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Genetic Engineering and Editing of Plants: An Analysis of New and Persisting Questions

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Abstract

Genetic engineering is a molecular biology technique that enables a gene or genes to be inserted into a plant's genome. The first genetically engineered plants were grown commercially in 1996, and the most common genetically engineered traits are herbicide and insect resistance. Questions and concerns have been raised about the effects of these traits on the environment and human health, many of which are addressed in a pair of 2008 and 2009 *Annual Review of Plant Biology* articles. As new science is published and new techniques like genome editing emerge, reanalysis of some of these issues, and a look at emerging issues, is warranted. Herein, an analysis of relevant scientific literature is used to present a scientific perspective on selected topics related to genetic engineering and genome editing.

Contents

1. INTRODUCTION

Genetically engineered plants and foods were rapidly adopted by many farmers in the United States and internationally after their introduction in the mid-1990's. But their acceptance by consumers has been mixed amid concerns about human and environmental health. The abundant and often contradictory information available in the media and on the Internet makes identifying fact-based information on the topic difficult. And genome-edited products reaching the market will likely raise some similar and some unique questions.

This review attempts to provide relevant scientific information from peer-reviewed sources to answer certain common questions regarding genetically engineered and genome-edited plants. It is written as a partial update to "Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues," parts I and II (99, 100), and readers seeking additional, although sometimes dated, information might refer to those articles.

The present analysis of evolving and emerging issues considers important topics, such as effects of genetic engineering and genome editing on herbicide use, biodiversity, international trade, and the future of genetic technologies in agriculture. However, the breadth of topics related to genetic engineering and genome editing does not allow for a complete representation of every important issue or inclusion of every study. Efforts were made, though, to accurately represent the range of scientific findings on these topics.

2. WHAT ARE THE DIFFERENCES BETWEEN GENETIC ENGINEERING AND GENOME EDITING?

Genetic engineering uses molecular biology tools to integrate DNA, termed recombinant DNA (rDNA), into an organism's genome in the laboratory. The rDNA being integrated may come from the same species, other varieties of the same species, different species, or even organisms from a different kingdom.

Scientists routinely use a common soil bacterium, *Agrobacterium tumefaciens*, to move rDNA into a plant cell. In nature, *Agrobacterium* transfers DNA from its tumor-inducing (Ti) plasmid into the plant genome for its own benefit. When a plant is being engineered, part of the *Agrobacterium* DNA is exchanged for the scientist's gene of interest. Another method of delivering DNA into a plant genome is through particle bombardment. This delivery utilizes a gene gun, which uses high pressure to propel DNA-coated metal (e.g., gold, tungsten) particles into cells, where the DNA dissociates from the particle and can be incorporated into the genome. rDNA is inserted into the genome at random locations, although genome sequencing after transformation can identify insertion locations and the DNA nearby.

In contrast, more recently developed genome editing techniques are used to edit DNA at precise, targeted genomic locations. To accomplish this, directions to a specific segment of DNA are delivered to the cell along with the materials required for editing. When those materials are successfully delivered and activated, genomic DNA is cut at the target site and changes, or edits, are made to the DNA during its repair. Two major differences between genetic engineering and genome editing are (*a*) the targeted, precise nature of genome editing and (*b*) the capacity for genome editing, in some cases, to edit the DNA without inserting foreign genetic material. A 2019 comprehensive review by Chen and colleagues (32) outlines current applications and future prospects for genome editing in greater detail.

2.1. Strategies for Inducing Site-Specific DNA Breaks for Genome Editing

Researchers took the first steps toward precision genome editing in the 1990s. Zinc finger nucleases (ZFNs) were made by combining two different protein domains: DNA-binding zinc fingers

and a DNA-cutting tool—or restriction endonuclease—called *Fok1* (30, 91). In theory, zinc fingers can be programmed to bind to almost any specific DNA sequence, facilitating a *Fok1*-induced double-stranded DNA (dsDNA) break. In reality, ZFN utility is constrained by the difficulty of engineering zinc fingers that bind to predictable, precise DNA targets (30, 80).

CRISPR-Cas: clustered regularly interspaced short palindromic repeats–CRISPR-associated protein

While the promise and constraints of ZFNs were being explored, plant scientists identified another approach to genome editing using DNA-binding transcription activator-like (TAL) effectors from the plant bacterial pathogen *Xanthomonas*. TAL effectors have sets of repeating amino acids (20, 88, 131), and small variations in these amino acid repeats determine DNA-binding specificity (18, 112, 132). These variations can be programmed to target specific DNA sequences. In 2010, TAL effector nucleases (TALENs), combining the TAL effector DNA-binding domains and the catalytic domain of *FokI*, were first reported to make dsDNA breaks at directed plant genomic loci (34).

In recent years, research attention and funds have largely turned to the CRISPR-Cas system for genome editing. CRISPR-Cas was discovered as an adaptive microbial immune system (11, 19, 23) where a short segment of DNA (protospacer) from an attacking phage is inserted into a special region of the microbe's DNA called the CRISPR array (11, 110, 126). Subsequent invasion by a phage activates the CRISPR array, initiating transcription of the segment of phage-derived DNA into guide RNA (gRNA) (23), which binds to the Cas protein (23). The gRNA/Cas complex detects the phage DNA through complementary binding, which causes Cas to cut the phage DNA, resulting in microbial resistance to the phage (11, 85, 110).

2.2. Genome Editing Can Occur During Repair of DNA Breaks

A simplified version of the CRISPR-Cas microbial immune system is used for genome editing. Researchers introduce the DNA-cutting Cas protein and a carefully designed gRNA into a cell where, together, they find a specific DNA sequence and induce a dsDNA break (85). When dsDNA breaks occur, either homology-directed repair (HDR) or nonhomologous end joining (NHEJ) can repair the break. HDR inserts a piece of DNA with significant homology to the loose ends at the break site into the genome. This piece of DNA is delivered to the cell with the other CRISPR machinery. On the other hand, NHEJ leaves the cell to repair the break without incorporating new DNA. NHEJ is error prone and sometimes results in DNA insertions or deletions that disrupt the reading frame and function of the resulting protein. More recently, CRISPR-Cas has been used to make single-stranded DNA nicks that allow for individual base editing without use of NHEJ or HDR (61, 95) (see Section 9).

3. HAS WIDESPREAD USE OF Bt CROPS LED TO INSECT RESISTANCE TO Bt TOXINS?

While commercial applications of genome editing of crops is in its infancy, crops modified using genetic engineering technologies have been commercially available in the United States since 1995. Insect resistance is one of the most widely engineered traits and is accomplished through the introduction of an insecticidal protein from the bacterium *Bacillus thuringiensis* (Bt). The proteins themselves have been used in commercial sprays by organic and conventional growers since 1938 to control insects that damage crops (135). The most widely used and most studied type of these insecticidal proteins are crystalline (Cry) proteins. After ingestion by an insect, Cry proteins are cleaved, yielding an active Bt toxin that binds specifically to proteins in the larval gut. This binding creates lethal pores in the insect's gut membranes (33, 62). Recent evidence suggests that noncleaved Cry proteins may also be toxic to insects (62, 151). There are hundreds of Bt toxins,

RESISTANCE TERMINOLOGY: TOLERANCE VERSUS RESISTANCE

There is a lack of continuity across disciplines as to the precise definitions of tolerance and resistance when referring to insects or weeds that can survive exposure to an insecticide, such as Bt, or an herbicide, such as glyphosate. Herein, we use the definition of resistance offered by Tabashnik and colleagues (150): genetically based decrease in susceptibility. We thus avoid use of "tolerance" for reader clarity.

including Cry proteins and vegetative insecticidal proteins (Vips), and each protein kills a narrow spectrum of insect species (29, 161).

To provide protection against insect pests and reduce reliance on insecticidal sprays, some crops have been genetically engineered to produce Bt toxins. Corn and cotton were the first commercially available Bt crops, released in 1996. Unlike Bt sprays, which are still widely used by organic farmers, Bt crops can kill, for example, insect pests that bore inside plant stems and are not accessible by sprays. Their use decreases pesticide applications (38, 92, 122) (see Section 3.4), and resulting regional pest suppression can help reduce insect damage to crops of neighboring nonadopters, such as organic growers (57).

3.1. Strategies to Manage and Slow the Evolution of Insect Resistance to Bt

Because Bt crops can significantly reduce insecticide use, preserving their efficacy through careful management is important. Even with such management, insect pests were expected to evolve resistance (see the sidebar titled Resistance Terminology: Tolerance Versus Resistance) to Bt toxins, given their widespread use. To delay insect resistance as long as possible, an insect resistance management strategy, based on high-dose refuge, was implemented in the United States and some other countries for plants with genetically engineered Bt traits. In this strategy, (*a*) plants are engineered to produce a high dose of Bt, such that 95% of insects heterozygous for a resistance trait die, and (*b*) refuges of non-Bt crops are planted nearby to maintain a susceptible insect population. This lowers the frequency of any resistance alleles that evolve in the population (108, 157). When sufficient refuge is planted, a rare insect with homozygous Bt resistance is likely to mate with the more abundant Bt-susceptible insects living on refuge plants. Thus, 95% of resulting heterozygous progeny should be killed by high-dose Bt plants, maintaining a low frequency of Bt resistance in the pest insect population (84, 149).

Putting more than one Bt trait in a single plant, or pyramiding, can delay the evolution of insect resistance and allows for smaller refuge areas (165, 175). The frequency of evolved homozygous resistance to a single-trait Bt plant is one in a million (10^{-6}), whereas developing homozygous resistance to two independent Bt traits is 10^{-12} (27). But, if an insect's resistance to one Bt trait confers partial resistance to a second Bt trait, pyramids lose some efficacy. Pyramids also lose efficacy when grown adjacent to single-trait Bt plants. Such field layouts enable sequential evolution of resistance (118, 174).

Heterozygous: having differences between copies of the same gene within a single organism

Allele: one version of a given gene where multiple versions are found in a population

Homozygous: having both or all copies of the same gene within a single organism be identical

3.2. Implementation of High-Dose Refuge and Pyramids to Delay Insect Resistance to Bt

To fulfill refuge requirements, growers may employ either structured refuges, where non-Bt crops are planted in blocks, or refuge-in-a-bag, in which Bt seed is mixed with 5–10% non-Bt seed. Refuge-in-a-bag allows farmers to comply with a refuge strategy without the extra time or planning required during planting, but it does have a drawback. With corn, which outcrosses,

pollination of a refuge non-Bt plant with Bt pollen creates kernels heterozygous for the Bt trait. Heterozygous Bt kernels then increase the mortality of homozygous susceptible insects and select instead for insects with modest/heterozygous Bt resistance, thereby increasing the frequency of resistance alleles in the population (168).

Regulatory bodies determine refuge requirements based on variables, such as a plant's specific Bt trait(s) and the frequency of insect resistance in the population. In the United States, the Environmental Protection Agency (EPA) controls refuge requirements for each Bt crop based on prevalence of insect resistance, growing region, and specific Bt trait(s). For example, since insects are less likely to evolve resistance to crops with multiple Bt traits, refuge requirements are lower compared to those for plants with a single Bt trait.

Australian refuge requirements were initially quite conservative. In the 1996–1997 growing season, an industry-wide cap of 10% Bt cotton was permitted, effectively creating a 90% refuge (43) because two cotton pests already had mild Bt resistance (43, 103). Furthermore, single-trait Bt cotton was removed from the market as soon as two-trait Bt cotton was available (43). Three-trait cotton is now available and was adopted for >90% of hectares in 2016–2017 in Australia (148).

3.3. Efficacy of High-Dose Refuge and Pyramids

High-dose refuge and pyramid strategies for maintaining insect susceptibility to Bt were initially quite successful, with only three cases of insect resistance reported worldwide by 2005 (148). But, by 2016, the number of reported cases of resistance increased to 16, representing seven unique insect species in five countries (148). Unsurprisingly, in 14 of these 16 cases, the Bt high-dose standard was not met (148).

For the high-dose refuge strategy to be effective, there must be widespread compliance with refuge requirements. Seed producers (registrants) in the United States are responsible for monitoring and reporting grower compliance to the EPA (117). Growers are contractually obligated to plant refuge, and if seed producers find a grower out of compliance for two consecutive years, they must subsequently decline to sell Bt seeds to that grower.

Despite these mandated programs, compliance has dropped over time. For example, compliance among growers of Bt corn used for European corn borer resistance decreased in the United States from 86% in 2002 to 78% in 2008 (155) and has continued falling. Bt corn growers in the southern United States complied at a rate of 42% in 2011 and only 19% by 2017 (156). Poor compliance makes evolved Bt resistance likely to spread more rapidly through insect populations, sacrificing long-term sustainability of Bt crops for short-term benefits.

3.4. Benefits of Using Bt Crops

Because Bt crops produce Cry proteins that are toxic to insects, growers spray less chemical insecticide on their crops (38, 92, 122). Between 1995 and 2009, the kilograms of active ingredient (AI) of insecticide applied to corn in the United States decreased from 8.5 million kg to 1.8 million kg AI (38); the AI applied to cotton decreased from 10.3 million kg to 2.1 million kg (38). While kilograms of AI fail to account for the type of pesticide, its potency, its effects on humans, off-target insects, or the environment, decreases of this magnitude surely benefit the environment.

Other benefits for users of Bt crops include increased yield and subsequent profits compared to nonadopters (56). Increased yields are especially important in developing countries due to food scarcity and undernourishment. An example can be seen in Bangladesh, where Bt brinjal (eggplant), the country's second largest crop, is now resistant to the eggplant fruit and shoot

borer (EFSB). Despite spraying insecticide up to 100 times per season on non–genetically engineered eggplant, farmers still lost 30–60% of their crop to EFSB (141). An India-based company, Maharashtra Hybrid Seed Company (Mahyco), transformed brinjal with the Bt gene for *Cry1Ac* (licensed from Monsanto) and, through a public-private partnership, passed the new Bt brinjal to the Bangladesh Agricultural Research Institute (BARI), which bred the Bt trait into nine common Bangladeshi brinjal lines (141). In 2015–16, the new lines were adopted by 250 farmers. This grew to 6,512 farmers in 2016–17 and 27,012 farmers in 2017–18 (141). In 2016–17, growers realized economic benefits with a net return per hectare that was six times greater than nonadopters' (141), and environmental benefits that decreased the number of insecticide applications from 41 to 11 during the crop's life span (129). To maintain these advantages, several steps should be taken to delay insect resistance. Since BARI lines only have one Bt gene, creating a pyramided line is advisable to maintain EFSB susceptibility. Additionally, effective education programs to encourage compliance with planting refuges are needed to delay development of resistance. Use of such genetically engineered varieties has the potential to decrease chemical reliance, increase grower profits, and enhance food security, but it requires strict stewardship practices.

4. DO GENETICALLY ENGINEERED HERBICIDE-RESISTANT CROPS LEAD TO HERBICIDE-RESISTANT WEEDS?

4.1. Mechanisms of Herbicide Resistance in Weeds

Herbicide-resistant (HR) (see the sidebar titled Resistance Terminology: Tolerance Versus Resistance) weeds emerge and increase in prevalence in response to selective pressure conferred by repeated use of a single herbicide. The first synthetic herbicides, 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4-dichlorophenoxyacetic acid (2,4-D), were discovered in the 1940s. Their discovery initiated decades of research focused on the difficult task of identifying new herbicides with different mechanisms of action, such as inhibition of amino acid production or photosynthesis (97).

The evolution of HR weeds was inevitable given a growing reliance on the repeated use of the same herbicides to control weeds. The first confirmed case of herbicide resistance was observed in a nursery in 1968 (before the development of genetic engineering technology), where the weed common groundsel evolved resistance to atrazine and simazine after ten years of annual or semiannual application (134).

Weeds can evolve resistance to herbicides in different ways (63). Sometimes, a single weed gene coding for a protein targeted by an herbicide mutates such that the herbicide can no longer bind to that target, rendering the weed resistant. Alternatively, a mutation in a transcription factor or promoter can cause higher expression of the protein targeted by the herbicide, necessitating higher herbicide doses to maintain effectiveness. In addition to developing resistance to an herbicide at its target site, weeds can also evolve resistance at nontarget genetic loci. For example, a weed might become better able to sequester the herbicide. Additionally, metabolic resistance can evolve via increased or altered activity of enzyme families that detoxify the herbicide, for example, endogenous cytochrome P450 monooxygenases, glucosyl transferases, or glutathione *S*-transferases (170). Low-dose applications of herbicide contribute to metabolic resistance because weeds with slight resistance to an herbicide might survive and reproduce. Metabolic resistance to one herbicide can, in some cases, also confer cross-resistance to other herbicides. For example, *Echinochloa phyllopogon*, a prevalent weed globally, is resistant to multiple, dissimilar herbicides (170 and references therein). Its resistance is due to higher activity of some metabolic enzymes, as evidenced by herbicide susceptibility after treatment with cytochrome P450 inhibitors. Combined metabolic and target site resistance can also co-occur (16). In a study of 30 diclofop-methyl resistant *Lolium*

rigidum populations in Western Australia, 70% possessed both target-site resistance and metabolic resistance (70).

4.2. Use and Effects of Herbicide-Resistant Crops

Many genetically engineered crops are engineered for resistance to specific herbicides. To date, genome editing has not been used to create commercial HR crops. This might be because HDR (see Section 2.2) is still fairly inefficient; thus, it is easier to genetically engineer herbicide resistance than it is to use genome editing. Many farmers have embraced the engineered varieties because they can chemically manage a wide array of weeds without damaging crops. Understanding the diverse mechanisms of herbicide resistance in weeds is needed to devise effective and sustainable weed management strategies in genetically engineered fields. Herbicides must be used at the right time, at the right dose, and with the correct crop rotations to maintain efficacy. Genetically engineered crops could enable farmers to use herbicides in these ways, but they can also encourage the continuous use of single herbicides if their crop variety of interest has only one herbicide-resistance trait. In this case, having that single resistance trait greatly reduces the duration of herbicide efficacy.

HR crops, used either singly or in combination with an insect-resistance trait(s), so-called stacked traits, comprised 88% of global genetically engineered crops in 2016–2017, based on land area (81). Most current HR crops are engineered for resistance to glyphosate (the AI of Roundup), a broad-spectrum, systemic herbicide. As a result, use of glyphosate-based herbicides in the United States increased 14.6-fold between 1995—the year before glyphosate-resistant crops were commercialized—and 2014 (17). In the first ten years of HR soybean cultivation (1996–2005), the rapid adoption of glyphosate-resistant soybeans and glyphosate-based herbicides in the United States resulted in a decrease from 19 herbicides to 1 that was used on more than 5% of soybean acreage (21).

One measure of pesticide impact is the Environmental Impact Quotient (EIQ), which considers a range of criteria related to farmworker, consumer, and environmental health and safety (96). EIQ is not an optimal comparator, because it uses a discreet number to express probabilities from many variables (94, 124), but it is still a useful indicator of overall impact of a pesticide. The EIQ of glyphosate is 15.3 (15), lower than that of some of the herbicides it replaced (e.g., cyanazine, 20.06) (93, 96, 122). Based on EIQ, glyphosate is a relatively benign herbicide environmentally.

Glyphosate was used regularly before genetically engineered crops. In fact, the first reported instance of glyphosate resistance was in an Australian orchard in 1996 where glyphosate was used to control weeds between trees (127), even though no genetically engineered crops had been grown on that site. By the beginning of 2005, the number of species of weeds with at least one instance of glyphosate resistance grew to 9 worldwide and subsequently increased to 43 globally by 2019 (21) (**http://www.weedscience.org**), correlating with increased use of glyphosate-based herbicides and glyphosate-resistant crops.

Some countries in South America, particularly Brazil and Argentina, have very high adoption rates of genetically engineered soybeans, maize, and cotton (81). In 2017, 97% of Brazil's soybeans and 100% of Argentina's soybeans and cotton were genetically engineered for herbicide resistance, often stacked with insect resistance (81). Of South America's 58 herbicide-resistant weeds reported in soybean fields by 2017, 26 had resistance to glyphosate (123 and references therein). Problematic glyphosate-resistant weeds include *Sorghum halepense* (Johnson grass), *Digitaria insularis*, *Conyza* spp., and *Lolium multiflorum* (123). Glyphosate-resistant *L. multiflorum* was reported in southern Brazil in 2003–2004, leading growers to use alternative mode-of-action herbicides, acetyl CoA carboxylase (ACCase) or acetolactate synthase (ALS) inhibitors, prior to sowing

genetically engineered soybean or corn crops. In 2010–2011, *L. multiflorum* with resistance to both glyphosate and ACCase inhibitors was reported, followed in 2017 with reports of *L. multiflorum* with resistance to both glyphosate and ALS inhibitors (123).

While there has clearly been a shift toward weed resistance to glyphosate, the overall evolution of species with resistance to a given herbicide group has been consistent since about 1980—15 years before genetically engineered crops were introduced commercially (**http://www.weedscience.org**). Since 1980, about 11 weed species each year have developed resistance to some herbicide (73). Nevertheless, the number of glyphosate-resistant weed species is increasing more quickly than weed species with resistance to other herbicides, with the exception of weeds resistant to ALS inhibitors (**http://www.weedscience.org**).

Weed shift can occur when a single mode of action of weed control is used over time, causing a change in the weed population (119). This derives from the fact that species with high susceptibility to a particular mode of control decrease over time, while species with any innate tolerance to that mode of control increase over time, potentially becoming more problematic and rendering that mode of control ineffective.

In the United States and South America, crops such as genetically engineered soybean are highly unlikely to outcross to a wild relative, because the cultivated crop is not grown near sexually compatible relatives. Other crops, like sorghum and rice, grow close to weedy relatives (although at the present time no commercial genetically engineered varieties of these crops are grown in the United States). Under such conditions, outcrossing of an HR crop with its neighboring weed facilitates easy transmission of herbicide resistance to the weed (see Section 6). There are strategies that could be used to overcome this challenge (64). One strategy is to transform cultivated HR crops with an additional genetic element that incurs a fitness expense to the weed but is neutral or beneficial to the cultivated crop (65).

4.3. Durable Weed Management

Using multiple methods of weed control prolongs a given method's efficacy. Crops with multiple herbicide resistances in the same plant, such as soybeans that have both glyphosate and glufosinate (e.g., Liberty) resistance, can be used to slow or prevent the evolution of HR weeds if growers alternate the use of the two herbicides. Herbicides with different modes of action are useful to growers, but their utility will be short-lived without care to slow the emergence of HR weeds. Alternative approaches to weed control can be used in concert with specific herbicides to preserve their efficacy (14). For example, integrated weed management supports the use of diverse weed control techniques, such as cover cropping, crop rotation, biological control, and tillage (73). Tillage is a strategy where soil is disrupted or overturned to prevent weed growth, but it needs to be used carefully since it can also be detrimental to soil health. While implementing integrated weed management practices may incur initial costs of time, money, and labor, over the longer term, delaying herbicide resistance in weeds will be worth it.

5. DO CROPS CREATED WITH GENETIC ENGINEERING AFFECT BIODIVERSITY, AND HOW MIGHT GENOME EDITING AFFECT BIODIVERSITY?

Crop diversity within and between farms increases valued ecosystem qualities, such as pollinator habitats and protection from diseases and pests. Adoption of genetically engineered varieties can decrease biodiversity if growers abandon diverse crops or crop rotations to grow the limited genetically engineered species and varieties. Between 1978 and 2012, regions of the United States

including the heartland, eastern uplands, and southern seaboard, which currently grow predominantly corn, soybeans, and/or cotton, saw crop diversity decrease (2). Although this decline began before genetically engineered crops were introduced, the availability of these higher-yielding, higher-profit-margin crops (92), once available, might have influenced growers' decisions. Concerns have also been raised over the effects of specific genetically engineered traits on biodiversity. For example, high adoption of Roundup Ready varieties of corn and soybean has led to increased use of glyphosate-based herbicides that could potentially affect growth of wild plants, animals, insects, or microbes. Additionally, Bt varieties, while toxic to specific targeted insects, should also be, and are, assessed for effects on nontarget organisms.

Broad use of genetically engineered crops can also contribute to biodiversity if, for example, it enables specialized crop rotations. Use of an herbicide on an HR row crop during one season can provide enough weed suppression on a given piece of land to enable subsequent growth of non–genetically engineered vegetable crops for which weeds were previously a prohibitive consideration (114). Genetically engineered plants might also increase biodiversity through insertion of resistance traits into varieties that had been abandoned due to biotic or abiotic stress susceptibility (12). However, regulatory costs associated with commercializing genetically engineered crops have limited their use in enhancing genetic diversity. In the United States, the current reduced regulations and associated costs of commercializing some genome-edited crops, compared to genetically engineered crops (see Section 8.1), might facilitate the introduction of edited plants with regionally beneficial adaptations or increased diversity.

5.1. Effects of Bt Crops on Nontarget Organisms

Bt crops take advantage of the precision of Bt proteins to protect plants from specific pests while not harming most nontarget insects (see Section 3). Although Bt proteins are highly specific in their interactions with proteins in the insect gut, it is possible for them to affect nontarget but susceptible insects if proteins in the nontarget insect's gut bear high similarity to those in the target insect's gut. Therefore, studies of the effects of Bt crops on nontarget organisms and on field organismal diversity are an important part of environmental risk assessments (for more on risk assessment methodology, see 136).

There are hundreds of Bt toxins that have highly specific insecticidal effects. A 2016 review of studies analyzing effects of different Bt toxins on nontarget organisms presented little evidence for adverse effects on nontarget insects (169 and references therein). For example, research on the effects of the specific Bt toxins Cry1Ac and Cry2A on the nontarget paddy grasshoppers, of Cry1Ac on honey bees, of Cry1Ab on aphids, and of Cry1Ab and Cry3Bb1 on earthworms found no meaningful evidence of harm. Conflicting results were presented for lady beetles, nematodes, and monarch butterflies. For lady beetles, the conclusion of negative effects of Bt Cry1Ab and Cry3Bb was suspect due to poor study design, given that mortality was not dose dependent for Cry1Ab and control insect populations had high mortality (3, 139). For nematodes, data on Bt Cry1.105, Cry2Ab2, and Cry3Bb1 indicated that effects were only seen at doses greater than what would be expected to be present in soil (76). Detailed studies have explored effects of Bt on monarch butterflies; for details, see the National Research Council report *Genetically Engineered Crops: Experiences and Prospects* (114). To summarize those studies, only one transgenic Bt maize variety, Bt176, seemed to pose a risk to monarchs because of exceptionally high Bt content; it was removed from the market (114). However, there is evidence that milkweed populations, which monarchs need to lay eggs, have declined as a result of increased use of herbicides on HR crops (125).

Some researchers have surveyed arthropod abundance and diversity in Bt and non-Bt fields. One study found no significant difference in arthropod abundance for Bt Cry1Ac cotton fields

versus non-Bt cotton fields; however, they did find decreased diversity in Bt plots (142). A field study of Bt Cry1Ie maize and its near-isogenic counterpart found no genotype-dependent changes in soil fauna diversity, abundance, or community composition (54). Another study analyzed arthropod diversity in postharvest fields with Bt rice straw versus non-Bt rice straw and found no consistent effect on diversity (9). Other studies analyzing overall arthropod diversity included an analysis of a likely alternative to Bt crops: non-Bt crops sprayed with insecticide. Comparing sprayed and unsprayed Bt Cry1Ab sweet corn and sprayed and unsprayed non-Bt sweet corn, researchers found that growing season, location, and insecticide treatment caused changes in arthropod diversity, but use of Bt versus non-Bt varieties did not (133).

Use of Bt crops to control one plant pest population could shift ecosystem niches and inadvertently cause impacts on secondary plant pests. For example, a primary pest might moderate the population of a secondary pest through competition in the absence of Bt. Introduction of Bt through GE crops or through insecticidal Bt sprays decreases the population of the primary pest, allowing the secondary pest population to grow. Consider that the territory of the western bean cutworm, not a target of Bt, has expanded since 2000 from the western US Great Plains to the eastern United States, possibly due to reduced competition from Bt-susceptible corn earworms (28 and references therein). In support of that observation, survival of western bean cutworm was much higher, when grown in the lab or a controlled field environment with corn earworm larvae that inhabited Bt crops, than on non-Bt crops (42). This suggests that suppression of corn earworm populations by Bt crops could have been one factor in the expansion of western bean cutworm territory (42).

Nontarget organisms that eat Bt plants could bring Bt into the wider agroecosystem, even if they themselves are unaffected, making multitrophic experiments important. In one study, spider mites and aphids were fed on maize plants expressing six Cry proteins, five of which were measurable with ELISA (147). Then, those spider mites or aphids were fed to lacewing larvae, lady beetle larvae, or juvenile spiders. All five measurable Bt proteins (Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, and Cry34Ab1) were detected in the lacewing larvae, lady beetle larvae, and juvenile spiders, demonstrating that Bt was passed up the food chain (147). However, the presence of these Bt proteins, even in combination, did not affect mortality, weight, or development of lacewing larvae, lady beetle larvae, or juvenile spiders (147). Another study on aphids came to a different conclusion, finding that lady beetles fed aphids from Cry3Bb maize did have lower survival rates than aphids fed on non-Bt plants or (notably) plots treated with tefluthrin, an insecticide (145).

Isogenic: having nearly identical genotypes; here, the difference between the two lines is the introduced gene

Secondary pest: a pest not targeted by a given insect control method (e.g., Bt) that becomes more prevalent when that method is used

ELISA (enzyme-linked immunosorbent assay): used to detect and quantify substances, such as proteins

Technical glyphosate: refers to the glyphosate compound itself, while glyphosate-based herbicides include additional compounds, such as adjuvants

5.2. Effects of Genetically Engineered Crops on Honey Bees

Western honey bees (*Apis mellifera*) are a culturally important part of modern food systems for both their role as important pollinators of food crops and their honey. The ambiguous cause of colony collapse disorder, where worker bees desert the colony, spurred research to identify culprits of this phenomenon. Studies of bees in Bt and conventional maize fields found that Bt pollen did not influence bee survival rate or body weight (41, 75) and thus was not a likely culprit in this disorder.

Another negative effect of genetically engineered crops on honey bees could result from the increased use of glyphosate on glyphosate-resistant genetically engineered crops. A 2014 study tested effects of a range of glyphosate levels on brood and adult bee mortality, finding no negative effects from technical glyphosate (152). It should be noted that some authors of this study were from companies that sell glyphosate-based herbicides. But, other work supports their findings, identifying no change in honey bee survival when exposed to a glyphosate-based herbicide (74). In a widely publicized 2015 study, bees fed sucrose with 10 mg/L of technical glyphosate took

more time to return to hives from a release point (10); however, with a sample size of eight bees in the affected group, robust conclusions cannot be drawn.

Phylum: a broad taxonomic group used to classify organisms

Mesocosm: a controlled outdoor experimental system that serves as a middle ground between field experiments and laboratory experiments

In 2018, some studies focused on possible effects of glyphosate on honey bee gut microbiomes (40, 113). In one study, total bacteria, as measured by 16S rDNA copies, were not consistently altered by technical glyphosate, although one bacterium, *Snodgrassella alvi*, had non-dose-dependent changes in abundance (113). In another study, it was found that gut microbial diversity decreased in honey bee larvae when their diet included 20 mg/L technical glyphosate, although the authors acknowledge this dose is "unlikely to be encountered in the field" (40, p. 7787).

5.3. Effects of Genetically Engineered Crops on Soil Microbial Communities

Plants have close associations with microorganisms growing near or within their roots, and as a result, proteins and metabolites are exchanged between the two. Thus, microbial communities near roots of genetically engineered plants have been studied for any effects that Bt proteins or glyphosate resistance proteins might have. Overall, Bt plants do not have a consistent, adverse effect on soil microorganism community structure or diversity (102, 106, 162). Also, effects of decomposing Bt plants after harvest have been tested for potential harm to soil microorganisms, but no significant changes in microbial communities were found (55, 105). Glyphosate-resistant plants, when compared to near-isogenic lines, did not affect the biomass or basal respiration rates of the soil microbial communities where they grew (8). In that study, shotgun sequencing showed that, at each of two research sites, genetically engineered varieties had higher species diversity, but changes in phylum relative abundance were always less than 1.5% (8).

Glyphosate inhibits the shikimic acid pathway in plants, and although animals do not have an equivalent pathway, some microbes do. Thus, the soil microbiome is likely to experience some effects when plants are sprayed with glyphosate, either through glyphosate landing on the soil surface or from its being exuded from plant roots into the rhizosphere, which is a region of soil in close proximity to plant roots (98). Researchers have tried to measure effects of glyphosate-based herbicides on soil-based and rhizosphere microbial communities. But the inherent complexity of measuring such effects across vast numbers of ecosystems with different microbial structures and environments makes drawing robust conclusions difficult. Furthermore, diverse methods and protocols have been used, making comparisons between available studies difficult. Despite those difficulties, an increase in microbial respiration or activity as measured, for example, by microbial production of CO_2 is consistently observed after application of glyphosate-based herbicides to plants or soil (24, 71, 109). Some research identified small changes to soil microbial community structure after treatment of plants or soil with glyphosate-based herbicides, although those changes are not consistent across studies and are small compared to factors like sample proximity to roots and geographic location (24, 68, 137, 138). For further review, see Reference 44.

5.4. Effects of Glyphosate in Aquatic Habitats

Some research suggests that glyphosate runoff can affect aquatic life. A realistic risk assessment of such effects must account for observed concentrations of glyphosate in waterways. Surveys of glyphosate concentration in groundwater, drinking water, surface water, streams, rivers, and/or lakes were done in regions of Mexico, the United States, Canada, Switzerland, and Argentina (7, 13, 39, 72, 130, 146). Highest glyphosate concentrations across many measurements in each study ranged from 0.00142 to 0.43 mg/L. Thus, reports of effects on aquatic life by glyphosate must be considered in the context of relevant concentrations. Experiments in mesocosms in Argentina found that with 8 milligram AI/L glyphosate formulation, populations of diatoms decreased while

populations of cyanobacteria increased (163). Juvenile *Galaxias anomalus*, a freshwater fish species, was held in aquaria and was unaffected by 0.36 mg AI/L glyphosate formulation. But, they suffered higher mortality rates when exposed to the combination of glyphosate and the parasite *Telogaster opisthorchis* (89), a situation that could occur in nature.

Given the likely concentration of glyphosate in bodies of water and the high doses needed to induce negative effects, glyphosate would seem to rarely have deleterious effects on aquatic populations. Nonetheless, efforts should continue to keep glyphosate out of waterways.

Transgene: a gene from the same or another organism inserted into an organism by recombinant DNA (rDNA) techniques

5.5. How Might Plant Genome Editing Affect Biodiversity?

Biodiversity is also important at the genetic level. Genetic variation is at the heart of plant breeding, but huge swaths of that genetic diversity have been lost through the process of domestication. Researchers are using genome editing to reintroduce that diversity. One approach is to edit genes in domesticated plants to have the alleles of their wild relatives. Another approach is to edit wild varieties for de novo domestication. For example, researchers have identified many mutations in domesticated tomatoes that make fruits more plentiful, tastier, and bigger in addition to being easier to grow, care for and harvest than their wild counterparts. De novo domestication might be employed to generate crops with practical traits for commercial production while maintaining the wide diversity of biotic/abiotic stress resistance, environmental adaptations, flavor, and nutrition of wild varieties (101, 176).

6. WHAT ARE THE ENVIRONMENTAL CONSEQUENCES OF TRANSGENES AND/OR TRANSGENIC PLANTS ESCAPING CULTIVATED AGROECOSYSTEMS?

Genes from crop plants commonly enter wild populations through pollen dispersal, and crop species themselves can become feral through seed dispersal and vegetative propagation (e.g., 78). Thus, before transgenic plants are grown in the environment, the likelihood of a specific transgene or transgenic crop becoming feral and the resulting environmental impacts should be evaluated.

Over a thousand free-living plant populations have been found to contain transgenes; however, it is not entirely clear how many are transient and how many persist (45). The establishment and persistence of escaped transgenes depend on the frequency of outcrossing events, the phenotype conferred by the transgene, and the fitness of the free-living, transgenic line in the environment (104). Environmental impacts might include outcompeting native species, increased weediness, and/or effects on crop quality and yield (77).

In 2011, a panel of experts expounded the potential risks of transgene flow from Bt-cowpea in West Africa (77). The panel agreed that transgene flow would almost certainly occur in areas where Bt-cowpea was planted near wild cowpea and therefore considered the potential environmental impacts. Bt could increase the fitness of wild cowpea by decreasing its susceptibility to the targeted pest. Concurrent increased fitness could make wild cowpea more consistently weedy in agricultural fields. If not properly contained, wild Bt cowpea might grow to maturity with the cultivated Bt cowpea in the field and get mixed in with the harvest, reducing that harvest's quality. In addition, if Bt provided a selective advantage to wild cowpea outside of agricultural fields, the abundance of other valued wild species in the ecosystem could possibly decrease. The panel judged these outcomes to be quite unlikely but suggested additional experimentation to further elucidate environmental impacts.

Many transgenes that have entered the environment to date have not caused environmental harm, but some have. For example, in Oregon, USA, in 2003, mature glyphosate-resistant creeping

bentgrass drying in the field met with strong winds, dispersing mature seeds great distances (171). Its pollen can also travel far, reaching a maximum distance of 21 km (164). Despite attempts to find and eliminate glyphosate-resistant creeping bentgrass, it has become prevalent in some counties in Oregon and Idaho (171, 172). In 2017, the US EPA approved special, localized use of glufosinate, an herbicide unrelated to glyphosate, to help mitigate the problem of canals being overgrown with glyphosate-resistant creeping bentgrass (45).

Precautionary principle: suggests that when some effects of a product are unknown, restraint should be used even in the absence of evidence of negative consequences

Multiple herbicide resistances in *Brassica napus*, which is used to make canola oil, present another issue. Conventional and transgenic *B. napus* varieties disperse large numbers of seed in the soil that are able to germinate in subsequent years and present themselves as weeds that growers must manage, just as they manage other weeds. As a wind-pollinated plant, *B. napus* also readily outcrosses, resulting in the spread of traits such as herbicide resistance—transgenic or otherwise. For example, planting three *B. napus* varieties, one with transgenic glyphosate resistance, one with transgenic glufosinate resistance, and one with nontransgenic imidazolinone resistance in close proximity resulted in volunteer *B. napus* with resistance to these three diverse herbicides in just 18 months (69). When *B. napus* accumulates multiple herbicide resistances in single plants, farmers have fewer options for managing feral populations. Because there are *B. napus* varieties with nontransgenic herbicide resistance, this problem is not solely brought about by genetically engineered varieties. However, use of genetically engineered crops has increased the number and acreage of herbicide-resistant varieties, thus increasing the opportunity for establishment of wild, herbicide-resistant *B. napus*.

Tracing the movement of most transgenes in a wild population or in a nontransgenic, cultivated population is straightforward using technologies like DNA sequencing or PCR, although costs of such analyses are not trivial and require trained professionals. Many edited genes will be much more difficult to track, specifically when the genome editing results in a simple mutation without inserted DNA fragments. Such edits are indistinguishable from naturally occurring mutations. From a scientific point of view, this indistinguishability suggests that these types of events need not be of more concern than those generated by naturally occurring mutation processes. However, additional considerations, such as economic risk, might be of importance based on domestic and international export and import pressures (see Section 7).

7. HOW IS THE INTERNATIONAL TRADE OF GENETICALLY ENGINEERED PLANTS AND FOODS REGULATED?

Global trade of genetically engineered crops is largely controlled by two international agreements. The first, The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), was created by the World Trade Organization in 1994 and delineates trading parameters for genetically engineered organisms (167). The second, The Cartagena Protocol on Biosafety to the Convention on Biological Diversity (The Protocol), focuses on biosafety and guidance for moving "living modified organisms" (140) between signatory countries (116). The Protocol was agreed to in 2000 and has been signed by many countries that are also members of the World Trade Organization. There is some tension between the SPS Agreement and The Protocol, particularly as relates to the precautionary principle. The SPS Agreement stipulates that restrictions on genetically engineered imports must be based on scientific evidence of risk to human, animal, or plant health (167). The Protocol allows restrictions to prevent "potential adverse effects," even when scientific evidence does not suggest the existence of such effects (140).

Developing and least developed countries can be influenced by their major export markets to embrace or eschew genetically engineered crops (66). For example, the European Union (EU) is one of the largest export destinations for many African countries. Because of strict EU

regulations, some African governments ban genetically engineered crops in part for economic reasons (35, 120), even in times of famine (107). Studies suggest that, despite such trade losses, adoption of genetic engineering technology would still benefit many developing countries (5, 31). A review of 27 peer-reviewed papers measuring or modeling the welfare effects of genetic engineering adoption reached a similar conclusion—adoption of genetic engineering technology generally yields economic benefits (67).

Mutation breeding: intentionally mutagenizing a seed's DNA to induce a desirable mutation(s)

However, individual players in different economies might have different results. Adoption of genetically engineered crops can increase global supplies and lead to price declines (22, 67). This can negatively affect producers unless an increase in production compensates for any decrease in price. Consumers stand to gain from these lower prices. Depending on various factors, producers who do not adopt the technology might be positively or negatively impacted by the entrance of genetically engineered crops to the marketplace. On the one hand, they may need to remain competitive with genetically engineered crops while not reaping their benefits (67). On the other hand, they might see price increases for non–genetically engineered products when importers highly regulate or ban genetically engineered crops, or when a separate market emerges for non–genetically engineered products (67).

Regardless of economic consequences, some developing and least developed countries might not grow genetically engineered crops because they lack the legislation and/or regulatory infrastructure required to complete standard safety and risk assessments before commercialization (1).

Some countries with genetically engineered import restrictions test imports to ensure compliance. Although such testing is relatively easy and straightforward for genetically engineered products (50), small DNA changes made with genome editing will be more difficult to test. Furthermore, differentiating DNA changes made with genome editing from DNA changes made by spontaneous mutations or mutation breeding will be impossible (60). Will countries with bans on genome-edited products accept the risk that imports from certain countries could have been genome edited, or ban imports from those countries outright (86)?

8. WHERE ARE GENETICALLY ENGINEERED CROPS GROWN INTERNATIONALLY, AND WHAT REGULATORY FRAMEWORKS GOVERN THEM?

As of fall 2018, 43 countries plus the EU have approved at least one genetically engineered crop variety for cultivation, food, and/or feed (79). Thirty crop species have at least one genetically engineered variety approved for cultivation, food, and/or feed in at least one country (79). How do individual countries make decisions about growing and/or importing genetically engineered crops?

8.1. United States

In the United States, genetically engineered products have been regulated on a case-by-case basis by a combination of the oversight of the US Department of Agriculture (USDA), the EPA, and/or the Food and Drug Administration (FDA), as outlined in the Coordinated Framework for Regulation of Biotechnology (53).

USDA's regulatory authority derives from its responsibility to monitor plant pests or anything that could act as a plant pest. The USDA must authorize outdoor growth of genetically engineered plant varieties during research and development and evaluate the potential of genetically engineered plants to pass transgenes into wild populations, to become weedy, to change plant metabolism, or to have other impacts.

If evaluation of data indicates a genetically engineered plant poses no risks, the USDA deregulates the variety, allowing it to be sold commercially without further oversight.

In March 2018, the USDA announced that it will not regulate genome-edited products that are not plant pests and contain no foreign DNA (see Section 2.2). Developers who are unsure about the regulatory status of a new edited variety can submit a letter of inquiry to the USDA, providing details about the variety and the technology used to develop it. As of April 2019, 77 such inquiries had been received. In the future, it is uncertain whether all companies will continue to submit these inquiries. If not, tracking genome-edited seed and distinguishing it from conventionally developed seed might be difficult.

In June 2019, the USDA proposed new rules to streamline its regulatory process for genetically engineered and genome-edited plants. The rules would exclude from regulation plants that could have been made with traditional breeding. If adopted, the rules will also base regulation on the familiarity of the USDA with the technology, trait, or event.

The FDA is responsible for food safety and, under the Federal Food, Drug, and Cosmetic Act, food producers are legally liable if their products cause harm. The FDA is not required to approve safety of a genetically engineered product before it enters the market; however, its rigorous consultation process is considered a de facto requirement by developers of genetically engineered crops (114). Products are assessed for potential allergenicity or toxicity, substantial equivalence of the genetically engineered food to its parental variety (excepting the introduced gene product), expression and activity of the engineered gene, and other criteria (160). The safety standard requires "a reasonable certainty that the substance is not harmful under the conditions of its intended use" (53). The FDA plans to release a draft guidance in late 2019 detailing its approach to genome-edited foods (158).

The EPA regulates pesticide use. Genetically engineered varieties with insecticidal traits, such as those producing Bt toxins (see Section 3), are themselves considered pesticides, and therefore the EPA must evaluate them. The EPA regulates how much of a given pesticide, like Bt, is acceptable in food, feed, and the environment, and all pesticides pass through an EPA registration process before distribution. Developers of genetically engineered plants with pesticidal properties must provide "extensive scientific data and information on the potential health and environmental effects of a pesticide" (53). There are consequences to the fact that genetically engineered plants are considered pesticides, e.g., any nursery propagating a genetically engineered Bt variety must be certified and registered as a pesticide producer.

On June 11, 2019, President Donald Trump signed an Executive Order requiring the USDA, FDA, and EPA to identify ways to streamline regulations under their jurisdictions, and to "exempt low-risk products of agricultural biotechnology from undue regulation" (153). It will take some time to see what regulatory changes result from this Executive Order. One step in that direction was taken on January 9, 2020, when the USDA, FDA and EPA announced the launch of the Unified Website for Biotechnology Regulation (**https://usbiotechnologyregulation.mrp.usda.gov/biotechnologygov/home/**), which streamlines information from these three agencies charged with overseeing regulation of agriculture biotechnology products and is addressing in part President Trump's Executive Order on modernizing the regulatory framework for these products.

8.2. Canada, Australia, and Brazil

In Canada, plants with a novel trait (PNT) are regulated in the same way, whether they are created by genetic engineering, mutagenesis, or traditional breeding (143). PNT regulations are triggered when plants have potential to cause environmental harm and when the trait is not currently present, or is present at a significantly different level, in plant populations cultivated

within Canadian borders (25). A significantly different level has not been defined, although many breeders assume that a 20–30% change in a gene's expression is the threshold for triggering PNT regulations (46, 143). Explicit guidelines might be useful for breeders using genome editing to change gene expression. Furthermore, an edited gene might be expressed at the same level but have decreased functionality due to an edit. Regulatory standards should become clearer as The Canada Food Inspection Agency and Health Canada, which oversee and evaluate PNTs, release decisions on plants submitted for their consideration.

In April 2019, Australia announced that plants (and animals) made with genome editing would not be regulated as long as they used NHEJ and did not use HDR (see Section 2). Its regulation of genetically engineered organisms is under the purview of the Office of the Gene Technology Regulator.

In Brazil, regulation of genetically engineered organisms by the National Biosafety Technical Committee (CTNBio) is based on risk assessment, but those decisions can be overturned for social or economic reasons by the National Biosafety Council (6). In January 2018, CTNBio announced it will not regulate plants created with "Precision Breeding Innovation" (e.g., genome editing) like it does traditional genetically engineered products (115). However, CTNBio must be consulted to affirm that Precision Breeding Innovation, not genetic engineering, was used to make the new variety (115). In 2018, a genome-edited variety of sugarcane was approved without passing through traditional genetic engineering regulations (144).

8.3. European Union

Some places take a more process-oriented or precautionary approach to regulation, like the EU. In the EU, the genetic engineering regulatory process begins with a notification to the designated authorities of a Member State (country), detailing the specific genetic modification, its intended use (for cultivation and/or food and feed, which dictates the specifics of the regulatory pathway), and safety information (36, 37). Then, the European Food Safety Authority (EFSA) issues an opinion, based on a risk assessment, that goes to the European Commission (EC). That body drafts a decision that is sent to a committee of representatives from each of the EU Member States for a vote, where, interestingly, the Member States have never reached a qualified majority for or against an EC draft decision (47). The next step is reconsideration by an Appeal Committee of Member States, where the usual vote is "no opinion" (e.g., 49). Their reasons cannot contradict the EFSA risk assessment without scientific evidence. A stalemate sends the final decision back to the EC, but regardless of the EC decision, any Member State can ban cultivation of genetically engineered varieties within its borders. Currently, only one genetically engineered variety, Bt maize, is authorized for cultivation in some EU countries, and currently it represents just 1.5% of EU land used for maize cultivation (48). Around 60 genetically engineered crop varieties are authorized for import as food or feed (not cultivation), and most of them are used for livestock feed. The few commercial foods with genetically engineered ingredients are, by law, labeled if they exceed 0.9%, and the genetically engineered crop and food require traceability (136).

In July 2018, the European Court of Justice ruled that ". . .organisms obtained by means of techniques/methods of mutagenesis constitute genetically modified organisms. . .," meaning that genome-edited seeds and products are subject to genetic engineering regulation, although products of mutation breeding are allowed by exemption (52). As a result, some plant breeding companies, large and small, have moved operations out of Europe (144). In November 2018, the EC's Group of Chief Scientific Advisors concluded that, based on a scientific perspective, this decision should be reconsidered (51). Further analysis of the EU regulatory system can be found in a 2019 review by Schiemann and colleagues (136).

8.4. China and India

Regulation of genetically engineered products in China is based on the 2001 "Regulations on the Administration of the Biosafety of Agricultural Genetically Modified Organisms" decree issued by the State Council (26). Every genetically engineered agricultural product must pass a safety assessment and receive a biosafety certificate and a license from the Ministry of Agriculture before commercial cultivation begins (26, 166). Only genetically engineered cotton and genetically engineered papaya are widely cultivated legally (166). Bt rice received a biosafety certificate but has not received a license. However, genetically engineered rice has been consistently found by EU officials in Chinese exports (50, 166).

In India, the Genetic Engineering Appraisal Committee (GEAC) within the Ministry of Environment, Forest and Climate Change (MoEF&CC) makes regulatory decisions on genetically engineered products, including approval of genetically engineered cotton in 2002. Although genetically engineered brinjal (eggplant) received GEAC approval in 2009, just prior to its release in 2010, MoEF&CC imposed an indefinite moratorium in response to public opposition (82, 128). [Bt brinjal has been used successfully in Bangladesh; see Section 3.4 (141).] The GEAC declared genetically engineered mustard (*Brassica juncea*) "safe for consumption" in May 2017, but remaining concerns about environmental safety resulted in additional GEAC-approved field studies to further study the issue (83). A decision on regulating genome-edited crops has not been announced.

More information about international regulation can be found in recent reviews (46, 136, 144).

9. WHAT IS THE FUTURE OF GENOME EDITING IN PLANTS?

The first genome-edited food ingredient to enter the US food supply chain was a soybean oil made from the Calyxt High Oleic Soybean. This soybean, edited with TALENs (see Section 2.1) to have reduced saturated and trans fats, was first sold to restaurants in the Midwest in early 2019. More than 17,000 acres of the new soybean variety were grown in 2018, and that number doubled to 34,000 acres in 2019. The Calyxt High Oleic Soybean went through the voluntary FDA consultation process, which raised no food safety issues (159). It also went through the USDA "Am I Regulated?" consultation process to determine its regulatory status. The USDA determined that because this soybean has no introduced genetic material and is not a plant pest, it is not regulated as a genetically engineered plant (58).

Publicly available "Am I Regulated?" consultation letters with the USDA provide a glimpse into what nontransgenic genome-edited varieties might soon be available to consumers (154). Genome-edited disease-resistant wheat and corn, antibrowning mushrooms and potatoes, easy-to-harvest tomatoes, and drought- and salt-tolerant soybeans have all received USDA clearance as nonregulated plants, although at this writing they had not passed through the FDA consultation process (154). Peer-reviewed literature can also provide clues to future edited products (86).

Other applications of genome editing in plants are still in the research and development pipeline. As described in Section 5.5, genome editing is being explored as a tool to increase crop genetic diversity. Another application is to combine favorable alleles of many genes into a single plant with relative speed. This is useful because many plant phenotypes, such as flavor, are impacted by many genes. Modern genetic tools allow researchers to identify suites of genes that contribute to a given quantitative trait. However, identifying and combining several beneficial alleles into a single plant is difficult with traditional plant breeding, requiring many crosses and backcrosses. Genome editing might make combining multiple rare, beneficial alleles in one plant more feasible. For a review of other developments in and applications of plant genome editing, see Reference 87.

As such products move through research, development, and commercialization pipelines, researchers continue to make technical breakthroughs to refine and improve the use of genome editing technology. One such technique is DNA base editing. As reviewed in Section 2.2, repair of double-stranded breaks by NHEJ generates small mutations, but the exact base substitution or indel cannot be programmed ahead of time. Although HDR allows for precise base substitution, it is less efficient. Base-editing techniques are being developed and refined that will make it possible to change specific base pairs without the use of HDR, increasing the efficiency of making such changes (61, 95).

Although the most common Cas endonuclease is Cas9, alternative Cas enzymes have been identified and will provide greater flexibility in targeting more genomic regions. For example, Cpf1, also known as Cas12, uses a T-rich protospacer-adjacent motif (PAM) while Cas9 uses a G-rich PAM (173). While Cas9 generates blunt DNA ends, Cpf1 makes an uneven cut, generating cohesive ends (173). It also cuts farther from the PAM and, in some instances, increases the efficiency of HDR (111). Cas13 targets and cuts RNA instead of DNA, and researchers leveraged the properties of Cas13 to transform *Nicotiana benthamiana*, a close relative of tobacco, with a Cas13-mediated defense system against *Turnip mosaic virus*, successfully suppressing the virus (4).

Another important area of CRISPR research is DNA-free genome editing. When the CRISPR machinery is delivered to cells as DNA, pieces of the plasmid can be integrated randomly into the plant genome. When that happens, the edited line must be backcrossed to eliminate this random insertion(s). In the DNA-free method, Cas ribonucleoproteins and gRNAs can be delivered directly to the cell, saving the time needed for backcrossing (90).

Doubtless, technical advances will continue to be made in genome editing as researchers learn to increase editing efficiency and predictability and employ genome editing in new contexts. As that happens, we will decide on international, national, local, and individual levels how, if, and when to use them.

10. ARE GENETIC ENGINEERING AND GENOME EDITING THE ONLY WAYS TO FEED A GROWING POPULATION?

Many challenges face our food systems. In 2015, an estimated 795 million people, or 10.9% of the worldwide population, were undernourished, down from 18.6% in 1991 (59). But there is wide variation among countries: 20% of people in African countries were undernourished; 12.1% in Asia; 5.5% in Latin America and the Caribbean versus 1.8% in the "developed" world (59). Many factors impact food security now and will continue to have effects in the future. Among them are the following. (*a*) Many smallholder farmers have persistent, significant yield gaps, where actual yield is substantially lower than potential yield. (*b*) The percentage of people farming is decreasing, and so farmers must grow enough to support expanding urban populations (121 and references therein). (*c*) Transportation infrastructure for food distribution is often lacking in developing and least developed countries. (*d*) In many areas, diets are shifting to incorporate more meat, which requires far greater inputs per calorie than plants. (*e*) A large proportion of food is lost or wasted at each step of production, from yield loss in diseased fields to consumer household waste. (*f*) Political stability has a large impact on a nation's food security. (*g*) Environmental conditions, such as drought, flooding, and other natural disasters, can disrupt the food supply for millions.

Genetic engineering and genome editing are tools that might help us address some of these complex issues. They can be used effectively to help plants maintain sufficient yields in the face of abiotic challenges, like drought and changing climates. They can make underutilized plant varieties with low yields or high disease susceptibility become more resilient. They can make specific plants a source of essential nutrients that are lacking in the diets of some populations. Despite their

promise, it is clear that not every issue can or should be solved with these technologies; many are societal problems that must be addressed by changing behavior and mindsets. Decisions to use, not to use, or how to use these tools should be made by informed stakeholders—including consumers and famers in collaboration with plant breeders. But the authors are keenly aware that a pathway or mechanism for that inclusive conversation is not clear. Using crops created through genetic engineering and genome editing cannot replace sustainable practices, such as cover cropping, crop rotation, or crop diversification. They can ideally be used in concert with these practices, serving as one tool of the many that farmers at all production levels can use to adjust to local conditions and challenges.

11. CONCLUDING REMARKS

Genetically engineered plants and foods can be used to decrease the use of insecticides, make plants more nutritious, and improve yields on smallholder farms. However, some uses of genetic engineering can have negative consequences, like the escape of the glyphosate-resistance transgene from creeping bentgrass into wild grass species. It is therefore necessary (and required by law) to evaluate the potential positive and negative consequences of any product of the technology before its release.

This review seeks to provide fact-based information on some of the positive and negative outcomes that may be associated with genetic engineering. Additionally, to the extent that data are available, it also uses currently available information to contextualize the potential future of genome-edited crops and foods. Still, given the same information, different backgrounds and value systems may lead people to different conclusions about when, how, or if such technologies should be used.

SUMMARY POINTS

1. In contrast to genetic engineering, genome editing is a technique that allows scientists to make small mutations or insert DNA into a plant genome at specific locations.
2. Genetically engineered Bt crops increase yields and reduce insecticide use, but to maintain efficacy, multiple Bt traits should be introduced along with use of the high-dose refuge strategy.
3. Each Bt used in crops is highly specific to its target insects; off-target effects are reported rarely and inconsistently.
4. HR crops, mostly glyphosate-resistant, are widely planted worldwide; however, 43 glyphosate-resistant weed species have emerged. Crop rotations, use of herbicides with different modes of action, and integrated weed management can help control and prevent HR weeds.
5. Effects of glyphosate on bees appears minimal. The likelihood of high glyphosate concentrations in aquatic environments is rare, although, if present, they could affect already stressed aquatic organisms. Bt plants have minimal adverse effects on soil microbe community structure or function compared to impacts of environments and locations.
6. Transgenes from genetically engineered plants do enter wild populations, but the degree to which they are problematic depends on circumstances, like whether the transgene confers a selective advantage in that population. Movement of edited genes will be more difficult to track.

7. Countries take different approaches to regulating GE plants and foods. With regard to edited varieties, some regulate as they do genetically engineered varieties, while others regulate edited plants without DNA insertions more like classically bred plants.
8. While only one edited food ingredient has entered the US food supply to date, others have received USDA clearance as nonregulated. There will be a range of future efforts, but some could focus on reintroducing crop diversity lost through domestication.
9. Worldwide, many challenges face our food supply. While engineering and editing might be helpful in addressing some issues, decisions on whether and how to use these tools should involve informed stakeholders, but the pathway for such a process is not clear.

FUTURE ISSUES

1. Public perception of genetic engineering will continue to affect the extent to which the technology is used to modify crops. Public perception of genome-edited crops is not yet clear.
2. Regulations of genome-edited plants continue to be finalized in different countries. Regulatory decisions will impact how, if, and when genome editing is used in different crops and for different traits.
3. Ongoing court cases might influence the future use of glyphosate and glyphosate-resistant crops. How farmers and seed companies will respond is unknown, but, as glyphosate is a relatively nontoxic herbicide, it is possible that more toxic herbicides could take its place.
4. While the authors call for inclusive conversations with informed stakeholders about how and when to use modern genetic techniques, there is as yet not a clear mechanism for such decision making, or what/who constitutes an informed stakeholder.

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