceived risk have led to strategies for broader communication and public education of the technology to clarify the true risk through communication from trusted independent sources.

Interestingly, survey results from regions where acceptance of GM food is low indicate lack of "perceived" benefits as an important factor leading to their mistrust (Gaskell et al. 2010; USDA FAS 2016d; WHO 2005). A study commissioned by WHO (2005) indicated that "people do not react so much to genetic modification as a specific technology, but rather to the context in which GMOs are developed and the purported benefits they are to produce." The survey conducted by the European Commission in 2010 appears to echo the same sentiment. The survey indicated that objections to GM food are related to concerns regarding safety in the context of a lack of perceived product benefit (Gaskell et al. 2010; USDA FAS 2016d). Modern medicines made from GMOs (bacteria and plants) are generally well received (WHO 2005), while GM foods continue to meet strong opposition in many parts of the world. Patients needing medical care place greater emphasis on the benefits of medicines. Modern medicines made from GMOs, such as insulin, growth hormones, and vaccines, come with added, but nevertheless important, benefits of affordability and availability.

Farmers who are dependent on abundant harvests view crop yield, efficacy, reduction in pesticide use, and overall input cost as primary benefits. First-generation GM crops with herbicide tolerance and/or insect resistance traits provided farmers economic benefits because of increased crop yield as a result of improved weed and

pest management as well as reduced input costs. Farmers worldwide in both developed and developing nations where they are free to choose often embrace GM crops. The findings from these early studies showed farmers benefited from the traits in the first-generation GM crops as evidenced by their rapid adoption (ISAAA 2016). Studies comparing yields of adopters and non-adopters showed that smallholder farmers in developing countries have benefited the most, especially in terms of yield, averaging 16% increase in yield for insect-resistant maize, 21% for herbicide-tolerant soybean, and 30% for insect-resistant cotton (Carpenter 2010, 2013). Yield improvement plus reduced input cost drove the value-added profitability which in turn brings social welfare gains.

However, first-generation GM crops did not project the potential benefits easily to consumers. Crop yield impacts commodity price which eventually impacts the food price consumers pay at stores. It has been estimated that consumers realize a significant portion of the total economic benefit of the first-generation GM crops (Carpenter 2013). GM crops containing IR traits provide consumers potential health benefits in addition to cost benefits. IR crops require lower insecticide usage than conventional crops which are impacted by insect pests (Shelton et al. 2002; Qaim et al. 2008). This, in turn, results in lower pesticide residues in food and water. In some circumstances, GM traits can also directly benefit human health. For example, traits such as IR maize controls mycotoxin contamination caused by insect damage to plant tissues (Wu 2006; Qaim et al. 2008). Insect damage predisposes maize tissue to mycotoxin contamination as insect pests create pores through which fungal spores enter maize kernels. Field studies have demonstrated that IR maize contains significantly lower levels of certain mycotoxins, which can cause adverse health effects in humans and livestock (Wu 2006 and references therein). If lack of perceived benefits is an important contributor to low-level acceptance, second-generation GM crops with nutrient quality traits might improve the image, and therefore acceptability, of GM food.

16.4.3 Regulatory and Policy Development

Progression in public acceptance of GM crops and food has been generally less well-received than the developers of these products had anticipated. During the period of 1996–2010, there was an overall downward trend in the percentage of GM food supporters in Europe (Gaskell et al. 2010). In China, public attitude has turned from largely neutral to negative (Jayaraman and Jia 2012; Li et al. 2016). Several food scares and crises in Europe and in China unrelated to GM crops negatively impacted public confidence in food safety and trust in the regulatory bodies charged to protect consumers. Facing increasing consumer skepticism, low level of public trust, pressure from organizations opposing crop biotechnology, and political requirements, authorities in EU and China in particular resorted to legislative changes and regulation enhancements aiming to bolster public confidence in the regulatory processes evaluating GM crops.

However, these new regulatory measures resulted in delayed regulatory approvals of otherwise safe products which result in far-reaching consequences including affecting new product development and innovation, limiting farmer access to useful technologies, and stymieing international trade of these products. In Europe, several major private European plant biotechnology companies relocated R&D operations to the USA because of the more favorable regulatory climate in the USA (USDA FAS 2016d). In 2013, BASF withdrew the application for authorization of phytophthora-resistant potato for food and feed uses, processing, and cultivation. In his assessment of regulatory triggers for products of biotechnology, McHughen (2016) asserted "Science must form the foundation for effective regulation but it is not and should not be the sole determinant of public regulatory policy. Other considerations, such as social policy, ethics, economics, etc., maybe constructed upon the scientific foundation, but they should not drive public policy in the absence of scientifically sound foundation, any more than science alone should direct policy in the absence of these other aspects." The following sections highlight the evolution of the regulatory and policy developments in EU and China and potential impact on the adoption of GM crops.

16.4.3.1 European Union

European environmental policies in 1970s established a regulatory policy that was based on the precautionary principle. This principle emphasizes an awareness of scientific uncertainty about potential negative effects resulting from a phenomenon, product, or process (Freestone and Hey 1996). The concept was later adopted in Directive 2001/18/EC concerning authorization for cultivation, the first major change to EU biotechnology legislation since 1990. Also included in the directive is mandatory post-market monitoring. In the subsequent years, the European Parliament and the Council of the European Union released several regulations, including (1) Regulation (EC) No 1829/2003 concerning authorization for import, distribution, or processing, (2) Regulation (EC) No 641/2004 on implementation of Regulation (EC) No 1829/2003, (3) Regulatory (EC) No 1946/2003 concerning transboundary movements of GMOs, and (4) Directive (EU) 2015/412 allowing member states to restrict or ban the cultivation of EU-authorized GM plants in their territories for nonscientific reasons. These regulations are complemented by 11 guidance documents released between 2005 and 2015. The complicated regulatory procedures and voluminous data requirements delay regulatory submission, risk assessment, and product approval. Delay in bringing products to market and high regulatory cost have particularly large ramifications on continuous innovation and participation by public institutions and small private companies in product development.

Since 1997, EC regulation on labeling requires that products intentionally containing GM ingredients must be labeled, whatever the level of GM content. In 2003, the European Parliament and the Council of the European Union released Regulation (EC) No 1830/2003 concerning traceability and labeling. The traceability and process-based labeling requirements for all food and feed derived from GM plants are

among the most demanding in the world. The labeling requirements are also applicable to highly refined processed oil and sugar in which no trace of introduced genetic material or protein can be detected. GM labeling was intended to give consumers the right to choose. However, amid the region's preference for "naturalness" and negative publicity from those who oppose GM food products, retailers started to avoid labeled food products to protect their market. Generally, requirements that demand traceability and identification of GM food products have a negative stigma attached that discourages their acceptance by retailers which in turn discourages farmer adoption. Romania was one of a handful of countries that adopted GM maize, the only approved GM crop, but farmers have chosen to grow a conventional variety in 2016, amid complex traceability rules (USDA FAS 2016d).

16.4.3.2 China

Facing a huge population and potential food shortages, China positioned agricultural biotechnology as one of the important strategic tools for food security. The government of China invested heavily in biotechnology research and seed development. In 2001, the State Council of China decreed a general policy for regulation of GMO biosafety titled "Regulations on Safety Administration of Agricultural Genetically Modified Organisms" which replaced the first biosafety regulation for agricultural biotechnology issued in 1993 (USDA FAS 2016a). Following the State Council Regulations, the Ministry of Agriculture (MOA) announced a series of implementing regulations. In 2009, MOA issued biosafety certificates for two Bt rice lines and GM maize expressing phytase, paving the way for production trials of GM products in China prior to commercialization. China was on the brink of commercializing a first genetically modified staple crop (rice) and a feed crop (maize) when public sentiment toward GM crops turned negative. Issuance of safety certificates for Bt rice and GM maize by MOA prompted outcries from professionals in humanities and social sciences who signed a public petition asking MOA to withdraw the biosafety certificates (Jia 2010). The petition states "the approval for the commercialization of GM rice and maize enables China to become the world's first country to plant a GM staple food, threatening the national safety." The petition represented one of the most high-profile challenges to China's policy toward the adoption of GM crops. In the following year, the news of golden rice tests in children provoked public outrage amid negative portrayal of the study intent by GMO opponents (Jayaraman and Jia 2012). The test, after a successful trial involving US adults, was designed to assess whether beta-carotene, a precursor of Vitamin A, would be converted efficiently to Vitamin A in children eating golden rice (Tang et al. 2012).

In 2016, MOA revealed a road map for commercialization of GM crops in China. The determined order of priority was as follows: cash crops not for food use, crops with input traits for feed and industrial use, food crops, and finally staple food crops including rice, wheat, and soybean (USDA 2016a). This order of priority indicated Bt rice commercialization would likely remain undetermined in the near future.

Also in 2016, MOA released a revised "Regulations on Safety Administration of Agricultural Genetically Modified Organisms" and guidelines pertaining to the conduct of risk assessment. China's regulatory procedures for GM crops for either cultivation or for importation for food and feed are complicated and lengthy.

This level of complexity has created a challenging environment toward successfully approving products in China. These requirements include approval from the country of origin prior to submission, in-country environment safety and food and feed safety studies, and multiple submissions and assessments of the same product. Presumably, the complicated process and additional requirements were intended to demonstrate the rigor of risk assessment and bolster public confidence in the regulatory system. However, by requiring data in excess of what is typically required for product approval in other nations, this bureaucratically complicated process may inadvertently reaffirm public perception that GM crops are inherently risky.

16.4.4 International Trade Concerns and Policy Decisions

Fear of trade-related impact and loss of access to export markets is another concern for adoption of GM crops (WHO 2005). In parts of Africa and Asia, there is a perception that avoiding cultivation of GM crops might give the region a marketing edge by guaranteeing that agricultural exports are "GM-free." This is especially true of European markets where consumer skepticism toward GM food is relatively high and regulatory climate is particularly challenging. A de facto moratorium imposed by the EU in 1998 on the importation of food products that might contain GMO followed by EU traceability and labeling rules implemented in 2003 did nothing but substantiate the concern that adoption of GM crops would result in a loss of European market access.

In the sub-Sahara region of Africa, the export risks are related to cash crops, including tea, coffee, sugar, bananas, and a wide range of horticultural products (Wafula and Gruére 2013). To date, no GM varieties for these African cash crops are available, nor will they likely become the main driver for commercial interest in the near future given the situation in Africa. Moreover, their fear is that the genetic elements in the GM crops might enter these indigenous crops. However, these fears are unfounded because these crops are not biologically compatible with these cash crops. Export risk was even cited as a reason for rejection of food aid during a famine situation in southern Africa in 2002 (WHO 2005; Gruére and Sengupta 2009). Several countries, including Zambia and Zimbabwe, were concerned that accepting food aid potentially containing GM maize could risk exports of organic vegetables and horticultural products to European markets. However, the perceived export risk is not fully supported by actual trade flows which show only a small trade volume of select products with countries outside Africa (Wafula and Gruére 2013).

In Asia, export concerns for fruits, in particular papaya, were heightened in Thailand when reports of possible gene escape from GM papaya field trials began circulating in 2005. Under pressure from exporters, the Thai Department of

Agriculture instituted a temporary moratorium on all GM field trials (Gruére and Sengupta 2009). In 2006, both the Vietnam food association and Thai rice exporters announced their decisions to ban the use of any GM rice coinciding with the widespread international rejection of US rice out of fear of GM contamination (Gruére and Sengupta 2009). These decisions were largely driven by the concern of rice exports to Europe and Japan. The Thai government responded to the decision by adopting a GM-free clause in the Thailand 2007–2011 rice strategic plan. Elsewhere in Asia, rice exporters in India supported a ban on GM rice for fear of losing market access to Europe and denounced GM rice field trials when the US rice situation was unfolding (Gruére and Sengupta 2009).

Anderson (2010) examined potential economic impacts of GM crop adoption in sub-Saharan Africa and Asia. The study considered several adoption scenarios of GM coarse grains, oilseeds, rice, wheat, and cotton by key countries with or without EU policy response as well as global full adoption. The analyses revealed economic welfare gains by countries willing to adopt GM crops and multiplication of economic gains if next-generation GM crops with traits alleviating nutritional deficiency were to be adopted. More importantly, economic benefits from GM crop adoption by countries in sub-Saharan Africa and Asia would not be greatly impacted by developed countries banning imports of agricultural products from the adopting countries.

16.4.5 Social Concerns

Social concerns involving agricultural biotechnology are complex. Among the concerns expressed by some is that GM crop adoption would put some consumers who assert their wish to maintain a GM-free diet in a position where they are unable to apply their values (Thompson 2000). This expressed concern leads to "right to know" so consumer preference can be considered when it comes to addressing a non-GM food choice. Currently, European regulation requires mandatory labeling of GM food products. The US law permits voluntary labeling of food products containing ingredients from GM crops, but labeling of GM foods is required only if the food has a nutritional or food safety property that is significantly different from what consumers would expect of that food (FDA 1992, 2015). There are important differences between the two labeling systems at technical and practical levels. Mandatory labeling is often used to warn consumers of specific health risks, while voluntary labeling is commonly used to differentiate products for marketing purposes (Qaim 2009). At a practical level, mandatory labeling requires food products containing any GM ingredients above a certain threshold for trace amounts to indicate their presence. There are no specific requirements for voluntary labeling. Labeling requires a system of market segregation and identity preservation which comes at a significant cost to the product. This layering of complex identification has implications on international trade of such products. In the EU, the high degree of complexity, uncertainty, and direct incurred cost because of the labeling and traceability rules provide no incentives to farmers to plant GM crops who are willing to adopt the technology (USDA FAS 2016d).

Despite nearly 20 years of cultivation of a variety of GM crops and intense research on the safety of those crops, there has yet to be any identified significant hazard directly linked to GM crops (Nicolia et al. 2013). Regardless, some remain concerned as to their safety (Verma 2013; Zilberman et al. 2013). Several of these concerns regard the linking of GM crops to potential adverse environmental impacts. The first of these concerns is the "weediness potential" in which engineered plants become agriculture weeds or invasive in natural habitats, displacing other crops or native plants. A second concern focuses on horizontal gene transfer, in which plant genes move into other organisms, genetically altering the compromised plant. Another identified concern is the potential for outcrossing between plants in which traits are transferred from GM crops to wild relatives. Finally, the impact on nontarget organisms, such as beneficial insects, which become exposed to insecticidal traits expressed in GM crops, has also been identified as an expressed concern (Shelton et al. 2002; WHO 2005; Verma 2013; Zilberman et al. 2013).

These concerns may have stemmed from non-GM related events and early preliminary studies involving GM plants. There are cases of non-GM human-released organisms, including plants and animals intended to be used as ornamentals or biological controls, that have become widely established and threaten indigenous organisms in many habitats worldwide (Stemke 2004). Preliminary environmental impact studies on nontarget organisms (Carpenter 2011) and the gene flow study concerning the wild relatives of maize in Mexico (Quist and Chapela 2001) heightened the awareness of potential negative impacts although the findings were unsupported upon further investigations (Carpenter 2011 and references therein). For example, although outcrossing with wild relatives is not unique to GM crops, concern for potential outcrossing of trait(s) from GM maize to traditional landraces and wild relatives of maize in Mexico evokes strong emotions (USDA FAS 2016e). A systematic review of the scientific literature spanning the years between 2002 and 2012 on GM crop safety has failed to detect any significant hazards through the use of GM crops (Nicolia et al. 2013). From a scientific point of view, potential outcrossing can be managed by spatial isolation as demonstrated by Baltazar et al. (2015). However, using the example of maize in Mexico, it is possible to illuminate the complexity involved. In Mexico, maize is a symbol of national heritage and holds culture and tradition values. These factors drive the reluctance to adopt GM maize for cultivation in Mexico, the center of origin for maize. This may explain why bringing GM crops into Mexico may not be easily overcome by local skepticism even if sound scientific persuasion is employed.

16.5 Conclusions

Ever since the development of transformation technology, scientists have made rapid progress. Transgenic traits have been generated in a large number of crops and have been adopted by farmers around the world. The main commercial crops have been soybean, maize, cotton, and canola with some acreage devoted to papaya and sugar beet cultivation. Most of the commercialized traits have been input traits that mainly

include herbicide tolerance and insect resistance. Development of agronomic and output traits have been challenging either due to complex gene interactions that need to be managed to confer such traits or due to poor public acceptance. So far, transgenic crops have been generated with random integration of the genes in the genome. With the advent of designed nucleases that can introduce double-strand breaks in the genome, a new generation of gene-edited products is being developed. With this technology, site-specific gene integrations, single locus gene stacking and genome editing have become a reality. A large number of traits have already been generated using these nucleases in lab-based experiments but have not been evaluated in the field yet. Since the technology can be used to edit endogenous genes to confer the desired traits, such products have the potential to be called non-transgenic and may not be as tightly regulated as the traditional transgenic crops. Though transgenic crops have enjoyed wide commercial success around the world, public perception will continue to affect the demand for such crops into the foreseeable future. Negative public perception in some countries has resulted in tougher policies resulting in prolonged product development timelines. Nevertheless, the science behind the development of genetically modified traits is strong. There is a need for academia and industry to do a better job in educating people so that they can better understand the technology and can make more informed decisions about their food choices.

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