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The End of the GMO? Genome Editing, Gene Drives and New Frontiers of Plant Technology

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Abstract: Improvements to agriculture will constitute one of the world's greatest challenges in the coming century. Political and social controversies, as well as complications of plant breeding, intellectual property, and regulation, have compromised the promised impact of genetically engineered – typically transgenic – crops designated as "GMOs." Genome editing is a new suite of molecular tools for assisting biologists identify genes that control agronomic traits such as drought tolerance and pest resistance, as well as to elucidate how expression of these genes is intertwined within the functional framework of the cell. This technology has recently gained momentum for its ability to accelerate the crop breeding process in an unprecedented fashion and expand the range of crop varieties with improved precision and lower costs. This review explains the basic concepts and provides examples of how genome editing could help address the United Nation's Sustainable Development Goals with respect to food, agriculture, and medicine. It concludes with a discussion of the potential social impact of genome editing and gene drive. These effects are contingent on the resolution of novel ethical and regulatory challenges that add new layers of complexity to societal questions of appropriate technology, in agriculture and beyond. We expect these questions to replace the irresolvable GMO debate.

Keywords: CRISPR, genome editing, gene drive, GMO, ethics, agriculture, sustainable development, climate change, human health, transgenics.

Introduction

This past summer, possibly the world's first meal consisting of genome-edited (CRISPR) foods was served up in Sweden by scientist Stefan Jannson (Zhang *et al.* 2016). The meal – "tagliatelle with CRISPRy fried vegetables" – was served with cabbage grown directly on Umeå University's campus. The Swedish Board of Agriculture ruled that as CRISPR-Cas genome-edited crops do not fall under the European Union's definition of a genetically modified organism (GMO), no special regulation was necessary. Similar rulings have occurred in the US and in Canada. If this trend continues, can we expect to see many more meals based on genome-edited crops across Europe and elsewhere in the future? This new and rapidly expanding form of technology, and impending public responses, may well force a fundamental re-evaluation of how best to develop tomorrow's food crops.

The genomics revolution that enabled modern agricultural biotechnology has been a source of optimism and controversy since its inception. Social and political resistance has prevented adoption and diffusion in many countries, in law if not in farmer practice (Herring and Paarlberg 2016). Innovations in crop genetic engineering have, where accepted, significantly increased the number and diversity of crop varieties and enhanced harvested yield, improved nutritional content, and conferred resistance to biotic and physical stresses (Collinge *et al.* 2010; Deikman *et al.* 2012). Genomic techniques have proved valuable in complementing conventional breeding methods. Genetically modified (GM) crops have demonstrated the potential to address malnutrition and to improve agronomic practices where other approaches fail, as with virus-resistance for example. Some biotech crops enable labour-saving strategies that allow farmers

additional time for other activities. At the same time, labour displacement has not proved so detrimental to the rural poor as first hypothesised and even shows potential for decreasing gender inequality under certain cropping conditions and village economy (Katage and Qaim 2012; Kouser *et al.* 2017). Crops with improved yields and improved resistance to pests, weeds, and environmental stresses such as drought and flooding can assist farmers who lack access to public safety-net mechanisms or reliable markets. Resilience to certain environmental shocks that result from climate change is one possible outcome (Cominelli and Tonelli 2010). While the first GM crops were bred for improved agronomic traits, agricultural biotechnology has developed crops with improved human health benefits as well (Bhutta *et al.* 2013).

As often in new technology, promises of potential have frequently outrun workable options on the ground for farmers. That situation may be changing dramatically. Over the past few years, a new technology known as genome editing has come to the forefront. Genome editing systems based on existing bacterial defence and repair pathways are being developed with applications in crop science, livestock improvement, and medicine (Montenegro 2016). In general, the technology is rapid, precise, and efficient compared to other means of developing desired characteristics in plants, i.e. transgenesis, chemical or radiation-induced mutagenesis, and conventional breeding. These attributes, coupled with relatively low costs and comparative freedom from regulatory encumbrances, have enabled genome editing to revolutionise basic molecular biology research and take it to an entirely new level.

Genome editing systems based on clustered regularly interspaced short palindrome repeats (CRISPR) or CRISPR-associated protein 9 (Cas9), for example, are now available in most research labs and exhibit forms of utility ranging from those as small as examining the function of a particular gene fragment to as large as the genome-wide mutagenesis screening of an entire crop for novel traits (Ding *et al.* 2016; Bortesi and Fischer 2015; Sauer *et al.* 2016). Furthermore, genome editing provides a plethora of applications in the crop sciences. Unlike transgenic plants, genome editing allows plant breeders to know exactly where a change has been made in the genome, leaves no trace of that process, and enables all copies of a particular gene to be altered within a plant at the same time. Moreover, crop genome editing shows signs of proving more socially acceptable than GMOs, and thus subject to fewer regulatory barriers, though ethical issues and property issues remain to be settled (Potrykus 2010; Perez-Massof *et al.* 2013).

The following review illustrates how genome editing fits into the broader frame of agricultural development. It describes how genome editing differs from and builds upon earlier achievements in genomics. Next, it provides examples of how genome editing is being applied today to improve traits for the world's major food crops. The use of "gene drive" as a mechanism to spread newly edited genomes rapidly, as well as examples of the use of genome editing for livestock improvement and for medical breakthroughs in human health are provided. The review ends with a discourse regarding the future of genome editing as a tool to address various challenges, and reciprocally, some social, economic, and ethical questions requiring coordinated responses in order to move forward.

Agricultural Development

One of our greatest challenges is ensuring adequate nutrition for farm and non-farm families with more sustainable and nutrient-rich crops. Both farm and non-farm families need more income and affordable, healthier diets. While approximately 800 million people today are undernourished (meaning they consume an inadequate number of calories per day), more than half of the world's population is malnourished (meaning they lack access to essential micronutrients such as vitamins and minerals required for human health) (FAO 2013). Today, food-insecure populations are concentrated in sub-Saharan Africa and South Asia. Although the proportion of people living in extreme poverty (on less than $1.25 a day) has decreased steadily over the past 20 years, these gains from rapid advances in GDP have yet to sufficiently reach the poorest of the poor. Indeed, in some instances, increases in population growth are faster than real gross domestic product (GDP) growth (Bazuin *et al.* 2011). The world's population is expected to swell to 9 or 10 billion within the next 30 to 40 years, and much of this increase is predicted to take place in poorer countries (International Food Policy Research Institute 2014).

This situation is confounded by climate change. Many of the world's poor lead precarious rural livelihoods at perpetual risk from environmental shocks such as floods or drought. Rising sea levels may increase salinisation of coastal agricultural areas, and rising temperatures and CO_2 levels will affect growth cycles and the types of crops that can be grown in a given area. These environmental shocks are predicted to become more dramatic and frequent with global warming

in the coming century (Global Nutrition Report 2014). It has been suggested by the Food and Agriculture Organisation (FAO) that agricultural productivity must double by 2050 to adequately feed the world. The UN's Sustainable Development Goals set out to address global poverty and hunger, with the mindset that lowering the number of people who live in extreme poverty (http://sustainable development.un.org) would enable people to improve their nutritional status by purchasing more fruit and vegetables, and thus gaining access to a broader spectrum of micronutrients.

India presents an example of the dilemmas of technical change in agriculture. Like other parts of Asia, India has known famine. The "Green Revolution" in India, as elsewhere, relied on new crop technology in which high-yielding dwarf crop varieties, developed by plant biologist Norman Borlaug and colleagues, were introduced (Long *et al.* 2015). These new crop varieties – primarily wheat and rice – were enhanced by synthetic inputs such as fertilizer and pesticides, as well as modern irrigation practices. Today, India maintains quite large buffer stocks and has become a major exporter of cereal crops (Aswath *et al.* 2016). These crop improvement strategies of the "green" revolution were widely accepted in ways the "gene" revolution involving GMOs were not (Harriss and Stewart 2015). Both India and China have experienced major successes in the use of biotechnology in cotton, but to date, have been reluctant to allow transgenic food crops because of social and political resistance (Herring and Paarlberg 2016). Will genome editing face similar obstacles or present new developmental opportunities in food production?

Agricultural Biotechnology

What is a "GMO"? There is much uncertainty among citizens and regulators as to where the line distinguishing one from the other varieties of plant breeding should be drawn. The genomics revolution in biology enabled new molecular plant breeding techniques to complement or supersede conventional plant breeding. Marker Assisted Selection (MAS) allows plant breeders to identify improved traits in plants more rapidly than is possible in conventional breeding (Barabaschi *et al.* 2016). Agricultural biotechnology can also include – in contrast to previous plant-breeding practices – manipulation of recombinant DNA to generate new or improved traits in plants. "Transgenic plants" – containing DNA from sexually incompatible species – form the core of both regulatory scrutiny and popular opposition to GMOs. These plants may have unique nutritional or agronomic traits resulting from recombinant DNA (rDNA) techniques (Kamthan *et al.* 2016), but their use is restricted in much of the world.

Misgivings about biotechnology often target the "unnatural" alteration of a crop's genome by rDNA. What most critics do not realise is that many varieties of crops available today have had their genomes altered by a technology that existed long before the advent of recombinant DNA. Derived from mutation research that originated in the 1930s, "mutagenesis breeding" involves the introduction of random mutations to plant cuttings using chemical or irradiation mutagenesis. Plant tissues expressing novel traits are then propagated from these mutation events into new varieties of crops (Barabaschi *et al.* 2016). Over 3,000 varieties of crops have been developed using mutagenesis breeding, including the popular ruby red grapefruit. According to the Mutant Variety Database (https://mvd.iaea.org), mutagenised plants face neither stigmatisation as GMOs nor special regulation. Indeed, foods that sell at premium prices for being labeled "organic" may be produced from mutagenised plants, in practice if not in purist theory (Nuijten *et al.* 2017).

In a broad sense, genetic engineering enhances the potential for introducing novel traits into crops through the manipulation of their genetic material, either by adding new genes or making small changes to pre-existing genes that are already part of the crop genome. New genetic material can be incorporated into the plant genome through several delivery methods, chiefly *Agrobacterium*-mediated transformation and particle bombardment (gene gun). In the US, GM or "transgenic" crops have been commercially available since 1996 (James 2013). One of the most well known examples of a transgenic crop is Golden Rice, which expresses β-carotene and was created philanthropically with the intent of alleviating vitamin A deficiency (VAD) in developing countries. Golden rice contains genes derived from different species, such as maize, which together contribute to a synthetic β-carotene pathway (Al-Babili *et al.* 2005; Beyer 2010). Golden rice can easily be distinguished from its conventional counterparts by its yellow hue, unlike many transgenic plants that defy easy detection, monitoring, or regulation. Yet golden rice has yet to make it to farmers' fields for a number of reasons: political, regulatory, and agronomic.

Transgenic crops have been engineered to address many of the world's most significant agricultural challenges, including insect resistance and herbicide tolerance (Ricroch and Henard-

Daman 2016). Today, nearly 90 per cent of all transgenic crops cultivated across the world are herbicide tolerant (James 2013). Herbicides can be sprayed on these crops without causing damage to the crop itself while the growth of neighbouring weeds is retarded. Insect resistance is the second most common trait generated in transgenic crops. Bt (an insecticidal protein from *Bacillus thuringiensis*) is used globally to prevent insect infestation. Insects that ingest the transgenic plant that expresses the precursor Bt protein are killed, while non-target insects that may reside near the crop but are not pests remain unharmed (Kumar *et al.* 2008).

Cisgenic crops are those that do not contain a transgene from another species, but rather a gene from a sexually compatible variety of the same plant, e.g., a blight-resistant Chinese chestnut with a blight-vulnerable American chestnut. Cisgenesis creates plants that express genes from closely-related plants and are also designed to regain useful genes that have been lost over years of conventional crop breeding. For example, the Wheat Stem Rust Initiative works toward designing cisgenic versions of wheat containing multiple resistance genes to the fungal pathogen Ugg99 from wheat relatives (Singh *et al.* 2015).

"Gene silencing" (RNA interference technology – or RNAi) could also be considered a form of genetic engineering that is proving increasingly useful for agriculture. Plants are engineered to express the antisense RNA version of a specific gene that may be part of the plant genome or part of an invading pathogen's genome, such as a virus. Expression of the targeted gene is then blocked by a phenomenon known as gene silencing. Genetically modified papaya that has been generated using this technology is resistant to papaya ringspot virus (RSV) by expressing an antisense RNA to the viral genome. This technology is responsible for having saved the papaya industry in Hawaii (Gonsalves 1998). China's small papaya sector is almost entirely based on this technology. Though the RSVR papaya has failed to gain wide market presence in many countries because of political resistance, farmers elsewhere have spread the technology informally and found it effective in fighting the fatal virus (Evanega and Lynas 2015).

Despite wide adoption, and evident usefulness to many farmers in many countries, the technologies described above have shown limitations that have disappointed some early expectations. This can be attributed to long delays from multi-year field trials and legal challenges that have had limited progress. Moreover, plant breeding, even with improved technologies, is invariably complex. Golden rice technology, for example, has experienced numerous challenges in breeding into land races and has yet to have the long-awaited impact on Vitamin A deficiency. To date, successful crops have mainly been those protecting harvest yield from biotic stress – weeds and pests. The frontier looks different with the advent of genome editing.

Genome Editing Technologies

Genome editing is the most recent technology to be developed for plant breeding. It has other applications as well. Genome editing does not require the introduction of new gene sequences; rather, it may direct only one or a few nucleotide changes within a plant genome (Rani *et al.* 2016; Mao *et al.* 2016). This fact changes the regulatory playing field that governs genetically modified organisms (GMOs) that involve the introduction of genes from other species. As a result, genome editing can offer advantages to, or even be used to complement, other forms of biotechnology. For example, genome editing can offer a more facile and versatile replacement for gene silencing, but can also be used in concert with this technology in certain instances that require more sophistication than either technology is capable of on its own, such as functional genomics studies. Genome-editing technologies can offer improvements to practically any organism. It has found a place in livestock development, veterinary science and even medicine. Different aspects of agricultural biotechnology are summarised in Appendix 1.

In general, genome editing utilises various defence strategies developed by bacteria to target specific sequences of DNA and cleave those sequences at targeted sites with nucleases, or enzymes that cut DNA. The technology is then able to make use of DNA repair mechanisms already found in the cells of all organisms, and by repairing the sites of cleavage, establish specialised changes that will be carried through the genome of the "edited" organism to subsequent generations.

Although genome editing technology is in the spotlight today, its emergence has been a long time coming, as new editing systems have been discovered over the past decade and the ability to apply this technology has become increasingly facile (Stella and Montoya 2016). Originating with the identification of mega-nucleases, the field underwent a rapid revolution through the characterisation of the clustered regularly interspaced short palindromic repeats (CRISPR)-

associated protein (Cas) system, which is easy to use, low in cost, and robust in application. CRISPR-Cas9 as a technology resulted in a quantum leap of progress in the plant sciences. That leap is only now being realised, both in research laboratories as well as in the field. Various technologies that fall under the umbrella of genome editing are presented in Appendix 2.

Genome Editing and Plants

The process by which a plant cell is edited is as follows: a target site for genome editing is designed and screened for potential off-target effects using computer software. The sgRNA representing that target site is synthesised and inserted into a CRISPR-Cas9 expression cassette containing the gene encoding Cas9 and the sequence of the sgRNA, each under the control of a specific promoter. The cassettes are delivered into plant cells using a variety of methods, ranging from *Agrobacterium*-mediated to biolistic (gene gun) delivery, and even through the use of plant viruses engineered as delivery vectors. Plant cells that have been transformed are then screened for the presence of the desired mutation, either by restriction enzyme analysis or by directly sequencing their genomes (Kumar *et al.* 2015; Rani *et al.* 2016).

The various genome-editing systems described above provide a straightforward method for rapid gene targeting within one to two weeks (Shan *et al.* 2014). Two major advantages are that genome editing is more rapid than both traditional breeding and transgenic approaches, and a selection process using marker sequences or genes is not necessary (Xing *et al.* 2014). Alterations in the genome can be detected quickly and inexpensively and selectable markers are not required as they are in marker-assisted selection or transgenesis, respectively (Kim *et al.* 2016). A single genome editing event can also offer the possibility of simultaneous targeting of multiple (stacked) traits within a single crop; these traits can be carried to all homologues within the plant's genome, which is no small feat and difficult to control using both traditional breeding and transgenesis (Luo *et al.* 2016; Raitskin and Patron 2016). While humans have a diploid genome (23 pairs of chromosomes), plants can have higher levels of polyploidy (for example, the wheat genome has six copies of each chromosome). It can be challenging for traditional plant breeders and molecular biologists who work with transgenic plants alike to ensure that every chromosome homologue contains the gene of interest and that it is expressed in an optimal fashion (Zhu *et al.* 2016).

As a result of these features, the regulatory path for genome-edited plants into the marketplace is far more straightforward than it is for transgenic crops. Since many of the tools required for genome editing come directly from common bacteria (often harboured within our own gastrointestinal tract) and no additional genetic material is added to the genome (unlike the process creating transgenic plants), the promise of global acceptance of genome edited crops by farmers and consumers alike is more likely to be realised. These features provide assurances to scientists that any advances they make to the technology and any forthcoming products are less likely to be left on the shelf or subject to attack; consequently, genome editing can be said to have blossomed overnight (Cardi *et al.* 2016).

At the moment, genome-editing technologies are being specifically optimised for all major crop types. Often a proof of concept is first sought through a demonstration that a previously well characterised gene can be edited in that crop in such a way that all homologues have been altered in the plant and that the alteration is inherited stably to the next generation (Khatodia *et al.* 2016).

Some of the traits that have been examined include those that are fundamental to crop improvement, such as flower or fruit size, colour, grain yield, herbicide tolerance, and pest resistance (Barakate *et al.* 2016). As more and more research groups perfect the conditions for successfully editing a particular crop type, attention will shift to the production of novel traits that can improve vigour, stress tolerance, yield, and nutritional content of crop varieties (Basak *et al.* 2015).

Genome editing is also being rapidly incorporated as a tool for scientists to learn even more about how plants cope with abiotic and biotic pressures. The knowledge gleaned from these studies can be used to generate a second generation of newly genome edited crop varieties that are even better able to manage in a rapidly changing environment (Liu *et al.* 2016, Nongpiur *et al.* 2016).

The next section provides examples of some of the traits that are under examination for economically important crops.

Wheat

Wheat is one of the major food crops in the world but can be difficult to work with due to its large (17 Gb) hexaploid genome. Kumar *et al.* (2015) used CRISPR-Cas9 to alter genes involved in amino acid and carotenoid biosynthesis in a wheat cell suspension culture as a proof of concept that large complex genomes could undergo genome editing successfully. The same authors were also able to use genome editing to delete a large gene fragment in the wheat genome. Zhang *et al.* (2016) edited the wheat gene responsible for grain length and weight using particle bombardment. Approximately 16 per cent of the mutants recovered had all six alleles simultaneously knocked out. Both hexaploid bread wheat and tetraploid durum wheat (used predominantly for pasta) were edited in this fashion. Another research group was able to successfully target genes involved in wheat shoot and root development traits (Wang *et al.* 2014). Simultaneous editing of three homologous alleles of the *mlo* gene led to a bread wheat variety that was resistant to powdery mildew, a disease that is a threat to food security (Huang *et al.* 2016).

Maize

CRISPR/Cas9 has been used to demonstrate that genome editing could have a direct impact on the production of maize crops with new, agronomically helpful attributes (Svitashev *et al.* 2015; Char *et al.* 2016). CRISPR-Cas9 was employed to target a number of different genomic regions in immature maize embryos by biolistic transformation. These regions include regulatory elements required for leaf development, male fertility genes, and genes involved in amino acid biosynthesis (with the idea of creating herbicide resistant plants for the latter). Reduction of the anti-nutrient phytase has also been generated using Zinc Finger Nuclease (ZFN) technology in maize (Shukla *et al.* 2009).

Shi *et al.* (2016) used CRISPR/Cas9 to generate novel variants of the ethylene response gene ARGOS8. Overexpression of ARGOS8 has been shown to improve grain yield under drought stress conditions. Several mutants generated using CRISPR/Cas9 were able to increase grain yield by five bushels per acre (approximately 336 kg per hectare) under stress conditions. The same plants experienced no yield loss under well-watered conditions, showing that genome editing can generate novel types of drought-resistant crops. Along the same lines, Qi *et al.* (2016) were able to change storage protein content in maize using CRISPR-Cas9.

Transcription activator-like effector nuclease (TALENs) have also been used as genome editing tools in maize. As a proof of concept, Char *et al.* (2015) have shown that mutations can be generated at the maize glossy2 (gl2) locus, responsible for the waxy layer on leaves. Furthermore, scientists at Dupont Pioneer have edited the Wx1 gene that creates “waxy corn” used for producing specialty starch for processed foods, adhesives, and high-gloss paper.

Genome editing can also be used to directly alter maize pathogens and thus identify what specific interactions cause infection, so that plants can be modified to become resistant to those interactions. For example, Schuster *et al.* (2016) used the CRISPR/Cas9 system to alter genes in the fungal maize pathogen *Ustilagomaydis*. The fungal mutants can then be tested for their ability to infect maize plants, and using this reverse genetics approach, the virulence genes of the pathogen can be identified and their function during infection determined. With this knowledge, new maize crops edited to resist fungal infection can be designed and generated.

Rice

Genome editing has been extensively used to modify rice for a number of purposes (Li *et al.* 2016a, 2016b; Xu *et al.* 2017). Blanvillain-Baufumé *et al.* (2016) used TALEN as a genome editing tool to examine bacterial leaf blight infection in rice. Targeted mutations in the plant gene involved in leaf blight infection were generated and the ability of proteins from a variety of different bacterial strains to bind to these rice mutants and promote infection was examined. A number of the genome edited rice plants showed resistance to several of these bacterial strains, demonstrating that while new plants that are resistant to *Xanthomonas* infection could be developed, the nature of that resistance could also be studied in detail via direct plant pathogen interactions.

Rice resistant to rice blast, a fungal pathogen, has been developed using CRISPR-Cas9 to alter a gene involved in the plant stress response (Wang *et al.* 2016, Wang and Qi 2016). By creating a variety of mutations in this gene, the selected plants were demonstrated to resist rice blast but displayed no difference when compared to wild type plants with respect to agronomic traits such

as plant height, leaf length, grain weight, and number. Another research group located in China used the CRISPR/Cas9 system to alter genes in rice responsible for enhanced grain number density and larger size, simultaneously. The results showed that CRISPR/Cas9 can modify stacked, multiple traits in a single cultivar (Li *et al.* 2016c).

Soybean

Genome editing technologies have also been employed for soybean. Du *et al.* (2016) used CRISPR/ Cas9 to alter soy flower size and colour. The genome editing technique for soybean has been further optimised through the development of an online web tool that quickly identifies a high number of potential CRISPR/Cas9 target sites (Michno *et al.* 2015). Another research group used CRISPR/Cas9 to develop herbicide tolerance in soy (Li *et al.* 2015). Other examples of genome editing in soybean can be found in Sun *et al.* (2015), Jacobs *et al.* (2015), and Cai *et al.* (2015).

Citrus

Citrus is an economically important slow-growing tree crop found worldwide. Over half of citrus grown commercially in the world is sweet orange. The genome of sweet orange has been successfully modified using CRISPR/Cas9 (Jia and Wang 2014). More recently, Duncan grapefruit has been edited by CRISPR/Cas9 for resistance to Citrus canker, one of the worst pathogens of citrus. The bacteria that produces citrus canker injects a protein into infected citrus plant cells that suppresses plant defence and promotes bacterial growth and canker development. This bacterial effector protein can turn on genes in the cell of the citrus plant that aid in tumour development and bacterial infection by binding directly to the promoter region of the plant DNA. By altering the sequence of this promoter region using genome editing, grapefruit plants resistant to this disease were developed (Jia *et al.* 2016).

Tomato

Tomato, another economically important crop, has been studied for its nutritional enhancement properties through alteration of the carotenoid pathway (Brooks *et al.* 2014). Recently, Pan *et al.* (2016) used the CRISPR/Cas9 system to target two genes responsible for altering the colour of tomato fruit. The frequency of mutation was high and albino phenotypes were observed in tomato for two generations, indicating that the mutations were stably inherited and exhibited no off-target effects. Another study conducted by Cermak *et al.* (2015) examined the use of CRISPR/Cas9 delivered by a geminivirus vector to overexpress anthocyanin in tomato, which turns the fruit a deep purple colour. Anthocyanin, a compound found in blueberries, is associated with reduced cardiovascular and cancer risks. Tomatoes are less expensive, globally available and easier to grow than blueberries, and thus providing similar nutritional benefits is desirable.

Genome-Edited Livestock

For the past few years, genome-edited livestock, including pigs, cattle, sheep, goats, and chickens have been coming to farms (Lillico *et al.* 2013; Proudfoot *et al.* 2015; Tan *et al.* 2016; Yao *et al.* 2016). The technology could have benefits with respect to both animal welfare and the environment. For example, Tan *et al.* (2013) have employed TALEN-based technologies to generate cattle that lack horns. The de-horning of cattle is of questionable ethics due to pain inflicted on the animal during the process. By changing the genome of cattle to one that is polled, the animals never develop horns and are thus spared this procedure. Another research group was able to use TALENs to knock out the gene that encodes a growth factor that acts as a negative regulator of skeletal muscle mass. The resulting animals generated far more meat on a smaller quantity of feed (Zhao *et al.* 2016; Jenko *et al.* 2015). Other groups are planning to generate chickens that produce only egg laying hens and cattle that produce only meat-delivering steers. Most recently, Chinese researchers have generated goats that produce cashmere wool more effectively, so that fewer animals can produce the same amount of wool on less land. New companies such as Recombinetics are exploring new ways to produce genome-edited animals for industrial livestock.

Genome editing can be utilised to rapidly generate animal disease model systems. For example, Tan *et al.* (2013) were able to generate pigs which could act as models for infertility and colon cancer, respectively. Pigs can be edited to grow human organs (Garry and Garry 2016). Gene drives (as explained below) could be created to slow the population growth of animal pests such as rats, or create disease-resistant livestock, such as pigs that are resistant to African Swine Fever, dairy cattle that are resistant to the parasite that causes sleeping sickness, or chickens that

are resistant to Avian flu virus. Using a genome editing approach, the overuse of antibiotics to maintain livestock health could be greatly reduced (Saey 2015).

Genome Editing and Human Health

The potential of genome editing to improve human health is only beginning to blossom. For example, CRISPR-Cas9 has been used as an approach to attack antibiotic-resistant bacteria (Waddington *et al.* 2016). Research involving genome editing has been used to address currently untreatable genetic diseases such as Duchenne's muscular dystrophy, as well as human pathogens such as HIV and hepatitis B virus (Yin *et al.* 2014; Benjamin *et al.* 2016; Mendell and Rodino-Klapac 2016).

Today, genome-editing studies have been conducted using cell culture and animal trials, including non-human primates, to realise authentic changes to disease status (Niu *et al.* 2014; Stone *et al.* 2016; Wang and Qi 2016; Zhao *et al.* 2016). For example, the genetic disease cystic fibrosis can potentially be eliminated by genome editing and has been shown to work so far both in human cell culture as well as in a mouse model. The defective gene involved in cystic fibrosis can be corrected in inducible pluripotent stem cells, indicating that this genetic disease could be cured before its onset and removed forever from subsequent generations. Direct correction of the mutation in adult diseased lungs is also under consideration. While corrections may not reach every single epithelial cell in the lung of an infected patient, the resulting mosaic of edited versus unedited cells may still be sufficient to greatly reduce or eliminate symptoms of the disease (Alton *et al.* 2016).

Genome editing could also be used in the future to treat hereditary movement disorders, including Huntington's and Parkinson's disease (Seah *et al.* 2015; Im *et al.* 2016). For example, deletion of the defective gene that is responsible for Huntington's disease in mice has been shown to prevent protein aggregation in the brain and thus disease symptoms (Talan 2015). Furthermore, genome editing may play a significant role in a variety of forms of cancer therapy (Yi and Li 2016). The fate of patients with difficult to treat mitochondrial diseases could potentially be improved using genome editing technologies (Fogleman *et al.* 2016). Some researchers believe that genome editing could offer improvements in medicine that have never been realised before. As of now, the technology is too new for adequate appraisal of either potential or social implications (Singh *et al.* 2016). Who will decide? Who will govern?

Genome Editing and Gene Drive: Hacking Evolution?

Gene drives introduce the most fundamental alterations of organisms, enhancing both potential benefits and potential risks. For example, gene drive enabled by genome editing is being considered as a means to stop the spread of mosquito-borne diseases such as malaria, dengue, and Zika. The concept of gene drive was first conceptualised in the 1960s by an entomologist who hypothesised that mosquito breeding programmes could be set up so that the male offspring could be favoured due to the identification of a male-producing factor that is expressed in the genome of some male mosquitoes. As a result, release of male mosquitoes harbouring this male producing factor could shift the sex ratio of the mosquito population so that the number of females was reduced to below the level required for efficient disease transmission (Hammond 2016; Wieczorek 2016). It is the advent of genome editing using CRISPR-Cas9 that has offered unprecedented opportunities to reduce mosquito populations (Gurr and You 2016).

Gene drives work by incorporating a system of biased inheritance so that the ability of a gene or genetic element to pass from parent to offspring through sexual reproduction becomes enhanced. As a result, the presence of this genetic element increases in frequency and spreads from one generation to the next until most or all members of a given wild population representing that species contain the same element. Unlike classical Mendelian inheritance, in which each offspring has a 50 per cent chance of inheriting a specific gene from one of their parents, gene drives dictate that most or all offspring will inherit a particular genetic trait that is under the control of gene drive technology. In the study of genome-edited mosquitoes, for example, genes that confer a recessive female sterility phenotype were disrupted. CRISPR-Cas9 gene drive constructs designed to target and edit each sterility gene and its homologue were inserted into the female sterility gene locus. This approach resulted in a massive increase of sterile females. Population modelling showed that this gene drive could be used to effectively target female reproduction (because only females bite humans) in a mosquito population (Reid and O'Brochochta 2016). The technology could also be extended to edit mosquitoes so that they are no longer able to transmit infectious diseases (Singer and Frischenecht 2016).

Gene drive technologies using CRISPR/Cas9 have given humans the potential to eradicate entire species from this planet. Profound ethical concerns are immediately apparent. What are the risks of gene drive with respect to human health and the environment? How will gene-driven suppression of specific species of mosquitoes or other pests alter the Earth's ecosystem as a whole? How do we as a national or global society decide when and where gene drive technologies are to be used? Who decides? The threat of Zika virus over the past year, for example, in South America and southern States of the US has instigated a public discussion on the benefits and risks of gene-driven mosquito technologies. The ecological discussion is extremely complex: the *Aedes aegypti* mosquito itself is an invasive species alien to the western hemisphere, in no real sense natural or critical to ecological integrity.

Gene drive technologies could suppress or eliminate invasive species that threaten biodiversity, eliminate weeds, or even alter pathogens that damage crops or carry diseases. Gene drive technologies could also introduce new traits to existing populations, and could possibly rescue or save endangered plant species – or resurrect extinct ones.

For example, in an effort to protect the biodiversity of native plant species in the United States, gene drives are being developed to suppress the spread of the non-indigenous spotted knapweed *Centaurea maculosa*. Originating in Eastern Europe, the spotted knapweed was introduced into the US in the 1800s. It spread rapidly, damaging ecosystems and causing soil erosion. A gene drive solution could spread through the knapweed population and several approaches could be taken. One of these would entail the suppression of a sex-determination gene, in a fashion analogous to the mosquito gene drive described above, which could lead to an imbalance in plant sex ratio and consequently a population crash (Langin 2014). Unlike mosquitoes, however, knapweed grows slowly and it is unclear how factors such as rate and distance of pollen spread in the wild would affect the gene drive process (National Academies Press 2016).

Another example of the use of gene drive in plants would be the elimination of pigweed from agricultural fields. This weed reproduces rapidly and has evolved resistance to glyphosate, one of the most widely used herbicides globally. Using gene drive technology, the glyphosate resistance trait could be reversed in pigweed, making it again susceptible to this widely used herbicide. Alternatively, a suppression drive that creates a biased sex ratio could be created in pigweed, resulting in a population collapse of this species (National Academies Press 2016).

Not only can genome edited crops be used in conjunction with gene drive to eradicate weeds, they can also be designed to eliminate pests. Gene drive crops that no longer act as hosts for insect and microbial (fungal, bacterial, and virus) pathogens could be designed. As scientists gain a further understanding of what specific proteins are involved in pathogen-host interactions, the employment of gene drive to disrupt these interactions could ensure that future generations of crops will no longer support pathogen growth.

There are some caveats to the use of gene drive. For example, the technology will not work on invasive plant species that do not sexually reproduce or which reproduce very slowly. It is possible too that gene drives may have to be re-applied over time, because plants undergo natural selection and lose the trait that has been introduced (Callaway 2017). Potential resistance of a few individuals in a given population to gene drive is also a possibility, and could lead to the eventual re-emergence of a population that is impervious to its further usage. On the other hand, gene drives could permanently change entire plant or animal communities within a relatively short period of time, for better or for worse. It is the unforeseen and perhaps irreversible consequence of destabilising current ecosystems that brings pause to the idea of applying gene drives without a binding social contract with all stakeholders across the globe.

Social Impact of Genome Editing

While there has been much excitement about the potential for using genome editing to solve current challenges in agriculture and medicine, the eventual and long-term impact of this technology will require very careful consideration (Singh *et al.* 2016). Would correcting defects in genomes of people who have incurable diseases such as cystic fibrosis, muscular dystrophy, Parkinson's or Huntington's disease resulting from an accident of birth not meet with universal acclaim? Would removing human diseases caused by vectors of otherwise unstoppable pathogens such as Zika virus not constitute obvious progress for the human species? Or do such transformations of nature exemplify the hubris of "playing God," leading to a slippery slope of ethical degeneration, and further to "designer babies" with enhanced traits and the permanent alteration of human evolution as a whole (Krishan *et al.* 2016)? Would making corrections in the genomes of disease-affected people not entice others to alter the genomes of their offspring as

embryos, for example, to target genes that are linked to cancer or to other chronic diseases (Regalado 2015; Benston 2016)? Is it not a short ethical jump for would-be parents to play an active role in determining their children's appearance, intelligence, and athletic abilities once the potential is proven (Sankar and Cho 2015; Shantharam 2016)?

As with all technological change, societies seek a balance between risk and utility through some acceptable social consensus. On the utility side of the equation, the potential of genome editing offers a quantum leap from transgenesis. The same is arguably true on the risk side of the equation once gene drives are on the table. There is no way to confidently predict the downstream effects of genome editing over multiple generations. For example, off-target effects of genome editing, meaning the editing of additional unintended sites on the genome, could result in dramatic changes to an organism's health not necessarily in the short term, but possibly in the long run, such as turning proto-oncogenes on, other essential genes off, or even creating new genetic defects. While CRISPR-Cas9 can be used to modify epigenetic effects, its use may also create new conundrums with unpredictable consequences. Long-term animal studies have not yet been completed and in any event would not conclusively settle the incremental risk of genome editing in humans (Vogel 2015). This is not pure speculation; Chinese scientists have begun experiments with editing human genomes (Liang *et al.* 2015). Finally, might nature resist being re-ordered as organisms develop resistance to alterations made by gene drives (Callaway 2017)?

These profound ethical questions for society have less dramatic analogues in agriculture: altering the course of evolution of both crops and pests fundamentally, for example by inducing resistance to viruses and other pathogens that reduce yields and farm incomes, or inducing resistance to drought in some plants and not others. Breeders could without question generate crops enhanced for disease resistance and improved nutritional content – an attractive consideration for our soon-to-be more crowded and hotter planet. Genome edited crops are simple to generate, low in cost to produce, and leave no trace of transgene backbone or selectable markers. The fact that technologies such as CRISPR-Cas9 are derived from the same bacteria that already naturally reside in the human gut make it difficult to claim that anything "foreign" has been included in the editing process. On average, only one or a few nucleotides are altered in many genome-edited crops, perhaps decisively differentiating them from "GMOs" (Paul and Qi 2016). In fact, as the first genome edited crops begin to attract public interest, there seems to be no consensus on how to classify them.

For example, non-browning mushrooms developed through genome editing technologies by the biotech company Calyxt entered the market with no serious disturbance or resistance from anti-GMO protestors (Waltz 2016). This trait was achieved by deleting a few nucleotides from the gene that causes browning within the mushroom's genome. No sequences of plant pests, such as viruses or bacteria that are often associated with GMOs, were included in the editing process.

Waxy corn has also been given the green light by the US regulatory system for commercialisation since no genetic material from a separate organism had been inserted into the plant genome (Unglesbee 2016; Ossola 2016).

Although genome-edited crops do not invoke the same regulations as GMOs, some could argue that it is too early to tell how edited crops and livestock would affect our ecosystems and environment. If we change the genomes of pigs for example, so that they were no longer susceptible to influenza virus, would there be unintended consequences down the line for how the virus evolves, and therefore for human health? The immediate benefits with respect to disease burden seem huge, but what would the ecological impact be in the long term? If we can generate plants that are able to tolerate a wide variety of herbicides, would this benefit the environment or not?

These questions can be compared to many of the concerns raised with respect to GM crops created with the use of existing technologies. A glance at current international policies regulating GMOs seems to be a good place to start.

The End of the GMO Debate?

Regulation of GMOs around the world roughly follows a conceptual divide between the United States and Europe (Paarlberg 2001). In the US, regulations favour a notion of substantial equivalence: permission to plant means that no additional risk can be perceived from the new traits introduced into the GM crop compared to its non-GM equivalent. In Europe the "precautionary principle" leans toward a position that there is insufficient evidence of the safety

of most GMOs, necessitating further studies to prove that no additional risk exists. Precaution has added many years to development timelines for GM crops that could be grown and sold in Europe, thus blocking research and development of crops that could have both local and global utility. One direct consequence is the under-representation of GM crops in sub-Saharan Africa, where new traits are sorely needed but restricted due to Africa's colonial history and trade dependency with Europe (Paarlberg 2008).

The result of these two conflicting perceptions of GMOs on grounds of risk – to food safety and the environment – has disrupted trade between the US and the EU, and as a result, among their trading partners. In addition to differences of risk assessment, a second objection to GMOs that divides the public is that of intellectual property and patents. Because relatively few firms dominate existing technology, many worry that GMOs enable monopolisation of the world's food system by multinational corporations. Whether or not one can patent a crop cultivar varies widely across nations, but objections are widespread. Would genome-edited plants face similar objections on grounds of property?

It is too early to tell how property systems will treat the innovations described above. Nevertheless, genome-edited crops are *a priori* almost certain to be less susceptible to the objections to biotechnology on grounds of monopoly built on intellectual property.

There are two reasons to expect greater acceptability of genome-edited crops. First, patents are national and need not be universally accepted; there is already variation across countries. Moreover, patents are continually challenged in courts: these are not determinant structures but playing fields on which contestants contend. In the United States, the long contest pitted a University of California Berkeley group against one at Harvard and MIT. The latter group seems to have won; the former will appeal. European patents will be years in the decision stage (Ledford *et al.* 2016; Ledford 2017; *Nature* Editorial 2017).

Secondly, the objection to property rights is that first movers attain a privileged position leading to oligopoly or monopoly. Genome-edited plants are less likely than GMOs to face this social problem. This is because the process is inexpensive and fast, requiring less capital, infrastructure, and staying power. Developers risk much less in terms of cost; more players would be able to compete on a more equal footing. The potential for a geographical concentration of the industry would also be reduced. But these advantages could be eroded, or eliminated entirely, by classification and regulation. The more heavily regulated genome-edited plants are, the more likely they are to be monopolised by firms with deep pockets, political heft, and compliance staff – in contrast to universities, small firms, and individuals who lack these resources, and countries with weaker bio-safety scientific capacity (Kolady and Herring 2014). Indeed, momentum in new technologies is emerging from university settings, not industrial life-science firms. Setting the regulatory bar too high would enable more monopoly and reduce competition and innovation, while simultaneously attaching stigma to the plants, as happened with GMOs. Removing obstacles of regulation and the stigma of the GMO from genome-edited crops would presumably draw more investment in agricultural development.

Will genome-edited plants be coded as "GMOs" or not? For the time being, Sweden, Canada, and the United States have decided to not classify genome-edited plants as GMOs. The reasoning is the absence of transgenesis in genome-edited crops: no "foreign" DNA need be involved. In this sense, genome-edited crops are more like precisely site-specific mutagenised plants than transgenic plants in which incorporation of a transgene is uncertain. Indeed, with the progress of synthetic biology, it becomes increasingly possible to synthesise a gene rather than to find, isolate, and transfer it from another species. These facts should remove much of the objections on grounds of "unnatural" plants.

However, like "GMOs," genome-edited cultivars vary. For example, several nucleotide substitutions or a small deletion in a plant genome, using genome-editing technology, closely resembles the breeding mutagenesis process described earlier and used for over half a century without any differences in regulation from conventional crops. A nuclease used in genome editing to cleave DNA resembles the effect of a chemical or irradiation mutagen used in mutagenesis breeding. Repair pathways employed by the cell for correcting double-stranded breaks in DNA caused by either process are identical. As a result of these similarities, crops edited in this fashion currently bypass the regulatory frameworks in many regions of the world (Wolf *et al.* 2016). Organic farmers can grow mutagenised crops, without labels or special regulatory approvals.

However, other genome-edited crops have undergone more substantial editing. Some of these

editing events may include the incorporation of hundreds or thousands of nucleotides through a template that can be added in conjunction with the nuclease. In this way, a single transgene can be added to the target site during the genome editing transformation process, resulting in the incorporation of what could very well be genetic material from another organism. The outcome of this breeding process could thus resemble a transgenic crop more than a simple product of mutagenesis (Jones 2015). Moreover, the genome editing transformation event can even be repeated to incorporate other transgenes, precisely into the same target site, in a stacked manner. Although crops developed using genome editing in this fashion differ from transgenic plants because the technology is much more precise and construct sequences derived from plant pathogens are lacking, the fact that heterologous sequences derived from other species can be added to the plant's genome suggests that the genome-edited crop has a lot more to it than just simply a new mutation.

The degree of regulatory oversight of genome-edited crops could depend on the type of DNA repair process used, the nature of the trait added, and the pre-existing regulatory structure of a particular country. There will be uncertainty, delay, and variance, but we can be fairly certain there will be no global standard soon. We can also be fairly certain that if a global standard is ultimately agreed to, it will lack means of enforcement and will further complicate international trade and intellectual property regimes.

Variance among genome-edited plants thus adds a further layer of difficulty in defining exactly a "GMO" (Jones 2015; Wolf *et al.* 2016). Are all genome-edited crops "GMOs," or some, or none? Do they all belong in the same category, or require disaggregation? By what criteria do we group and split new cultivars? In the absence of demonstrated hazards, how is risk assessed differentially? This conceptual morass suggests the end of the GMO as a workable frame for regulating plant breeding (Johnson 2015).

Nature does not code plants as GMOs or not GMOs – these are purely political conventions based on social mobilisation and regulatory precedents. These human constructions vary over time and space. Indeed, nature makes its own transgenic and mutagenised plants, completely indifferent to how societies might codify them (Kyndt *et al.* 2015). We can confidently predict that there will be significant controversy over how to classify and regulate or normalise genome-edited crops. Whatever the outcome in particular places or times, it is unlikely to be consistent, generalisable or enforceable. There is already great incoherence and inconsistency in the concept of "GMO," making it "practically impossible to define" in law or biology (Johnson 2015). The dominant criterion has been cross-species transfers of genetic materials – transgenesis. Genome-editing technologies have greater utility, broader applicability, less potential for monopoly, and evidently universal applicability compared to transgenic technology – more democratic access on a more level playing field.

Conclusions

Caribou Biosciences, a company founded by the University of Berkeley scientist and CRISPR pioneer Jennifer Doudna, is preparing to initiate field trials on varieties of corn and wheat edited for drought resistance (Montenegro 2016). Cibus, a San Diego based company, has used a novel form of genome editing to produce the first commercially available genome-edited crop *SU Canola*™, a herbicide resistant form of rapeseed that has received regulatory approval in Canada. Other agronomic traits under development by both of these companies include increased crop yield, disease tolerance, the production of healthier oils, and tolerance to high salinity.

Genome editing technologies hold the promise of crop and livestock improvement and even of curing patients of what have been up to now incurable diseases. The applications are vast and the human condition as a whole could be changed by genome editing. CRISPR-Cas9 as a genome editing platform, for example, has proved to be flexible across species, has high multiplexing potential, though as yet indeterminate intellectual property constraints. Since the technology leaves no sign of transgenesis, plants generated by genome editing are not considered to be GMOs and thus do not provoke the political and social energy that often accompanies biotechnology in agriculture. While inexpensive and relatively simple to implement, genome editing still has some drawbacks, including off-target effects and our inability to conclude what the long-term impact of this technology will be over many generations. Concerns regarding deliberate changes that genome editing can make to the course of human evolution seem for now to belong within the pages of a science fiction novel; however, so did many modern technologies at some point in history.

The immediate issue is that risk assessment guidelines to address environmental and human

health effects lag far behind the rapid adoption of the technology in research labs around the world, outpacing bio-security frameworks for responsible regulation. More daunting still is that any workable mechanism for enforcing guidelines on a global scale is hard to conjure. One emergent agreement among practitioners is that genome editing be prohibited in germ lines, as results would otherwise be permanent over generations, altering evolution in unknowable ways. Yet how could such an agreement be enforced? Who would decide? One proposal has been to write restrictions into patents – the "ethical license" – as the Harvard group did in licensing to Monsanto (Guerrini *et al.* 2017). But then how do patents get enforced? Patent laws are national, and idiosyncratic, not global. Bio-property in transgenic seeds has proved virtually impossible to enforce internationally (Herring 2007).

While CRISPR-Cas9 technology becomes more effective and easier to use, research on other editing systems such as mega-nucleases are in the pipeline and will soon offer an even more diverse toolkit for scientists (Lambert *et al.* 2016). The term GMO – variously defined – is becoming ambiguous, more a normative and political construct than a biologically meaningful one. Genome editing as a whole thus challenges existing governmental regulatory structures designed to manage differences among organisms bred for new traits by different technologies (Esvelt 2016). It is not a reach to predict the end of the GMO as a cornerstone of regulating agricultural technology and flashpoint of conflict restricting progress. Genome editing offers a new frontier for plant technology that is unprecedented but brings along with it unprecedented challenges, particularly with the advent of gene drives. How these challenges are faced and dealt with will affect our world for generations to come.

References

Al-Babili, S., and Beyer, P. (2005), "Golden Rice: Five Years on the Road, Five Years to Go?" *Trends in Plant Science*, vol. 10, no. 12, December, pp. 565–73.▲

Alton, E. W., Boyd, A. C., Davies, J. C., Gill, D. R., Griesenbach, U., Harrison, P. T., *et al.* (2016), "Genetic Medicines for CF: Hype Versus Reality," *Pediatric Pulmonology*, vol. 51, no. S44, October, pp. S5–S17.▲

Aswath, C. R., Krisnaraj, P. U., and Padmanaban, G. (2016), "Why India Needs Biotechnology to Ensure Food and Nutrition Security," *Genetically Modified Organisms in Food*, 1st Edition Production, Safety, Regulation and Public Health, Elsevier.▲

Barabaschi, D., Tondelli, A., Desiderio, F., Volante, A., Vaccino, P., Valè, G., *et al.* (2016), "Next Generation Breeding," *Plant Science*, vol. 242, January, pp. 3–13.▲

Barakate, A., and Stephens, J. (2016), "An Overview of CRISPR-Based Tools and Their Improvements: New Opportunities in Understanding Plant-Pathogen Interactions for Better Crop Protection," *Frontiers in Plant Science*, vol. 7, June 1.▲

Basak, J., and Nithin, C. (2015), "Targeting Non-Coding RNAs in Plants with the CRISPR-Cas Technology is a Challenge yet Worth Accepting," *Frontiers in Plant Science*, vol. 6, November 19.▲

Bazuin, S., Azadi, H., and Witlox, F. (2011), "Application of GM Crops in Sub-Saharan Africa: Lessons Learned from Green Revolution," *Biotechnology Advances*, vol. 29, no. 6, November-December, pp. 908–12.▲

Benjamin, R., Berges, B. K., Solis-Leal, A., Igbinedion, O., Strong, C. L., and Schiller, M. R. (2016), "TALEN Gene Editing Takes Aim on HIV," *Human Genetics*, vol. 135, no. 9, September, pp. 1059–70.▲

Benston, S. (2016), "CRISPR, a Crossroads in Genetic Intervention: Pitting the Right to Health Against the Right to Disability," *Laws*, vol. 5, no. 1, March.▲

Beyer, P. (2010), "Golden Rice and 'Golden' Crops for Human Nutrition," *New Biotechnology*, vol. 27, no. 5, Novermber 30, pp. 478–81.▲

Bhutta, Z. A., Salam, R. A., and Das, J. K. (2013), "Meeting the Challenges of Micronutrient Malnutrition in the Developing World," *British Medical Bulletin*, vol. 106, pp. 7–17.▲

Blanvillain-Baufumé, S., Reschke, M., Solé, M., Auguy, F., Doucoure, H., Szurek, B., *et al.* (2016), "Targeted Promoter Editing for Rice Resistance to Xanthomonasoryzaepv Oryzae Reveals Differential Activities for SWEET14-Inducing TAL Effectors," *Plant Biotechnology Journal*, August 19.▲

Bono, J. M., Olesnicky, E. C., and Matzkin, L. M. (2015), "Connecting Genotypes, Phenotypes and Fitness: Harnessing the Power of CRISPR/Cas9 Genome Editing," *Molecular Ecology*, vol. 24, no. 15, August, pp. 3810–22.▲

Bortesi, L., and Fischer, R. (2015), "The CRISPR/Cas9 System for Plant Genome Editing and Beyond," *Biotechnology Advances*, vol. 33, no. 1, January-February, pp. 41–52.▲

Brooks, C., Nekrasov, V., Lippman, Z. B., and Van Eck J. (2014), "Efficient Gene Editing in Tomato in the First Generation Using the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated9 System," *Plant Physiology*, vol. 166, no. 3, November, pp. 1292–7, available at doi: 10.1104/pp.114.247577, viewed on April 26, 2017.▲

Callaway, E. (2017), "Gene Drives Thwarted by Emergence of Resistant Organisms," *Nature News*, January 31.▲

Cai, Y., Chen, L., Liu, X., Sun, S., Wu, C., Jiang, B., *et al.* (2015), "CRISPR/Cas9-Mediated Genome Editing in Soybean Hairy Roots," *Public Library of Science One*, vol. 10, no. 8, August 18.▲

Cardi, T., and Neal Stewart Jr., C. (2016), "Progress of Targeted Genome Modification Approaches in Higher Plants," *Plant Cell Reports*, vol. 35, no. 7, July, pp. 1401–16.

Čermák, T., Baltes, N. J., Čegan, R., Zhang, Y., and Voytas, D. F. (2015), "High-Frequency, Precise Modification of the Tomato Genome," *Genome Biology*, vol. 16, no. 232, November 6.

Char, S. N., Unger-Wallace, E., Frame, B., Briggs, S. A., Main, M., Spalding, M. H., *et al.* (2015), "Heritable Site-Specific Mutagenesis Using Talens in Maize," *Plant Biotechnology Journal*, vol. 13, no. 7, September, pp. 1002–10.

Char, S. N., Neelakandan, A. K., Nahampun, H., Frame, B., Main, M., Spalding, M. H., *et al.* (2016), "An Agrobacterium-Delivered CRISPR/Cas9 System for High-Frequency Targeted Mutagenesis in Maize," *Plant Biotechnology Journal*, August 11.

Collinge, D. B., Jørgensen, H. J., Lund, O. S., and Lyngkjaer, M. F. (2010), "Engineering Pathogen Resistance in Crop Plants: Current Trends and Future Prospects," *Annual Review of Phytopathology*, vol. 48, pp. 269–91.

Cominelli, E., and Tonelli, C. (2010), "Transgenic Crops Coping with Water Scarcity," *New Biotechnology*, vol. 27, no. 5, November 30, pp. 473–7.

Deikman, J., Petracek, M., and Heard, J. E. (2012), "Drought Tolerance through Biotechnology: Improving Translation from the Laboratory to Farmers' Fields," *Current Opinion in Biotechnology*, vol. 23, no. 2, April, pp. 243–50.

Ding, Y., Li, H., Chen, L. L., and Xie, K. (2016), "Recent Advances in Genome Editing Using CRISPR/Cas9," *Frontiers in Plant Science*, vol. 7, no. 703, May 24.

Du, H., Zeng, X., Zhao, M., Cui, X., Wang, Q., Yang, H., *et al.* (2016), "Efficient Targeted Mutagenesis in Soybean by TALENs and CRISPR/Cas9," *Biotechnology*, vol. 217, January 10, pp. 90–7.

Esvelt, K. (2016), "Gene Editing Can Drive Science to Openness," *Nature*, vol. 534, no. 7606, January 8, p. 153.

Evanega, Sand, and Lynas, M. (2015), "Dialectic of the Pro-Poor Papaya," in Ronald J. Herring (ed.) *The Oxford Handbook of Food, Politics, and Society*, Oxford University Press, New York and Oxford.

Food and Agriculture Organisation (FAO) (2013), *The State of Food Insecurity in the World*, Executive Summary.

Fogleman, S., Santana, C., Bishop, C., Miller, A., and Capco, D. G. (2016), "CRISPR/Cas9 and Mitochondrial Gene Replacement Therapy: Promising Techniques and Ethical Considerations," *American Journal of Stem Cells*, vol. 5, no. 2, August 20, pp. 39–52.

Garry, M. G., and Garry, M. G. (2016), "Humanised Organ Generating Animals," *Regenerative Medicine*, vol. 11, no. 7, pp. 617–9.

Global Nutrition Report (2014), "Actions and Accountability to Accelerate the World's Progress on Nutrition," International Food Policy Research Institute, Issue Brief 82, November.

Gonsalves, D. (1998), "Control of Papaya Ringspot Virus in Papaya: A Case Study," *Annual Review of Phytopathology*, vol. 36, pp. 415–37.

Guerrini, C. J., Curnutte, M. A., Sherkow, J. S., and Scott, C. T. (2017), "The Rise of the Ethical Licence," *Nature Biotechnology*, vol. 35, no. 1. January 19, pp. 22–4, available at doi: 10.1038/nbt.3756, viewed on May 2, 2017.

Gurr, G. M., and You, M. (2016), "Conservation Biological Control of Pests in the Molecular Era: New Opportunities to Address Old Constraints," *Frontiers in Plant Science*, vol. 6, January 12, p. 1255.

Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., *et al.* (2016), "CRISPR-Cas9 Gene Drive System Targeting Female Reproduction in the Malaria Mosquito Vector Anopheles Gambiae," *Nature Biotechnology*, vol. 34, no. 1, January, pp. 78–83.

Harriss, J., and Stewart, D. (2015), "Science, Politics and the Framing of Modern Agricultural Technologies," in R. Herring (ed.), *The Oxford Handbook of Food, Politics and Society*, Oxford University Press, Oxford and New York.

Herring, R. (2007), "Stealth Seeds: Biosafety, Bioproperty, Biopolitics." *Journal of Development Studies* 43:1.

Herring, R., and Paarlberg, R. (2016), "The Political Economy of Biotechnology," *Annual Review of Resource Economics*, vol. 8, pp. 397–416, Palo Alto.

Huang, S., Weigel, O., Beachy, R., and Li, J. (2016), "A Proposed Regulatory Framework for Genome-Edited Crops," *Nature Genetics*, vol. 48, pp. 109–11.

Im, W., Moon, J., and Kim, M. (2016), "Applications of CRISPR/Cas9 for Gene Editing in Hereditary Movement Disorders," *Journal of Movement Disorders*, vol. 9, no. 3, September, pp. 136–43.

International Food Policy Research Institute (IFPRI) (2014), *Global Hunger Index*, International Food Policy Research Institute and Concern Worldwide, Bonn, Washington D. C. and Dublin.

Jacobs, T. B., LaFayette, P. R., Schmitz R. J., and Parrott, W. A. (2015), "Targeted Genome Modifications in Soybean with CRISPR/Cas9," *BMC Biotechnology*, vol. 15, no. 16, March 12.

James, Clive (2013), "ISAAA Brief 46: Executive Summary Global Status of Commercialised Biotech/GM Crops," The International Service for the Acquisition of Agri-Biotech Applications.

Jenko, J., Gorjanc, G., Cleveland, M. A., Varshney, R. K., Whitelaw, C. B., Woolliams, J. A., *et al.* (2015), "Potential of Promotion of Alleles by Genome Editing to Improve Quantitative Traits in Livestock Breeding Programmes," *Genetics Selection Evolution*, vol. 47, no. 55, July 2.

Jia, H., Orbovic, V., Jones, J. B., and Wang, N. (2016), "Modification of the PthA4 Effector Binding Elements in Type I CsLOB1 Promoter Using Cas9/sgRNA to Produce Transgenic Duncan Grapefruit Alleviating

XccΔpthA4:dCsLOB1.3 Infection," *Plant Biotechnology Journal*, vol. 14, no. 5, May, pp. 1291–301.

Jia, H., and Wang, N. (2014), "Targeted Genome Editing of Sweet Orange Using Cas9/sgRNA," *Public Library of Science One*, vol. 9, no. 4, April 7.

Johnson, N. (2015), "It is Practically Impossible to Define 'GMOs'," *Grist*, available at http://grist.org/food/mind-bomb-its-practically-impossible-to-define-gmos/, viewed on May 3, 2016.

Jones, H. D. (2015), "Future of Breeding by Genome Editing is in the Hands of Regulators," *GM Crops Food*, vol. 6, no. 4, pp. 223–32.

Kamthan, A., Chaudhuri, A., Kamthan, M., and Datta, A. (2016), "Genetically Modified (GM) Crops: Milestones and New Advances in Crop Improvement," *Theoretical and Applied Genetics*, vol. 129, no. 9, September, pp. 1639–55.

Kathage, J., and Qaim, M. (2012), "Economic Impacts and Impact Dynamics of Bt (Bacillus thuringiensis) Cotton in India," *Proceedings of the National Academy of Sciences*, vol. 109, no. 29, pp. 11652–56.

Khatodia, S., Bhatotia, K., Passricha, N., Khurana, S. M., and Tuteja, N. (2016), "The CRISPR/Cas Genome-Editing Tool: Application in Improvement of Crops," *Frontiers in Plant Science*, vol. 7, no. 506, April 19.

Kim, H., Kim, S. T., Ryu, J., Choi, M. K., Kweon, J., Kang, B. C., *et al.* (2016), "A Simple, Flexible and High-Throughput Cloning System for Plant Genome Editing via CRISPR-Cas System," *Journal of Integrative Plant Biology*, vol. 58, no. 8, August, pp. 705–12.

Kolady, D., and Herring, R. (2014), "Regulation of Genetically Engineered Crops in India: Implications of Uncertainty for Social Welfare, Competition and Innovation," *Canadian Journal of Agricultural Economics*, vol. 62, no. 4, pp. 471–90.

Kouser, S., Abedullah, and Qalm, M. (2017), "Bt Cotton and Employment Effects for Female Agricultural Labourers in Pakistan," *New Biotechnology*, vol. 34, January, pp. 40–6.

Krishan, K., Kanchan, T., and Singh, B. (2016), "Human Genome Editing and Ethical Considerations," *Science and Engineering Ethics*, vol. 22, no. 2, April, pp. 597–9.

Kumar, S., Chandra, A., and Pandey, K. C. (2008), "Bacillus thuringiensis (Bt) Transgenic Crop: An Environment Friendly Insect-Pest Management Strategy," *Journal of Environmental Biology*, vol. 29, no. 5, September, pp. 641–53.

Kumar, V., and Jain, M. (2015), "The CRISPR–Cas System for Plant Genome Editing: Advances and Opportunities," *Journal of Experimental Botany*, vol. 66, no. 1, pp. 47–57.

Kyndt, T., Quispe, D., Zhai, H., Jarret, R., Ghislain, M., Liu, Q., *et al.* (2015), "The Genome of Cultivated Sweet Potato Contains *Agrobacterium* T-DNAs with Expressed Genes: An Example of a Naturally Transgenic Food Crop," *Proceedings of the National Academy of Sciences*, available at http://www.pnas.org/content/112/18/5844.full, viewed on May 3, 2017.

Lambert, A. R., Hallinan, J. P., Shen, B. W., Chik, J. K., Bolduc, J. M., Kulshina, N., Robins, *et al.* (2016), "Indirect DNA Sequence Recognition and Its Impact on Nuclease Cleavage Activity," *Structure*, vol. 24, no. 6, June 7, pp. 862–73.

Languin, K. (2014), "Genetic Engineering to the Rescue Against Invasive Species?" *National Geographic*, July 18.

Ledford, H. (2017), "Why the CRISPR Patent Verdict Isn't the End of the Story," *Nature*, February 17, available at doi: 10.1038/nature.2017.21510, viewed on May 3, 2017.

Ledford, H. (2016), "Gene-Editing Surges as US Rethinks Regulations," *Nature*, vol. 532, pp. 158–9.

Li, J., Meng, X., Zong, Y., Chen, K., Zhang, H., Liu, J., *et al.* (2016a), "Gene Replacements and Insertions in Rice by Intron Targeting Using CRISPR-Cas9," *Nature Plants*, vol. 2, no. 10, September 12.

Li, M., Li, X., Zhou, Z., Wu, P., Fang, M., Pan, X., *et al.* (2016b), "Reassessment of the Four Yield-related Genes Gn1a, DEP1, GS3, and IPA1 in Rice Using a CRISPR/Cas9 System," *Frontiers in Plant Science*, vol. 7, no. 377, March 30.

Li, T., Liu, B., Chen, C. Y., and Yang, B. (2016c), "TALEN-Mediated Homologous Recombination Produces Site-Directed DNA Base Change and Herbicide-Resistant Rice," *Journal of Genetics and Genomics*, vol. 43, no. 5, May 20, pp. 297–305.

Li, Z., Liu, Z. B., Xing, A., Moon, B. P., Koellhoffer, J. P., Huang, L., *et al.* (2015), "Cas9-Guide RNA Directed Genome Editing in Soybean," *Plant Physiology*, vol. 169, no. 2, October, pp. 960–70.

Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zheng, Z., *et al.* (2015), "CRISPR/Cas9-Mediated Gene Editing in Human Tripronuclear Zygotes," *Protein Cell*, vol. 6, no. 363, available at doi: 10.1007/s13238-015-0153-5, viewed on May 3, 2017.

Lillico, S. G., Proudfoot, C., Carlson, D. F., Stverakova, D., Neil, C., Blain, C., *et al.* (2013), "Live Pigs Produced from Genome Edited Zygotes," *Scientfic Reports*, Oct 10, vol. 3:2847.

Liu, D., Hu, R., Palla, K. J., Tuskan, G. A., and Yang, X. (2016), "Advances and Perspectives on the Use of CRISPR/Cas9 Systems in Plant Genomics Research," *Current Opinion in Plant Biology*, vol. 30, April, pp. 70–7.

Long, S. P., Marshall-Colon, A., and Zhu, X. G. (2015), "Meeting the Global Food Demand of the Future by Engineering Crop Photosynthesis and Yield Potential," *Cell*, vol. 161, no. 1, March 26, pp. 56–66.

Luo, M., Gilbert, B., and Ayliffe, M. (2016), "Applications of CRISPR/Cas9 Technology for Targeted Mutagenesis, Gene Replacement and Stacking of Genes in Higher Plants," *Plant Cell Reports*, vol. 35, no. 7, July, pp. 1439–50.

Mao, Y., Botella, J. R., and Zhu, J. K. (2016), "Heritability of Targeted Gene Modifications Induced by Plant-Optimised CRISPR Systems," *Cellular and Molecular Life Sciences*, September 27.▲

Mendell, J. R., and Rodino-Klapac, L. R. (2016), "Duchenne Muscular Dystrophy: CRISPR/Cas9 Treatment," *Cell Research*, vol. 26, no. 5, May, pp. 513–4.▲

Merkert, S., and Martin, U. (2016), "Targeted Genome Engineering Using Designer Nucleases: State of the Art and Practical Guidance for Application in Human Pluripotent Stem Cells," *Stem Cell Research*, vol. 16, no. 2, March, pp. 377–86.▲

Michno, J. M., Wang, X., Liu, J., Curtin, S. J., Kono, T. J., and Stupar, R. M. (2015), "CRISPR/Cas Mutagenesis of Soybean and Medicagotruncatula Using a New Web-Tool and a Modified Cas9 Enzyme," *GM Crops Food*, vol. 6, no. 4, pp. 243–52.▲

Montenegro, M. (2016), "CRISPR is Coming to Agriculture - with Big Implications for Food, Farmers, Consumers, and Nature," *Ensia*, available at http://ensia.com/voices/crispr-is-coming-to-agriculture-with-big-implications-for-food-farmers-consumers-and-nature/, viewed on May 3, 2017.▲

National Academies Press (2016), "Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values," available at https://www.nap.edu/catalog/23405/gene-drives-on-the-horizon-advancing-science-navigating-uncertainty-and Aligning Research with Public Values, viewed on May 3, 2017.▲

Nature Editorial (2017), "Editorial," available at http://www.nature.com/news/gene-editing-in-legal-limbo-in-europe-1.21515, viewed on May 3, 2017.▲

Niu, Y., Shen, B., Cui, Y., Chen, Y., Wang, J., Wang, L., *et al.* (2014), "Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos," *Cell*, vol. 156, no. 4, February 13, pp. 836–43.▲

Nongpiur, R. C., Singla-Pareek, S. L., and Pareek, A. (2016), "Genomics Approaches for Improving Salinity Stress Tolerance in Crop Plants," *Current Genomics*, vol. 17, no. 4, August, pp. 343–57.▲

Nuijten, E., Messmer, M. M., and Lammerts van Bueren, E. M. (2017), "Concepts and Strategies of Organic Plant Breeding in Light of Novel Breeding Techniques," *Sustainability*, vol. 9, no. 18, available at doi: 10.3390/su9010018, viewed on May 3, 2017.▲

Ossola, A. (2016), "CRISPR-Modified Corn May Soon be Ready for Market," *Popular Science*, September 6.▲

Paarlberg, R. L. (2001), *The Politics of Precaution: Genetically Modified Crops in Developing Countries*, Johns Hopkins University Press, Baltimore.▲

Paarlberg, R. L. (2008), *Starved for Science: Keeping Biotechnology Out of Africa*, Harvard University Press, Cambridge.▲

Pan, C., Ye, L., Qin, L., Liu, X., He, Y., Wang, J., *et al.* (2016), "CRISPR/Cas9-Mediated Efficient and Heritable Targeted Mutagenesis in Tomato Plants in the First and Later Generations," *Scientific Reports*, vol. 6, no. 24765, April 21.▲

Paul, J. W., and Qi, Y. (2016), "CRISPR/Cas9 for Plant Genome Editing: Accomplishments, Problems and Prospects," *Plant Cell Reports*, vol. 35, no. 7, July, pp. 1417–27.▲

Pérez-Massot, E., Banakar, R., Gómez-Galera, S., Zorrilla-López, U., Sanahuja, G., Arjó, G., *et al.* (2013), "The Contribution of Transgenic Plants to Better Health through Improved Nutrition: Opportunities and Constraints," *Genes*, vol. 8, no. 1, January, pp. 29–41.▲

Potrykus, I. (2010), "Regulation Must be Revolutionised," *Nature*, vol. 466, no. 7306, July 29, p. 561.▲

Proudfoot, C., Carlson, D. F., Huddart, R., Long, C. R., Pryor, J. H., King, T. J., *et al.* (2015), "Genome Edited Sheep and Cattle," *Transgenic Research*, vol. 24, no. 1, February, pp. 147–53.▲

Qi, W., Zhu, T., Tian, Z., Li, C., Zhang, W., and Song, R. (2016), "High-Efficiency CRISPR/Cas9 Multiplex Gene Editing Using the Glycine tRNA-Processing System-Based Strategy in Maize," *BMC Biotechnology*, vol. 16, no. 1, August 11, p. 58.▲

Quétier, F. (2016), "The CRISPR-Cas9 Technology: Closer to the Ultimate Toolkit for Targeted Genome Editing," *Plant Science*, vol. 242, January, pp. 65–76.▲

Raitskin, O., and Patron, N. J. (2016), "Multi-Gene Engineering in Plants with RNA-Guided Cas9 Nuclease," *Current Opinion in Biotechnology*, vol. 37, February, pp. 69–75.▲

Rani, R., Yadav, P., Barbadikar, K. M., Baliyan, N., Malhotra, E. V., Singh, B. K., *et al.* (2016), "CRISPR/Cas9: A Promising Way to Exploit Genetic Variation in Plants," *Biotechnology Letters*, August 29.▲

Regalado, A. (2015), "CRISPR Gene Editing to Be Tested on People by 2017, Says Editas," *MIT Technology Review*, November 5.▲

Reid, W., and O'Brochta, D. A. (2016), "Applications of Genome Editing in Insects," *Current Opinion in Insect Science*, vol. 13, February, pp. 43–54.▲

Ricroch, A. E., and Hénard-Damave, M. C. (2016), "Next Biotech Plants: New Traits, Crops, Developers and Technologies for Addressing Global Challenges," *Critical Reviews in Biotechnology*, vol. 36, no. 4, August, pp. 675–90.▲

Sander, J. D., and Joung, J. K. (2014), "CRISPR-Cas Systems for Editing, Regulating and Targeting Genomes," *Nature Biotechnology*, vol. 32, pp. 347–55.▲

Sankar, P. L., and Cho, M. K. (2015), "Engineering Values into Genetic Engineering: A Proposed Analytic Framework for Scientific Social Responsibility," *American Journal of Bioethics*, vol. 15, no. 12, pp. 18–24.▲

Sauer, N. J., Mozoruk, J., Miller, R. B., Warburg, Z. J., Walker, K. A., Beetham, P. R., *et al.* (2016), "Oligonucleotide-Directed Mutagenesis for Precision Gene Editing," *Plant Biotechnology Journal*, vol. 14, no. 2, February, pp. 496–502.

Schuster, M., Schweizer, G., Reissmann, S., and Kahmann, R. (2016), "Genome Editing in *Ustilagomaydis* Using the CRISPR-Cas System," *Fungal Genetics and Biology*, vol. 89, April, pp. 3–9.

Seah, Y. F., El Farran, C. A., Warrier, T., Xu, J., and Loh, Y. H. (2015), "Induced Pluripotency and Gene Editing in Disease Modelling: Perspectives and Challenges," *International Journal of Molecular Sciences*, vol. 16, no. 12, December 2, pp. 28614–34.

Saey, T. H. (2015), "Gene Drives Spread Their Wings," *Science News*, vol. 188, no. 12, p. 16.

Shan, Q., Wang, Y., Li, J., and Gao, C. (2014), "Genome Editing in Rice and Wheat Using the CRISPR/Cas System," *Nature Protocols*, vol. 9, no. 10, October, pp. 2395–410.

Shantharam, S. (2016), "Genome Editing: The Benefits and the Ethics," *Magazine*, July 15.

Shi, J., Gao, H., Wang, H., Lafitte, H. R., Archibald, R. L., Yang, M., *et al.* (2016), "ARGOS8 Variants Generated by CRISPR-Cas9 Improve Maize Grain Yield under Field Drought Stress Conditions," *Plant Biotechnology Journal*, July 21.

Singer, M., and Frischknecht, F. (2016), "Time for Genome Editing: Next-Generation Attenuated Malaria Parasites," *Trends Parasitology*, October 25, pp. S1471-4922(16)30168-4.

Shukla, V. K., Doyon, Y., Miller, J. C., DeKelver, R. C., Moehle, E. A., Worden, S. E., *et al.* (2009), "Precise Genome Modification in the Crop Species Zea Mays Using Zinc-Finger Nucleases," *Nature*, vol. 459, no. 7245, May 21, pp. 437–41.

Singh, A., Chakraborty, D., and Maiti, S. (2016), "CRISPR/Cas9: A Historical and Chemical Biology Perspective of Targeted Genome Engineering," *Chemical Society Reviews*, September 14.

Singh, R. P., Hodson, D. P., Jin, Y., Lagudah, E. S., Ayliffe, M. A., Bhavani, S., *et al.* (2015), "Emergence and Spread of New Races of Wheat Stem Rust Fungus: Continued Threat to Food Security and Prospects of Genetic Control," *Phytopathology*, vol. 105, no. 7, July, pp. 872–84.

Stella, S., and Montoya, G. (2016), "The Genome Editing Revolution: A CRISPR-Cas TALE Off-Target Story," *Bioessays*, vol. 38, no. 1, July, pp. S4–S13.

Stone, D., Niyonzima, N., and Jerome, K. R. (2016), "Genome Editing and the Next Generation of Antiviral Therapy Infection," *Human Genetics*, vol. 135, no. 9, September, pp. 1071–82.

Sun, X., Hu, Z., Chen, R., Jiang, Q., Song, G., Zhang, H. *et al.* (2015), "Targeted Mutagenesis in Soybean Using the CRISPR-Cas9 System," *Scientific Reports*, vol. 5, no. 10342, May 29.

Svitashev, S., Young, J. K., Schwartz, C., Gao, H., Falco, S. C., and Cigan, A. M. (2015), "Targeted Mutagenesis, Precise Gene Editing, and Site-Specific Gene Insertion in Maize Using Cas9 and Guide RNA," *Plant Physiology*, vol. 169, no. 2, October, pp. 931–45.

Talan, J. (2015), "News from the Society for Neuroscience Annual Meeting: Gene Editing Techniques Show Promise in Silencing or Inhibiting the Mutant Huntington's Disease," *Neurology Today*, vol. 15, no. 22, November 19, pp. 14–16.

Tan, W., Carlson, D. F., Lancto, C. A., Garbe, J. R., Webster, D. A., Hackett, P. B., *et al.* (2013), "Efficient Nonmeiotic Allele Introgression in Livestock Using Custom Endonucleases," *Proceedings of the National Academy of Sciences USA*, vol. 110, no. 41, October 8, pp. 16526–31.

Tan, W., Proudfoot, C., Lillico, S. G., and Whitelaw, C. B. (2016), "Gene Targeting, Genome Editing: From Dolly to Editors," *Transgenic Research*, vol. 25, no. 3, June, pp. 273–87.

Unglesbee, E. (2016), "Brave New Crops," *Progressive Farmer*, nos. 1–2, October.

Vogel, G. (2015), "Bioethics: Embryo Engineering Alarm," *Science*, vol. 347, no. 6228, March, p. 1301.

Waddington, S. N., Privolizzi, R., Karda, R., and O'Neill, H. C. (2016), "A Broad Overview and Review of CRISPR-Cas Technology and Stem Cells," *Current Stem Cell Reports*, vol. 2, pp. 9–20.

Waltz, E. (2016), "Gene-Edited CRISPR Mushroom Escapes US Regulation," *Nature*, vol. 532, no. 7599, April 21, p. 293.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., *et al.* (2014), "Simultaneous Editing of Three Homoeoalleles in Hexaploid Bread Wheat Confers Heritable Resistance to Powdery Mildew," *Nature Biotechnology*, vol. 32, no. 9, September, pp. 947–51.

Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., *et al.* (2016), "Enhanced Rice Blast Resistance by CRISPR/Cas9-Targeted Mutagenesis of the ERF Transcription Factor Gene OsERF922," *Public Library of Science One*, vol. 11, no. 4, April 26.

Wang, F., and Qi, L. S. (2016), "Applications of CRISPR Genome Engineering in Cell Biology," *Trends in Cell Biology*, vol. 26, no. 11, November, pp. 875–88.

Wang, M., Glass, Z. A., and Xu, Q. (2016), "Non-Viral Delivery of Genome-Editing Nucleases for Gene Therapy," *Gene Therapy*, Oct 31.

Wieczorek, A. (2016), "CRISPR-Cas9 and Gene Drives: The Risks and Benefits of Game-Changing Technology," *Biotech In Focus*, Cooperative Extension Service, University of Hawaii at Manoa, July, Issue 52.

Wolf, J. D., Wang, K., and Yang, B. (2016), "The Regulatory Status of Genome-Edited Crops," *Plant Biotechnology Journal*, vol. 14, no. 2, pp. 510–18.

Xing, H. L., Dong, L., Wang, Z. P., Zhang, H. Y., Han, C. Y., Liu, B., *et al.* (2014), "A CRISPR/Cas9 Toolkit for Multiplex Genome Editing in Plants," *BMC Plant Biology*, vol. 14, no. 327, November 29.

Xu, R., Wei, P., and Yang, J. (2017), "Use of CRISPR/Cas Genome Editing Technology for Targeted Mutagenesis in Rice," *Methods in Molecular Biology*, vol. 1498, pp. 33–40.

Yao, J., Huang, J., and Zhao, J. (2016), "Genome Editing Revolutionises the Creation of Genetically Modified Pigs for Modeling Human Diseases," *Human Genetics*, vol. 135, no. 9, September, pp. 1093–105.

Yee, J. K. (2016), "Off-Target Effects of Engineered Nucleases," *The FEBS Journal*, vol. 283, no. 17, September, pp. 3239–48.

Yi, L., and Li, J. (2016), "CRISPR-Cas9 Therapeutics in Cancer: Promising Strategies and Present Challenges," *Biochimica et Biophysica Acta*, vol. 1866, no. 2, September 15, pp. 197–207.

Yin, H., Xue, W., Chen, S., Bogorad, R. L., Benedetti, E., Grompe, M., *et al.* (2014), "Genome Editing with Cas9 in Adult Mice Corrects a Disease Mutation and Phenotype," *Nature Biotechnology*, vol. 32, no. 6, June, pp. 551–3.

Zhang, D., Li, Z., and Li, J. F. (2016), "Targeted Gene Manipulation in Plants Using the CRISPR/Cas Technology," *Journal of Genetics and Genomics*, vol. 43, no. 5, May 20, pp. 251–62.

Zhang, S. (2016), "CRISPR Could Usher in a New Era of Delicious GMO Foods," *The Atlantic*, September 19.

Zhang, Z., Mao, Y., Ha, S., Liu, W., Botella, J. R., and Zhu, J. K. (2016), "A Multiplex CRISPR/Cas9 Platform for Fast and Efficient Editing of Multiple Genes in Arabidopsis," *Plant Cell Reports*, vol. 35, no. 7, July, pp. 1519–33.

Zhao, X., Ni, W., Chen, C., Sai, W., Qiao, J., Sheng, J., *et al.* (2016), "Targeted Editing of Myostatin Gene in Sheep by Transcription Activator-like Effector Nucleases," *Asian-Australasian Journal of Animal Science*, vol. 29, no. 3, March, pp. 413–8.

Zhu, C., Bortesi, L., Baysal, C., Twyman, R. M., Fischer, R., Capell, T., *et al.* (2016), "Characteristics of Genome Editing Mutations in Cereal Crops," *Trends in Plant Science*, September 16.

Appendix 1

Table 1 *Categories of agricultural biotechnology*

Technology	Description	Regulatory status	Example
Mutagenesis breeding	Random mutations introduced into genome via chemical or irradiation mutagenesis	None required	Ruby red grapefruit
Transgenesis	Introduction of novel traits by delivering DNA from a different organism to the target organism	Regulated in countries that permit its sale	Golden rice (Beyer 2010)
Cisgenesis	Introduction of a trait by delivering DNA from similar, sexually compatible species	Regulated in countries that permit its sale	American chestnut
RNA interference (RNAi)	Introduction of antisense RNA corresponding to a gene from an organism or from an invading pathogen of that organism	Regulated in countries that permit its sale	RSVR papaya
Genome editing	Targeted nuclease, in conjunction with the cell's DNA repair machinery, makes small one or a few nucleotide changes within an organism's genome	Currently unclear	Swedish cabbage

Source: https://mvd.iaea.org, Beyer (2010), Singh *et al.* (2015), Evanega and Lynas (2015), and Zhang *et al.* (2016).

Appendix 2

Genome Editing Technologies

Mega-nucleases: The first tools to be used for genome editing, mega-nucleases are naturally occurring enzymes found in bacteria. One single region on the mega-nuclease recognises and binds to relatively long DNA sequences (14-40 nucleotides long), then cleaves the DNA (Yee 2016; Zhu *et al.* 2016). Since all the activities are located within one protein domain, it is difficult to separate the targeting and DNA cutting functions of mega-nucleases, and thus it is impossible to programme the nuclease to target new sites on the genome for cleavage. Since the sequence recognition sites for mega-nucleases that have been identified so far do not occur naturally in the plant genome, there are limits to how useful they are for genome editing in crops.

Zinc Finger Nucleases (ZFN): Zinc finger nucleases are hybrid proteins consisting of a DNA binding domain (consisting of three or four binding modules, with each module recognising a specific segment of DNA) that has been fused to a nuclease domain, which creates a DNA break (Wang *et al.* 2016; Zhu 2016). ZFNs can be cumbersome to design and can have some off-target effects, meaning that they can bind to additional unintended sites and cleave DNA at locations other than the one desired. Another disadvantage of using ZFN is the high cost

of licensing the technology.

TALENs: As a technology, TALENs utilise the transcriptional activator-like effector (TALE) protein derived from the bacteria *Xanthomonas* as its DNA binding domain. This TALE DNA binding domain is fused to a nuclease domain (Benjamin *et al.* 2016). Since the target recognition sequence is larger for TALENs than for ZFNs, TALEN-based technologies display fewer off-target effects, meaning that the DNA binding domain binds exactly to the target site and nowhere else on the genome. A drawback to the use of TALENs is the difficulty of assembling the DNA binding domain (Merkert and Martin 2016).

CRISPR/Cas9: CRISPR-Cas9 has rapidly become the main tool for genome editing in plant science research laboratories. Discovered first in a common bacterium found in the intestinal tract, CRISPR-Cas9 is composed of a ribo-nucleoprotein complex containing both a CRISPR (clustered regularly interspaced short palindromic repeat) sequence of RNA and a Cas (CRISPR-associated) protein that protects bacteria from invading bacteriophage DNA (Bono *et al.* 2015; Quetre 2016).

For a long time, short DNA repeats that are interspaced with sequences containing homology to virus sequences (known as CRISPR loci) have been observed in the genomes of bacteria. Adjacent to these virus sequences are genes encoding a series of Cas proteins (Wang and Qi 2016). CRISPR loci and Cas proteins play a unique role in the bacteria's defence mechanism against invading pathogens; the bacteria can recognise a particular virus that infects the cell based on homology with one of its CRISPR loci. The relevant sequence can then be used as guide RNA to direct the Cas system to destroy the invading virus by destroying its genetic material. Cas9 is a protein within the Cas repertoire which can actually cleave DNA at the target site proposed by the CRISPR loci.

Researchers soon discovered that Cas9 could be easily adapted for use in genome editing and began to make their own versions of CRISPR synthetic guide RNA (sgRNA) that could be targeted to any sequence of any organism. The CRISPR RNA molecule is able to guide the nuclease to a specific DNA target site, at which the Cas9 nuclease performs its cleavage function (Sander and Joung 2014). Since Cas9 is efficient at causing a highly specific cleavage event within a target sequence of about 20 nucleotides, it is much easier to create sgRNAs than it is to form specific binding domains on proteins that ZFN or TALEN-based technologies require. The cell's repair machinery then makes the desired permanent change in the genome. The technology is versatile, available, and easy to use. While some off-target cleavage was originally reported upon the first applications of CRISPR-Cas9, this has been substantially reduced by altering the Cas9:sgRNA ratio and also by using computer software that assists in sgRNA design and reduces the potential for off-target effects.

In addition to its use as a genome editing tool, the targeting function of CRISPR-Cas9 has made it an effective tool at localising gene expression. This can be achieved by linking an inactivated version of Cas9 to a fluorescent protein. Furthermore, Cas9 can be fused to proteins that activate or suppress a variety of genes, and targeted to any regulatory element on a genome.