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Regulatory Logics and Politics of Agricultural Biotechnology Diffusion[1]

Ronald J. Herring (rjh5@cornell.edu)

Cornell University: Government, International Agriculture

Abstract

There is no robust and parsimonious explanation for differences in diffusion of agricultural biotechnology across countries or across time. Variables that delineate common political rifts in international trade and politics fail to explain variation. The one constant is a risk-utility balance, filtered through structures of regulatory mechanisms and their associated political ecology: Agriculture vs Environment ministries, eg. This paper assesses in a preliminary way political prospects of new technologies for genetic engineering of agricultural plants, especially CRISPR-Cas9. The frontiers of plant breeding are moving away from transgenesis as dominant form of plant breeding and test of what requires special regulation -- a 'GMO' or 'LMO.' New regulatory constructs treat gene-edited plants more as mutagenized crops -- in a targeted rather than random way. Since the thousands of mutagenized agricultural plants have been immune to regulation as 'GMOs' – and can be 'organic' - this political battle for definition will shape the future diffusion of agricultural – and other – biotechnologies globally, marking the end of pointless battles over the 'GMO.' Because of the utility of new techniques for consumers and farmers alike – unlike transgenic plants for the most part – as well as for the environment and human health, the risk-utility balance is being fundamentally altered.

1. Introduction: The End of the GMO?

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 CRISPR-Cas genome-edited crops do not fall under the EU's definition of a genetically modified organism (GMO); no special regulation was necessary. Similar rulings have occurred in the U.S. and Canada. If this

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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, will force a fundamental re-evaluation of how to regulate tomorrow's food crops – and much else.

The genomics revolution that enabled modern agricultural biotechnology has been a source of optimism and controversy since its inception: suicide seeds and silver bullets. 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 to complement conventional breeding methods. Genetically modified (GM) crops have also demonstrated potential to address malnutrition and to improve agronomic practices where other approaches fail: virus-resistance is a prominent example. Some biotech crops enable labor-saving strategies that allow farmers additional time for other activities. At the same time, labor displacement has not proved so detrimental to the rural poor as first hypothesized and there is even some evidence of potential for decreasing gender inequality under certain cropping conditions and village economy (Katage and Qaim 2012; Kouser et al., 2017). Crops with improved yield 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 genetically modified crops were bred for improved agronomic traits, agricultural biotechnology has pursued as well crops with improved human health benefits (Bhutta et al., 2013).

As often in new technology, promises of potential have freqently 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 defense and repair pathways within cells 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: transgenesis, chemical or radiation-induced mutagenesis and conventional breeding. These attributes, coupled with relatively low cost and comparative freedom from regulatory encumbrances, have enabled genome editing to revolutionize basic molecular-biology research and take it to an entirely new level.

Genome editing systems based on clustered regularly interspaced short palindrome repeats (CRISPR)/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 large ethical issues and property questions 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 some of humanity's greatest challenges, and, reciprocally, some social, economic and ethical questions requiring coordinated responses to move forward.

2. Agricultural development

The greatest challenge is adequate nutrition for both farm and non-farm families alike, with more sustainable, nutrient-rich and affordable crops. Even farm families often far below threshold incomes for access to adequate nutrition. Both farm and non-farm families need more

income, affordable food and healthier diets. While approximately 800 million people today are undernourished (meaning that they consume an inadequate number of calories per day), more than half of the world's population is malnourished (meaning that they lack accessibility 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 but are found everywhere. Although the proportion of people living in extreme poverty (on less than $1.25 a day) has decreased steadily over the past twenty years, these gains from rapid advances in GDP have yet to reach sufficiently 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 thirty to forty years, and much of this increase is predicted to take place in poorer countries (International Food Policy Research Institute, 2014).

The 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 salinization 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 FAO that agricultural productivity must double by 2050 to feed the world adequately. Last year's 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 fruits and vegetables, and thus 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 food-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 use of biotechnology in cotton, but have to date been reluctant to allow commercialization of food crops because of social and political resistance (Herring and Paarlberg 2016). Will genome editing face similar obstacles or present a new world of developmental opportunities in food production quite different from those facing 'GMOs'?

3. Agricultural biotechnology

What is a 'GMO'? There is much uncertainty among citizens and regulators as to where the bright line distinguishing varieties of plant breeding one from the other should be drawn (Johnson 2015). 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 are restricted in much of the world.

Misgivings about biotechnology often target the 'unnatural' alteration of a crop's genome by rDNA. What most consumers do not realize 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 1930's, '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). Mutagenized plants face neither stigmatization as GMOs nor special regulation. Indeed foods that sell at premium prices for

being labeled ‘organic’ may be produced with mutagenized plants, in practice if not in purist theory (Nuijten, Messmer, and Lammerts van Bueren, 2017).

Genetic engineering in a broad sense 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 United States, genetically modified (GM), or ‘transgenic’ crops have been commercially available since 1996 (ISAAA, 2014). 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% of all transgenic crops cultivated across the world are herbicide tolerant (ISAAA, 2014). Herbicides can be sprayed on these crops without causing damage to the crop itself while the growth of neighboring weeds is retarded. Insect resistance is the second most commonly used 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 which 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 being 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) also could 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 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. Long delays from multi-year field trials and legal challenges have 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. Multi-gene traits such as yield have proved more elusive. The frontier looks different with the advent of genome editing.

4. Genome Editing Technologies

Genome editing does not require the introduction of new gene sequences, separating it decisively from transgenic plants. 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 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. Moreover, since genome-editing technologies can offer improvements to practically any organism, not only plants can be altered. Genome editing has found a place in livestock development, veterinary science and – perhaps most importantly for normalization of the technology – human health and medicine.

In general, genome editing utilizes various defense strategies developed by bacteria to target specific sequences of DNA and cleave those sequences at targeted sites with nucleases -- enzymes that cut DNA. The technology is then able to make use of DNA repair mechanisms found already in the cells of all organisms, and, by repairing the sites of cleavage, establish specialized 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 characterization 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; applications are only now becoming realized both in research laboratories as well as in the field. Various technologies which fall under the umbrella of genome editing are presented in Appendix 1).

5. 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 synthesized 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 harbored within our own GI tracts) 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 realized. 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 has virtually blossomed overnight (Cardi et al., 2016)

At the moment, genome-editing technologies are being specifically optimized for all major crop types. Often a proof of concept is first sought out through the demonstration that a previously well characterized 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/fruit size, color, 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 will improve vigor, 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 then be used to generate a second generation of newly genome edited crop varieties that are even better able to manage in a rapidly changing world (Liu et al., 2016, Nangpiur et al., 2016). The next section provides examples of some of the traits that are under examination for economically important crops.

5a. 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., (2014) 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% 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 is resistant to powdery mildew, a disease that is a threat to food security (Huang et al., 2016).

5b. Maize. CRISPR/Cas9 has been used as a tool 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 maize immature 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 antinutrient phytase has also been generated using 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 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.

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 *Ustilago maydis*. 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.

5c. Rice. Genome editing has been extensively used to modify rice for a number of purposes (Li et al., 2016a, b, Xu et al., 2017). The authors 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 by using CRISPR-Cas9 to alter a gene that is involved in the plant stress response (Wang et al., (2016a, b). 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., 2016).

5d. Soybean. Genome editing technologies have also been employed for soybean. Du et al., (2016), used CRISPR/ Cas9 to alter soy flower size and color. The genome editing technique for soybean has been further optimized 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).

5e. 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 which produced citrus canker injects a protein into infected citrus plant cells that suppresses plant defense 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 tumor 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 were developed that were resistant to this disease (Jia et al., 2016).

5f. 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 color 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.

6. Genome-edited livestock

For the past few years, genome edited livestock, including pigs, cattle, sheep, goats and chickens have been coming to today's 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 thus are spared from 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 utilized 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, for example, or to create disease-resistant livestock, such as pigs which are resistant to African Swine Fever, dairy cattle which are resistant to the parasite that causes sleeping sickness, or chickens which 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).

7. 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, Mendall et al., 2016).

Today, genome-editing studies have been conducted using cell culture and animal trials, including non-human primates, to realize authentic changes to disease status (Niu et al., 2014, Stone et al., 2016, Wang and Qi, 2016, Zhou et al., 2016). For example, the genetic disease cystic fibrosis (CF) 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 CF 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 et al., 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 realized before. As of now, the technology is too new for adequate appraisal either of potential or of social implications (Singh et al., 2016). Who will decide? Who will govern?

8. 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 conceptualized in the 1960's by an entomologist who hypothesized that mosquito breeding programs could be set up so that male offspring could be favored due to the identification of a male-producing factor that is expressed from the genome of some male mosquitoes. As a result, release of male mosquitoes harboring 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 was 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% 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 modeling 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 of the benefits and risks of gene-drive mosquito technologies. The ecological discussion is devilishly 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, or eliminate weeds, and/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 (vide woolly mammoth project).

For example, in an effort to protect the biodiversity of native plant species in the US, gene drives are being developed to suppress the spread of the non-indigenous spotted knapweed *Centaurea maculosa*. Originating from Eastern Europe, the spotted knapweed was introduced into the United States in the 1800s. It spread rapidly, damaging ecosystems and causing soil erosion. A gene drive solution could spread throughout 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, that 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 for the use of gene drive in plants would be the elimination of pigweed (*Amaranthus palmeri*) 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 could genome edited crops be used in conjunction with gene drive to eradicate weeds, they can also be designed to eliminate pests. Gene drive crops could be designed that no longer will act as hosts for insect and microbial (fungal, bacterial and virus) pathogens. 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 reapplied over time, because plants will 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 destabilizing current ecosystems that brings pause to the idea of applying gene drives without a binding social contract with all stake-holders across the globe at the table.

9. 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 the human suffering 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,’ inducing a slippery slope of ethical degeneration, leading 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 people who were previously doomed from birth 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, genome editing offers a quantum leap from transgenesis in potential. The same is arguably true on the risk side of the equation once gene drives are on the table. There is no way to predict confidently 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 incomes in the field, or inducing resistance to drought in some plants and not others becomes conceivable. We 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 which already naturally reside in the human gut makes 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 via 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 commercialization 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 impact 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 be the ecological impact in the long term? If we can generate plants that are able to tolerate a spectrum of herbicides, what would be the net effect on environmental sustainability? How would we know?

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

10. **The End of the GMO Debate?**

Regulation of GMOs around the world roughly follows a conceptual divide between the US and Europe (Paarlberg 2001): permissive or precautionary. In the US, regulations favor 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 local and global utility. There is no universal standard for how cautious is cautious enough. 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 or risk – to food safety and environment -- has disrupted trade between the US and the EU and, as a result, among their trading partners. In addition of differences of risk assessment, a second objection to GMOs that divides publics is that of intellectual property and patents. Because relatively few firms have dominated existing technology, many worry that GMOs enable monopolization 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 the objections to biotechnology on grounds of monopoly built on intellectual property.

There are two reasons to expect more acceptability of genome-edited crops compared to 'GMOs.' First, patents are national and need not be universally accepted to invite investment in research and development: less is at risk. Moreover, patents are in no sense permanent rules of the game but are continually challenged in courts: these are not structures but playing fields on which contestants contend. In the US, the long contest of genome editing technology 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, 02/22/2017). It seems likely that the lower cost of entry and shortened development time will generate more players with a more competitive playing field. Monopoly or oligopoly will be far harder to establish or sustain. Concentration in the industry is bound to fall, and the dominance of internationally traded commodities such as cotton and maize will likely recede in favor of a broader palette of transformation of now-ignored crops.

Second, the objection to property rights is that first movers attain a privileged position leading to oligopoly or monopoly. First movers are favored by high capital requirements. Genome edited plants are less likely than are 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. Potential for industry concentration in the current major players geographically would also be reduced.

However, 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 monopolized 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 biosafety scientific capacity (Herring and Kandlikar 2009). 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 a stigma to the plants, as happened with GMOs. Removing obstacles of regulation and stigmata of the GMO from genome-edited crops would presumably draw more investment in agricultural development (Kolady and Herring, 2014).

Will genome-edited plants be coded as ‘GMOs’ or not? Sweden, Canada and the US are saying, so far, no. 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 mutagenized plants than transgenic plants in which incorporation of a transgene is uncertain. Indeed, with the progress of synthetic biology, it becomes increasingly possible to synthesize a gene or sequence rather than to find, isolate and transfer it from another species. These facts should remove much of the objections on grounds of ‘unnatural’ plants that violate the order of species on Noah’s ark.

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 – an a natural part of the plant's cellular machinery. As a result of these similarities, crops edited in this fashion currently bypass the regulatory frameworks of many regions of the world (Wolf et al., 2016). Organic farmers can grow mutagenized crops, without labels or special regulatory approvals, and do, all over the world.

In contrast to these minimally edited genomes, 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.[2] 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. To complicate matters further, does it matter if the sequences actually come from living material or are synthesized *de novo*?

The vector sum of regulatory politics may end up splitting rather than lumping genome-editing technologies. In that case, the degree of regulatory oversight of genome-edited crops in these latter cases could depend on the type of DNA repair process used, the nature of the trait added and the pre-existing regulatory structure for any 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.

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[2] We often use the essentializing and biologically meaningless short-hand of 'human genes' and 'fish genes' – eg in the attack on 'fish genes in tomatoes' – when in fact genetic instructions are constant regardless of organism in which they appear. Many 'human genes' are the same as those in not only simians, but bananas and protozoa as well.

The variance among genome-edited plants described above adds another layer of difficulty in defining what exactly is 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 go lumping and splitting new cultivars? In the absence of demonstrated hazard, how is risk assessed differentially? If there are no traces of transgenic material or transformation, how could a regulatory regime possibly claim to be practicable? This conceptual and practical morass suggests the end of the GMO as a workable frame for regulating plant breeding (Johnson 2015).

Because the category 'GMO' *itself* is artificial, in no sense natural, or recognized by nature, its boundaries are not robust for any significant predictive purpose in agriculture. Nature does not code plants as GMOs or not GMOs – these are purely political conventions based on social mobilization and regulatory precedents. These human constructions vary over time and space. Nature does not care, and makes its own transgenic and mutagenized plants, completely indifferent to how our species codifies them (Kyndt et al 2015).

We can confidently predict that there will be significant controversy over how to classify and regulate or normalize genome-edited crops. Or what regulatory structures will be allocated authority to decide their fate in the market. Ministries of Environment and their political bases tend to lean toward precaution, Ministries of Agriculture toward permissive or promotional stances. Because CRISPR is so versatile as a platform, we expect regulation to be product not process, based. Whatever the regulatory forum or politics in particular places or times, the legal status of CRISPR-derived products is unlikely to be consistent, generalizable or enforceable. Like the problem with transgenes in oils or processed products, traceability will present a huge hurdle.

We can less confidently predict the destabilization, perhaps disappearance, of the GMO as a regulatory construct. There is now 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. Objections to the 'GMO' include dominance by multinational firms capable of

investing resources and leaping over regulatory bars that discourage competition. Genome-editing technologies have less potential for monopoly, greater utility, broader applicability, and evidently universal applicability -- more democratic access on a more level playing field. Transgenic technologies will wither away, and with them the 'GMO.'

Conclusions: After the GMO

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 emotional 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; 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 biosecurity frameworks for responsible regulation. More daunting, any workable mechanistm 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, Curnutte, Sherkow and Scott, 2017)

Ethical license would seem a sensible solution but has two great flaws. First, it is not clear that so versatile and available a technology will necessitate patents before deployment. Second, suppose a patent contains an ethical license: how do patents get

enforced? Patent laws are national, and idiosyncratic, not global; battles over interpretations in the courts go on for years, especially in technically complex platforms (consider lines of code in gaming platforms). Even with transgenic plants, bio-property in seeds has proved virtually impossible to enforce internationally (Herring 2007).

While CRISPR-Cas9 technology becomes more effective and easier to use, research into 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 – always arbitrary in definition -- is becoming even more problematic as a basis for regulation and trade; it is even more decisively 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).

There is no robust and parsimonious explanation for differences in acceptance and rejection of agricultural biotechnology across countries or across time. Variables that delineate common political rifts in international trade and politics fail to explain variation (Herring and Paarlberg 2016). The one constant is a risk-utility balance, but of course risk means different things to different segments of civil society, as does utility. So the vector sum is filtered through structures of regulatory mechanisms and their associated political ecology: Agriculture vs Environment ministries, eg. Opposition has targeted unnatural plants contaminated by genetic sequences from other species, including viruses and bacteria. Regulation of ‘the GMO’ has restricted the market and dimmed development, leaning toward internationally traded commodities developed by very large firms. The risk-utility balance for urban consumers has been negative, though the risk itself is hypothetical, not hazard-based.

This paper has shown a fundamental alternation of the risk-utility balance. Just as rDNA transgenic drugs are regulated not as GMOs but as normalized pharmaceuticals, precisely becuas of their risk/utility balance, so too will new techniques shift that political equation. Moreover, the frontiers of plant breeding are moving away from transgenesis, which has been the dominant regulatory test of what constitutes a plant that requires special regulation -- a 'GMO' or 'LMO.' Biologically, and so far legally, gene-edited plants are closer to mutagenized crops – common and considered GMOs nowhere – though mutagenized in a targeted way, not by scrambling the genome with unknown random consequences. Since mutagenized agricultural plants have been

immune to regulation as ‘GMOs,’ this political battle for definition will shape the future diffusion of agricultural – and other -- biotechnology globally. Because of the utility of new techniques for consumers and farmers alike – unlike transgenic plants for the most part – as well as for the environment and human health, the risk-utility balance is being fundamentally altered.

It is then 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 with unprecedented challenges, particularly with the advent of gene drives. We expect these legitimate and consequential questions -- practical, legal and ethical -- to replace the irresolvable and largely pointless ‘GMO’ debate.

Appendix 1. Genome Editing Players (by Kathleen Hefferon)

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 recognizes and binds to relatively long DNA sequences (14- 40 nucleotides long), then cleaves the DNA (Yee, 2016, Zhu et al., 2016). Since all of 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 program 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.

4b. 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 recognizing 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.

4c. TALENs As a technology, TALENs utilize 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.,m 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 TALENsS is the difficulty of assembling the DNA binding domain (Merkert and Martin, 2016).

4d. 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 ribonucleoprotein 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 recognize 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 localizing 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.

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